

Chapter 5

Epidemiological Evidence: IgE, Allergies, and Hematopoietic Malignancies

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Abstract This chapter presents an overview of recent epidemiologic studies that address the association between atopic diseases and IgE and the risk of hematopoietic cancers, including a discussion of potential biological mechanisms. The strongest and most consistent (inverse) associations have been observed between allergic conditions and childhood leukemia, especially acute lymphoblastic leukemia. The cumulative evidence for an inverse association between atopy and non-Hodgkin lymphoma is suggestive, but the inconsistency of the associations with specific types of atopic conditions, the lack of any associations observed in most cohort studies, and the correlation between timing of diagnosis and decreasing IgE levels warrant further clarification. No consistent evidence has been found supporting an association between atopy and either plasma cell neoplasms or Hodgkin lymphoma.

There are several potential mechanisms by which allergic conditions could contribute to the risk of hematopoietic cancers. These include features of allergic responses (IgE, mast cell products) that may mediate enhanced antitumor immune responses, as well as allergy-associated changes in immune responses that inhibit immune cell activation, resulting in less exposure to DNA mutating and potentially oncogenic activities.

5.1 Introduction

This chapter presents an overview of recent epidemiologic studies that address the association between atopic (e.g., allergic) conditions and IgE and the risk of hematopoietic cancers, as well as a discussion of potential biological mechanisms. Since many hematopoietic cancers are malignancies that originate from cells of the immune system, including B and T cells, there are unique potential interactions

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within the immune system environment present in allergic individuals that may contribute to the etiology of these cancers.

Hematopoietic cancers, or cancers that arise from blood cells, include myeloid and lymphoid leukemias and Hodgkin and non-Hodgkin lymphomas (NHLs), as well as plasma cell malignancies (Table 5.1). These cancers reflect and parallel the entire developmental spectrum of the cells of the hematopoietic system. For example, most leukemias are cancers that arise from progenitor cells for lymphocytes, monocytes, and other white blood cells; lymphomas represent cancers that arise from mature lymphocytes; and plasma cell malignancies represent cancers that correspond to terminally differentiated B lymphocytes. A brief description

Table 5.1 Incidence and incidence rates of the major hematological cancers, 2000–2005, SEER^a

	Males		Females		Total	
	No. cases	AAIR ^b	No. cases	AAIR ^b	No. cases	AAIR ^b
Leukemias						
Acute lymphocytic (ALL) ^c	3,937	1.74	3,060	1.35	6,997	1.55
ALL < 14 years at diagnosis	2,107	4.18	1,696	3.53	3,803	3.86
Chronic lymphocytic (CLL) ^d	10,175	5.64	6,890	2.85	17,065	4.04
Acute myeloid (AML) ^e	9,287	4.94	7,763	3.27	17,050	3.97
Chronic myeloid (CML) ^f	2,827	1.43	2,180	0.92	5,007	1.15
Non-Hodgkin lymphoma (NHL) ^g	38,609	20.07	33,237	14.04	71,846	16.73
Hodgkin lymphoma (HL) ^h	6,683	3.06	5,588	2.43	12,271	2.72
<15 years at diagnosis	332	0.66	217	0.45	549	0.56
15–45 years at diagnosis	3,703	3.62	3,387	3.42	7,090	3.52
45+ years at diagnosis	2,648	3.83	1,984	2.41	4,632	3.06
Multiple myeloma and plasmacytoma ⁱ	12,969	7.05	11,035	4.62	24,004	5.66

Software: Surveillance Research Program, National Cancer Institute SEER*Stat software (www.seer.cancer.gov/seerstat) version 6.4.4.

^aSurveillance, Epidemiology and End-Results Program of the National Cancer Institute (www.SEER.gov).

^bAge-adjusted incidence rates/100,000 population at risk, age-adjusted to the 2000 US standard population age distribution standard (United States Census P25–1130).

^cInternational Classification for Diseases for Oncology Third Edition morphology codes 9835–9837.

^dInternational Classification for Diseases for Oncology Third Edition morphology codes 9823.

^eInternational Classification for Diseases for Oncology Third Edition morphology codes 9840, 9861, 9866, 9867, 9870–9874, 9891, 9895–9897, 9910, 9920, 9930–9931.

^fInternational Classification for Diseases for Oncology Third Edition morphology codes 9863, 9875–9876.

^gInternational Classification for Diseases for Oncology Third Edition morphology codes 9670–9719, 9727–9729.

^hInternational Classification for Diseases for Oncology Third Edition morphology codes 9650–9667.

ⁱInternational Classification for Diseases for Oncology Third Edition morphology codes 9731–9732, 9734.

of the biological origin is included, along with a review of the epidemiology relevant to atopic conditions, for each of the major types of hematopoietic neoplasms.

Hematopoietic cancers are unique in that the cell of origin for many of these cancers are the cells that make up the immune system—the same cells involved in immunoregulation, mediation of allergic responses, and antitumor immunity. Therefore, the link between allergy and hematopoietic cancers may be significantly more complex than that seen between allergy and other forms of cancer because the immunoregulatory mechanisms that promote, or inhibit, allergic responses may also induce immune cell activities that protect from, or promote, the development of hematopoietic cancers.

5.2 Methods of Review

We used a wide range of search terms in our review of papers to identify all epidemiologic studies that examined the association between the following conditions and the risk of hematopoietic cancer: atopy, allergy, asthma, hay fever, eczema, atopic dermatitis, and IgE. All of these conditions are associated (to varying degrees) with a Th2-skewed immune response and elevated IgE levels. However, it is important to note that certain conditions are heterogeneous, notably asthma, and include non-allergic subtypes that may not be associated with elevated IgE levels. Only a handful of studies measured IgE or other objective indicators of atopy [1–4]. Most epidemiological studies have not focused solely on allergy and IgE, and the exposures are thus usually assessed as one in a long series of questions such as “Have you ever been diagnosed with...” or “Have you ever experienced [symptoms of allergy],” therefore misclassification due to imprecise exposure measurement is common. We generally excluded studies that used hospital-based controls since bias due to control selection would be likely in such studies, except when these studies were included in larger pooled analyses in combination with studies that used population controls. We also excluded case–control studies with less than 100 cases, and cohort studies with less than 10 expected cases, since these would be unlikely to have adequate power to detect a reasonable association. Finally, because acquired immune deficiency syndrome (AIDS) is not representative of the normal immune response, we have excluded studies of patients with AIDS-related hematopoietic malignancies except for those that were included in a comprehensive population-based study (e.g., [5]).

Epidemiologic studies included in this review fall into two main categories—cohort and case–control studies. In cohort studies, a sample of subjects is chosen based on exposure status and is followed over time for observance of outcomes. The measure of risk is the relative risk (RR), also known as the rate ratio; it is simply the proportion of subjects who develop the outcome among the exposed divided by the proportion of subjects who develop the outcome among the unexposed. Other measures of effect used in cohort studies include the standardized mortality (SMR)

or incidence (SIR) ratios, in which the number of deaths or new cases occurring in the sample of subjects is compared to that expected in the general population of the same demographic composition. Cohort studies are less subject to recall bias since the exposures are documented prior to the onset of disease, but they are expensive to conduct, they may be less powerful for the study of rare diseases such as hematopoietic malignancies, and the exposure may change during the follow-up period.

Case-control studies are the other major type of etiologic study. Here, cases are selected and controls for comparison are chosen, ideally from the same source population as the cases; they may or may not be matched to the cases on age, sex, and various demographic or other factors. The exposure is then measured retrospectively in the cases and controls by self-report, medical record review, and/or physiological measurement, and the prevalence of the exposure between the groups is compared. The measure of risk in case-control studies is the odds ratio (OR), which is the close approximation to the RR when certain assumptions are met (e.g., the outcome is rare). The OR measures the likelihood that cases were exposed relative to the likelihood that controls were exposed. An OR or RR of 1.0 signifies no association at all between the exposure and the outcome; an OR or RR greater than 1.0 indicates that the exposure is more common among those affected and is compatible with an increased risk of developing the outcome, and an OR or RR less than 1.0 indicates that the exposure is less common among those affected by the disease and therefore is consistent with a protective effect. By convention, estimates representing SIRs and SMRs are multiplied by 100, and thus 100 signifies no link between exposure and outcome.

The preferred measure of statistical significance for epidemiological studies is the 95% confidence interval (CI), which is the range of values that would include the true risk estimate (e.g., OR or RR) 95 times, were the same study to be repeated 100 times. Another way to interpret the 95% CI is that there is 95% certainty that the true risk estimate falls within the interval. If 1.0 falls within the 95% CI range, then a 1.0 OR or RR signifying a null association cannot be ruled out. The advantage of using the 95% CI over a simple *p*-value is that more information is provided, especially when no link is demonstrated. When the 95% CI range is very wide, the decrease in precision may be due to a small sample size. Conversely, when the sample size is very large, the range becomes very narrow providing more confidence in a positive finding (when 1.0 is outside the range) or a negative finding (when 95% of the possible values are all close to 1.0). Throughout the tables, any 95% CI that excludes the null hypothesis (therefore consistent with a finding of statistical significance of $p > 0.05$) is indicated by boldface type.

5.3 Leukemias

Leukemias are cancers that are characterized by the abnormal proliferation of white blood cells in blood and bone marrow. They are a heterogeneous group with

different pathologic and cellular characteristics, etiology, age of onset, and clinical outcome. Acute leukemias are distinguished by increased numbers of immature blood cells, while chronic leukemias are characterized by elevated numbers of more mature abnormal blood cells. Leukemias also can be categorized by the normal type of blood cell that they correspond to, with lymphocytic leukemias corresponding to lymphocytes and myelogenous leukemias to cells that form granulocytes. Broad classifications are usually made for use in epidemiologic studies according to the cell of origin (myeloid or lymphoid) and the clinical course (acute or chronic), each of which has distinct epidemiologic and etiologic risk patterns [6]. There are numerous clinical subclassifications based on cell-surface markers and chromosomal rearrangements, many of which are correlated with prognosis but not necessarily etiology. Many early studies conducted prior to the availability of the technology to distinguish these subtypes simply combined all types of leukemia under one heading, obscuring any subtype-specific effects. Where subtypes have been examined separately, some general patterns emerge (Tables 5.2 and 5.3).

Childhood acute lymphoblastic leukemia (ALL) has been inversely linked to various atopic conditions in four questionnaire-based studies (from parent interviews) [7–10]. Two studies based on Swedish and British national medical record databases found a decreased risk linked to asthma [11, 12] and a number of other atopic conditions [12]. One medical record-based study [13] found a positive association between allergic and atopic conditions and childhood ALL based on a small number of cases relative to the other studies.

Hay fever specifically was linked to a decreased risk of childhood ALL in all four studies that examined this exposure [8–10, 12]. Eczema was inversely associated with risk in two [9, 10] out of the five [8–10, 12, 13] studies. Note that risk estimates were below 1.0 but the 95% CI did not exclude 1.0 [8, 12]. Only one of seven studies found that asthma was negatively associated with ALL [7], and six found no association [8–13].

Allergies to specific substances have not been consistently associated with ALL [8–10, 13]. Two groups examined the much less common childhood acute myelogenous leukemia (AML) and reported no association with any type of atopy [9, 12]. In summary, some forms of atopy were consistently associated with a decreased risk of childhood ALL, but not AML.

The evidence on adult leukemia subtypes is mixed. Adult ALL, which is quite rare, (Table 5.1), has generally not been linked to allergic conditions (Table 5.3), excepting penicillin allergy [14]. AML was inversely associated with an increasing number of allergies in a small case-control study [15]. Hay fever has been inversely linked to AML in one study [16]; a borderline increased risk was found in another [17]. No association has been found between asthma [11, 16] or unspecified allergies [16] and adult leukemias. Only one study has examined chronic myelogenous leukemia (CML) with null results for asthma [11].

Table 5.2 Cohort studies examining atopic conditions and risk of hematopoietic cancers

Author	Type of study	Source of subjects	Measure of risk	Exposure validation	Disease validation	Cancer type and subtype	Exposure	Relative risk (95% CI)
Bourget et al. [58]	Prospective cohort (14,407 individuals)	National Health and Nutrition Examination Survey (NHANES)	Rate ratio	Questionnaire	Hospital records and death certificates	Multiple myeloma (n = 18)	1 Atopic condition 2+ Atopic conditions	1.7 (0.3, 10.6) 3.4 (0.0, 285.8)
Kallen et al. [31]	Retrospective linkage study n = (64,346 individuals)	Swedish National Hospital Discharge Registry, 1969–1983	Standardized mortality ratio	Hospital record	Swedish National Death Index	NHL (n = 104) Hodgkin lymphoma (n = 23) Multiple myeloma (n = 39)	Asthma	61 (50.6, 74.0) 68 (45.5, 102.5) 38 (28.3, 51.8)
Mills et al. [59]	Prospective cohort (n = 34,000 individuals followed for 6 years)	Seventh-Day Adventists (California)	Rate ratio	Questionnaire and medical record if hospitalized	Cancer registry linkage or hospital record	Multiple myeloma (n = 23)	Asthma Hay fever Allergic reaction to: Medications Chemicals Bee sting Poisonous plants History of any allergy	0.8 (0.1, 6.1) 1.5 (0.5, 4.6) 1.2 (0.4, 3.6) 1.3 (0.2, 10.1) 1.8 (0.4, 7.7) 1.1 (0.4, 3.4) 1.7 (0.7, 4.0)
Soderberg et al. [22]	Prospective cohort of Swedish twins (n = 16,539 individuals followed for mean of 23 years)	Birth records in Sweden	Rate ratio	Questionnaire	Cancer registry linkage and death registry linkage	Multiple myeloma (n = 78) NHL (n = 112)	Asthma, hay fever or hives Hay fever Eczema Childhood eczema Asthma, hay fever or hives Hay fever Eczema Childhood eczema	0.7 (0.4, 1.4) 0.9 (0.5, 1.7) 0.6 (0.2, 2.0) 0.5 (0.1, 3.9) 1.1 (0.7, 1.8) 1.3 (0.8, 2.2) 1.3 (0.6, 2.6) 2.3 (1.0, 5.3)

Table 5.2 (continued)

Author	Type of study	Source of subjects	Measure of risk	Exposure validation	Disease validation	Cancer type and subtype	Exposure	Relative risk (95% CI ^a)
Tumer et al. [32]	Prospective cohort of healthy volunteers (n = 1,102,247)	ACS cohort	Relative risk	Questionnaire	National Death Index	CLL (n = 67)	Asthma, hay fever or hives	0.8 (0.4, 1.6)
							Hay fever	0.7 (0.3, 1.6)
							Eczema	1.2 (0.5, 3.1)
							Childhood eczema	-
						Multiple myeloma (n = 2,162)	Asthma	1.0 (0.7, 1.4)
							Hay fever	0.9 (0.8, 1.1)
							Asthma + hay fever	1.0 (0.7, 1.4)
							Asthma or hay fever	1.0 (0.8, 1.1)
						NHL (n = 4,2780)	Asthma	1.1 (0.9, 1.3)
							Hay fever	1.0 (0.9, 1.1)
Vesterinen et al. [33]	Retrospective cohort (n = 77,952) (Sweden)	National database linkage	Standardized incidence ratio	National Pharmacy Database	National Cancer Registry	Leukemia (n = 3,782)	Asthma + hay fever	0.9 (0.7, 1.2)
							Asthma or hay fever	1.0 (0.9, 1.1)
							Asthma	0.8 (0.6, 1.0)
							Hay fever	1.0 (0.9, 1.1)
						Males: Multiple myeloma (n = 22)	Asthma + hay fever	0.8 (0.6, 1.1)
							Asthma or hay fever	0.9 (0.8, 1.0)
							Asthma medications	1.1 (0.7, 1.6)
						NHL (n = 28)		
								0.9 (0.6, 1.4)
						Females: Multiple myeloma (n = 16)		
								0.8 (0.5, 1.1)
							Asthma medications	0.5 (0.3, 0.9)
						NHL (n = 37)		1.0 (0.7, 1.4)
								0.9 (0.6, 1.2)

^a95% confidence interval.

Table 5.3 Case-control studies examining atopic conditions and risk of leukemia

Author	Design	Sample size cases/controls	Response rate	Source of subjects/criteria	Disease validation	Exposure validation	Leukemia subtype	Exposure	Exposed cases/ exposed controls	Odds ratio (95% CI) ^a
Becker et al. [19]	Frequency- matched multi- center European study	Cases: 414 Controls: 2,458	Cases: 87.7% Controls: 68.5%	Cases: identified from hospitals and physicians Controls: population- based in 2 studies; hospital-based in 5 studies.	Pathology reports	Questionnaire	CLL ^b (n = 414)	Respiratory allergy Food allergy Medicine allergy	60/375 17/171 52/322	1.0 (0.8, 1.4) 0.6 (0.4, 1.1) 1.0 (0.7, 1.4)
Cooper et al. [14]	Frequency matched	Cases: 748 Controls: 637	Cases: 83% in first recruitment 95% in second recruitment Controls: 80%	Cases: age 18–79 participants in trials Controls: population-based	Pathology reports	Questionnaire	Adult AML ^c (n = 624)	Hay fever Allergies to: bee or wasp Penicillin No. of allergies: 1 2 Asthma Eczema Hay fever Allergies to: bee or wasp Penicillin No. of allergies: 1	94/96 31/32 38/39 141/155 76/58 38/54 27/26 23/96 5/32 12/39 28/155	1.0 (0.7, 1.4) 1.0 (0.6, 1.7) 1.0 (0.6, 1.7) 0.9 (0.7, 1.2) 1.3 (0.9, 2.0) 0.7 (0.5, 1.2) 1.1 (0.6, 2.0) 1.0 (0.6, 1.8) 0.8 (0.3, 2.1) 2.2 (1.04, 4.40) 0.9 (0.6, 1.5)

Table 5.3 (continued)

Author	Design	Sample size cases/controls	Response rate	Source of subjects/criteria	Disease validation	Exposure validation	Leukemia subtype	Exposure	Exposed cases/ exposed controls	Odds ratio (95% CI ^a)		
Doody et al. [17]	Individually matched	Cases: 133	NA	Kaiser Permanente (HMO) Cases: all regional hospitals, national clinical trials and cancer registries Controls: general practice rolls, matched to case by practice	Medical record	Medical record	AML ^c 15+ years	2	15/58	1.1 (0.6, 2.2)		
		Controls: 162						Asthma	10/54	0.9 (0.4, 1.9)		
	Individually matched	Cases: 940			NA	Cases: all regional hospitals, national clinical trials and cancer registries Controls: general practice rolls, matched to case by practice	Medical record	Questionnaire by mother, validated by medical record	ALL ^d (< 14 years) (<i>n</i> = 839)	Eczema	9/26	1.7 (0.7, 4.2)
		Controls: 1,337								Hay fever	17/8	2.5 (1.0, 6.1)
Hughes et al. [12]	Individually matched	Cases: 133	NA	Kaiser Permanente (HMO) Cases: all regional hospitals, national clinical trials and cancer registries Controls: general practice rolls, matched to case by practice	Medical record	Medical record	AML ^c 15+ years	Eczema	9/15	0.6 (0.3, 1.4)		
		Controls: 162						Probable asthma	69/135	1.0 (0.7, 1.3)		
	Individually matched	Cases: 940			NA	Cases: all regional hospitals, national clinical trials and cancer registries Controls: general practice rolls, matched to case by practice	Medical record	Questionnaire by mother, validated by medical record	ALL ^d (< 14 years) (<i>n</i> = 839)	Use of asthma medications	65/129	1.0 (0.7, 1.3)
		Controls: 1,337								Probable hay fever	15/60	0.5 (0.3, 0.8)
Doody et al. [17]	Individually matched	Cases: 133	NA	Kaiser Permanente (HMO) Cases: all regional hospitals, national clinical trials and cancer registries Controls: general practice rolls, matched to case by practice	Medical record	Medical record	AML ^c 15+ years	2	15/58	0.5 (0.3, 0.9)		
		Controls: 162						fever medications				
	Individually matched	Cases: 940			NA	Cases: all regional hospitals, national clinical trials and cancer registries Controls: general practice rolls, matched to case by practice	Medical record	Questionnaire by mother, validated by medical record	ALL ^d (< 14 years) (<i>n</i> = 839)	Probable eczema	217/433	0.9 (0.7, 1.1)
		Controls: 1,337								Use of eczema medications	60/156	0.7 (0.51, 0.97)
Hughes et al. [12]	Individually matched	Cases: 133	NA	Kaiser Permanente (HMO) Cases: all regional hospitals, national clinical trials and cancer registries Controls: general practice rolls, matched to case by practice	Medical record	Medical record	AML ^c 15+ years	At least one atopic condition	260/523	0.9 (0.7, 1.1)		
		Controls: 162						Use of medication for at least one atopic condition	125/288	0.8 (0.6, 1.0)		
	Individually matched	Cases: 940			NA	Cases: all regional hospitals, national clinical trials and cancer registries Controls: general practice rolls, matched to case by practice	Medical record	Questionnaire by mother, validated by medical record	ALL ^d (< 14 years) (<i>n</i> = 839)	Probable asthma	13/135	1.1 (0.6, 2.2)
		Controls: 1,337								Use of asthma medications	13/129	1.2 (0.6, 2.2)

Table 5.3 (continued)

Author	Design	Sample size cases/controls	Response rate	Source of subjects/criteria	Disease validation	Exposure validation	Leukemia subtype	Exposure	Exposed cases/ exposed controls	Odds ratio (95% CI) ^a
Melbye et al. [20]	Frequency matched	Cases: 3,055 Controls: 3,187	Eligible cases: 81 % Eligible controls: 71 %	Cases: hospitals and cancer registry Controls: population registers	Histopathology review and/or pathology report	Questionnaire	CLL ^b	Allergic rhinitis or hay fever Childhood eczema Asthma Elevated IgE Any allergy Asthma Pollen, dust, dander Food, drug, bee Eczema Hay fever Neuro- dermatitis Asthma Eczema Hives Food or drug allergy Allergic reaction to insects and other unspecified substances	161/815	0.9 (0.8, 1.1)
Rosenbaum et al. [8]	Frequency matched	Cases: 255 Controls: 760	Case: 71 % Control: 55 %	Cases: tumor registry at multi center medical center Controls: birth certificate registry	Pathology report	Questionnaire	ALL ^d < 15 years		60/275 43/372 34/153 7/31 8/47	0.9 (0.7, 1.2) 0.4 (0.3, 0.5) 0.6 (0.4, 0.9) 0.6 (0.3, 1.4) 0.5 (0.21, 0.97)
Schulz et al. [9]	Population- based individually matched case- control study (3 centers)	Cases: 1,294 Controls: 2,957	Cases: 80.5–83.6 % Controls: 68.1–71.0 %	Cases: German Childhood Cancer Registry Controls: census-based	Cancer registry	Questionnaire	ALL ^d < 14 years (<i>n</i> = 1,130)		16/58 36/208 38/192 7/35 25/97 9/19 40/123 13/46	0.7 (0.4, 1.3) 0.5 (0.3, 0.7) 0.5 (0.3, 0.7) 0.6 (0.3, 1.4) 0.6 (0.39, 0.99) 1.3 (0.6, 2.9) 0.8 (0.6, 1.2) 0.6 (0.3, 1.1)

Table 5.3 (continued)

Author	Design	Sample size cases/controls	Response rate	Source of subjects/criteria	Disease validation	Exposure validation	Leukemia subtype	Exposure	Exposed cases/ exposed controls	Odds ratio (95% CI ^a)
Severson et al. [15]	Frequency matched	Cases: 164 Controls: 133	Cases: 69.5% Controls: 65.2%	Cases: acute non- lymphocytic leukemia from Cancer Surveillance System (SEER), Age 20–79 Controls: group matched by sex and 5-year age group.	Pathology report	In person interview	Adult AML ^c	Hay fever	11/208	1.0 (0.5, 2.1)
								Neuro- dermatitis	9/192	0.8 (0.4, 1.7)
								Asthma	0/35	-
								Eczema	5/97	0.8 (0.3, 2.1)
								Hives	0/19	-
								Food or drug allergy	3/123	0.6 (0.2, 1.9)
								Allergic reaction to insects and other unspecified substances	3/46	0.9 (0.3, 3.3)
								Any allergy	31/79	0.4 (0.2, 0.6)
								Penicillin	8/16	0.4 (0.2, 1.1)
								Sulfa drugs	3/6	0.4 (0.1, 1.8)
								Other antibiotics	3/9	0.3 (0.1, 1.1)
								Other drugs	6/18	0.3 (0.1, 0.8)
								Dust	10/35	0.3 (0.1, 0.5)
								Eggs/feathers	3/15	0.2 (0.1, 0.6)
								Foods	5/30	0.2 (0.1, 0.4)
								Pollen	12/44	0.2 (0.1, 0.5)
								Bee/insect stings	3/15	0.2 (0.1, 0.6)
								Dogs/cats	15/2	0.1 (0.1, 0.4)
								Other allergies	39/10	0.2 (0.1, 0.5)

Table 5.3 (continued)

Author	Design	Sample size cases/controls	Response rate	Source of subjects/criteria	Disease validation	Exposure validation	Leukemia subtype	Exposure	Exposed cases/ exposed controls	Odds ratio (95% CI) ^a
Spector et al. [13]	Individually matched	Cases: 180 Controls: 718	N/A	Kaiser Permanente (HMO)	Pathology reports	Medical records	ALL ^d < 6 years	No. of allergies:		
								1	19/15	0.7 (0.3, 1.6)
								2	17/6	0.4 (0.1, 1.1)
								3	43/10	0.2 (0.1, 0.5)
								Allergy Dx > 1-year prior	48/173	1.2 (0.8, 1.9)
								Allergy Dx < 1-year prior	21/53	1.8 (1.02, 3.33)
								No. of allergies Dx > 1-year prior:		
								1	34/132	1.1 (0.7, 1.8)
								2	9/33	1.4 (0.6, 3.2)
								3	5/8	2.9 (0.9, 2.6)
								No. of allergies Dx < 1-year prior:		
								1	20/48	1.9 (1.1, 3.6)
								2	1/5	0.6 (0.1, 5.9)
								Asthma Dx > 1-year prior	18/57	1.6 (0.9, 2.9)
								Asthma Dx < 1-year prior	13/21	3.1 (1.4, 7.0)
								Atopy	19/45	2.2 (1.2, 4.2)

Table 5.3 (continued)

Author	Design	Sample size cases/controls	Response rate	Source of subjects/criteria	Disease validation	Exposure validation	Leukemia subtype	Exposure	Exposed cases/ exposed controls	Odds ratio (95% CI) ^a
								Dx > 1-year prior		
								Atopy Dx < 1-year	5/9	3.8 (1.0, 14.3)
								Eczema prior	20/62	1.1 (0.6, 2.0)
								Dx > 1year prior		
								Eczema	0/14	∞
								Dx < 1-year prior		
								Food, drug, bee allergy	8/44	0.7 (0.3, 1.5)
								Dx > 1-year prior		
								Food, drug, bee allergy	4/14	1.2 (0.4, 4.4)
								Dx < 1-year prior		
								Pollen, dust, dander	3/10	1.1 (0.3, 5.2)
								Dx > 1-year prior		
								Pollen, dust, dander	0/0	-
								Dx < 1year prior		
							B-cell ALL ^d	Allergy Dx > 1-year prior	44/158	1.2 (0.8, 1.9)

Table 5.3 (continued)

Author	Design	Sample size cases/controls	Response rate	Source of subjects/criteria	Disease validation	Exposure validation	Leukemia subtype	Exposure	Exposed cases/ exposed controls	Odds ratio (95% CI) ^a
								Allergy Dx < 1-year prior	15/39	1.7 (0.9, 3.3)
								No. allergies		
								Dx > 1-year prior:		
								1	31/121	1.1 (0.7, 1.8)
								2	9/29	1.5 (0.7, 3.5)
								3	4/8	2.4 (0.7, 8.5)
								No. allergies		
								Dx < 1-year prior:		
								1	14/35	1.7 (0.9, 3.5)
								2	1/4	0.7 (0.1, 7.3)
								Asthma	18/57	1.6 (0.9, 3.0)
								Dx > 1-year prior		
								Asthma	10/16	3.4 (1.3, 8.7)
								Dx < 1-year prior		
								Atopy	16/41	2.0 (1.03, 3.96)
								Dx > 1-year prior		
								Atopy	4/7	3.7 (0.9, 16.2)
								Dx < 1-year prior		
								Eczema	19/62	1.1 (0.6, 2.0)
								Dx > 1-year prior		

Table 5.3 (continued)

Author	Design	Sample size cases/controls	Response rate	Source of subjects/criteria	Disease validation	Exposure validation	Leukemia subtype	Exposure	Exposed cases/ exposed controls	Odds ratio (95% CI ^a)
Wen et al. [10]	randomly selected controls			catchment area Controls: randomly selected from census and health system			(n = 261) Adult myeloid (n = 313)	Hay fever Eczema Allergies Hay fever	Not given	1.1 (0.6, 1.9) 0.8 (0.4, 1.4) 0.7 (0.4, 1.2) 0.5 (0.2, 0.9)
	Individually matched	Cases: 1,842 Control: 1,986	Case: 92% Controls: 76.5%	Cases: Children's Cancer Study Controls: RDD ^f	Medical record, slide review	Telephone interview with mother of case	ALL ^d < 15 years	Asthma Hay fever Food, drug allergies Eczema Hives Any condition No, of atopic conditions	135/176 86/150 327/487 91/145 136/166 545/746	0.8 (0.6, 1.0) 0.6 (0.5, 0.8) 0.7 (0.6, 0.8) 0.7 (0.5, 0.9) 0.9 (0.7, 1.2) 0.7 (0.6, 0.8)
	Frequency matched; females only	Cases: 601 Controls: 717	Cases: 54% of total cases; 72% of eligible cases	Cases: Connecticut Tumor Registry (population- based) Controls: RDD ^f and Medicare/ Medicaid	Histologically confirmed by registry	Questionnaire	CLL ^b	1 2+ Eczema	Not given Not given 5/62	0.8 (0.7, 0.9) 0.6 (0.5, 0.7) 1.0 (0.4, 2.6)
			Controls: 69% 47% for Medicare/ Medicaid							

^a95% confidence interval; ^bchronic lymphocytic leukemia; ^cacute myelogenous leukemia; ^dacute lymphocytic leukemia; ^echronic myelogenous leukemia; ^frandom digit dial.

The most common adult-onset leukemia is chronic lymphocytic leukemia (CLL), which has been recently reclassified together with small lymphocytic NHL based on the common origin from a mature, but naïve (no antigen contact), B-lymphocyte [18]. Prior to this reclassification, when considered as a separate entity apart from NHL, CLL risk was not found to be associated with allergy, eczema, or asthma in either case-control [11, 19–21] or cohort [22] studies. However, when physiological measures were used (IgE levels), a decreased risk was reported [20].

5.4 Non-Hodgkin Lymphoma

Lymphomas are solid collectively tumors of lymphocyte origin and are the most common hematopoietic cancers, representing the fifth most common cancer, in terms of incidence rate, and the sixth in terms of cancer deaths, among both men and women in the United States {<http://apps.nccd.cdc.gov/uscs>} (Table 5.1). Most lymphomas originate from mature B lymphocytes, cells that have undergone the initial developmental stages and somatic DNA changes that result in the formation and expression of cell-surface Ig molecules, (the B-cell receptor for antigen). However, this broad classification represents epidemiologically, pathologically, clinically, and etiologically heterogeneous groups of cancers, consisting of Hodgkin lymphoma and plasma cell tumors (see sections below), T cell and natural killer cell lymphoma, and various types of B-cell NHL, including follicular lymphoma, diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma, and Burkitt lymphoma [23, 24]. While some NHLs are infected with Epstein–Barr virus (EBV), the great majority of the B-cell NHL seen in immunocompetent persons are EBV-negative.

Several types of B-cell NHL reflect different stages of B-cell differentiation, originating from naïve B cells, as well as B cells that have undergone various molecular changes that occur after initial encounter with antigen and exposure to helper T cells in secondary lymphoid organs [18]. As these activation-promoting interactions occur in the germinal centers of lymph nodes and other lymphoid organs, these cells are referred to as post-germinal center B cells. The germinal center reaction, which occurs in lymph nodes and other secondary lymphoid organs after initial encounter of B cells with antigen, involves rapid B-cell proliferation and somatic DNA changes that result in both changes in the isotype of the Ig that is produced by these cells (from IgM to IgG or other isotypes), as well as enhanced antigen-binding affinity. These DNA-modifying processes consist of Ig class switch recombination (CSR), which involves the recombination of the Ig variable region segment in Ig heavy chain genes (*IgH*) from IgM to another isotype (i.e., IgG), with the looping out and excision of intervening DNA; and somatic hypermutation (SHM), the active mutation of the variable region of Ig genes, which, when combined with selection by antigen, results in the production of antibodies with enhanced antigen-binding affinity. Errors in both *IgH* CSR and SHM are believed to lead to the seminal molecular lesions for many forms of B-cell lymphoma, as well as other mature B-cell cancers. Translocations/mutations involving other oncogenes, such as *BCL6*, are involved in

other forms of NHL (diffuse large B-cell lymphoma) and are thought to result from errors in SHM.

The association between atopy and NHL has been examined in 14 case-control studies and four cohort studies meeting our criteria, some with [19–21] and some without [5, 11, 16, 17, 22, 25–33] the inclusion of CLL patients (Tables 5.2 and 5.4). The majority used self-reported history as a measure of exposure. Nine case-control studies and one cohort study found a decreased risk of NHL linked to at least one allergic condition [5, 19, 20, 25, 27–31, 34], but the specificity of the exposure was not consistent across the studies. Hay fever or allergic rhinitis was inversely associated in three studies [20, 27, 34], but no association was observed in seven studies [16, 17, 19, 21, 22, 26, 32]. Two general population-based studies [19, 29] and one study of human immunodeficiency virus (HIV) sero-negative homosexual men [5] found food allergies to be inversely linked, but no such link was reported in five other studies (in men and women generally [25–27, 30] and in HIV sero-positive homosexual men [5]). Eczema was inversely associated with NHL in two studies [25, 28], positively associated in one [22], and no statistically significant association was observed in five others [16, 17, 20, 21, 27]. Inconsistent results have also been reported for allergies to specific antigenic substances [5, 26, 27, 29, 30], allergy shots, and asthma [11, 20, 21, 27, 28] (Tables 5.2 and 5.4).

As described above, NHL constitutes a heterogeneous and sometimes progressive group of lymphoid malignancies with subtypes corresponding to the stage of B- or T-cell development of the neoplastic clone. Major subtypes may have different descriptive risk patterns, which would suggest different etiological pathways [23, 24]. Thus examination by subtype is desirable, although often limited by power. Using cases classified by the World Health Organization (WHO)/Revised European-American Classification of Lymphoid Neoplasms (REAL) classification system [18], we [27] found that a history of any allergy, excluding allergy to medications, was inversely linked to DLBCL but not to follicular lymphoma. Conversely, eczema was associated with an increased risk of follicular but not DLBCL [27]. No link between self-reported allergic conditions and these common NHL subtypes was seen in two other studies using the same classification system [20, 21]. However, in an earlier study based on the Working Formulation, reclassified into the WHO/REAL classification, Holly and Bracci [35] reported a negative association between plant allergy and both DLBCL and small lymphocytic subtypes, and between animal allergy and DLBCL. These investigators also reported an increased risk of small lymphocytic and follicular lymphoma linked to hives [35]. T-cell lymphoma (mycosis fungoides) was assessed in a single small study and found to be inversely linked to metal, food allergy and to positive skin prick tests [36].

Two cohort studies attempting to assess the relationship between physiologic measures of atopy and NHL had insufficient power [1, 2]. In a large case-control study, Melbye et al. reported an inverse link between high serum IgE levels and total NHL and CLL subtypes in particular [20]. In a parallel analysis, they conducted a nested case-control study examining IgE levels measured from blood samples collected decades earlier as part of a national pregnancy cohort study [20]. An inverse

Table 5.4 (continued)

Author	Design	Sample size cases/controls	Response rate	Source of subjects/criteria	Disease validation	Exposure validation	NHL subtype	Exposure	Exposed cases/ exposed controls	Odds ratio (95% CI) ^a
Bernstein et al. [25]	Individually matched	Cases: 619 Controls: 619	Cases: 67% Controls: 72%	Cases: population-based cancer registry Controls: neighborhood controls	Pathology reports	Questionnaire	NHL (excluding leukemia)	Eczema Allergy to nuts/ berries	33/52 44/61	0.6 (0.4, 0.96) 0.7 (0.5, 1.1)
								Allergy to insect stings Asthma, hay fever, allergies to: eggs, milk, other foods, animal furs, pollen/dust, fabric, soaps/ perfumes, jewelry/ metals, detergents, cleaning com- pounds, aerosol sprays, stain removers, other allergies	47/64	0.7 (0.4, 1.0)
										All non- significant (<i>p</i> > 0.05)

Table 5.4 (continued)

Author Design	Sample size cases/controls	Response rate	Source of subjects/criteria	Disease validation	Exposure validation	NHL subtype	Exposure	Exposed cases/ exposed controls	Odds ratio (95% CI ^a)
Bracci Frequency et al. matched [34]	Cases: 1,593 Controls: 2,515	Cases: 72% Controls: 78%	Cases: Northern California Cancer Center rapid case ascertainment Controls: RDD ^c of Medicare, Medicaid, frequency matched by age (5year), sex, residence	Pathology report	Questionnaire	NHL (excluding leukemia)	Allergic rhinitis	276/691	0.7 (0.6, 0.8)
Briggs Population et al. based cases; [26] Randomly selected controls	Cases: 952 Controls: 1,691	Cases: 88.1% Controls: 83.1%	Cases: 8 population cancer registries Controls: RDD ^c , by age, region of cancer registry	Histopathological slide review	Questionnaire	NHL (excluding leukemia)	Any allergies: No. allergies: 1 2 3+ Plants Dust Food Animals Medication Insect bite or sting Specific chemical Class of compound Tobacco snoke Fabrics Hay fever	248/476 133/271 60/121 55/84 141/258 84 /166 40/79 37/64 62/112 8/13	1.0 (0.8, 1.2) 0.9 (0.7, 1.2) 0.9 (0.6, 1.2) 1.2 (0.9, 1.8) 1.0 (0.8, 1.3) 0.9 (0.7, 1.2) 1.1 (0.7, 1.6) 1.1 (0.7, 1.7) 1.0 (0.7, 1.4) 1.1 (0.5, 2.8)
Cozen Frequency et al. matched [27]	Cases: 1,321 Controls: 1,057	Cases: 59% Controls: 44%	Cases: population-based cancer registries	Pathology reports	Questionnaire	NHL (excluding leukemia)		11/8 5/20 6/7 7/11 149/155	2.7 (1.1, 7.0) 0.5 (0.2, 1.2) 1.6 (0.5, 4.8) 1.2 (0.4, 3.1) 0.7 (0.5, 0.9)

Table 5.4 (continued)

Author	Design	Sample size cases/controls	Response rate	Source of subjects/criteria	Disease validation	Exposure validation	NHL subtype	Exposure	Exposed cases/ exposed controls	Odds ratio (95% CI) ^a
Controls: RDD ^c and HCFA ^d <65 y/o										
								Food allergies	78/82	0.8 (0.5, 1.1)
								Animal allergies	74/63	0.9 (0.6, 1.3)
								Insect allergies	48/42	0.9 (0.6, 1.4)
								Dust allergies	119/105	0.9 (0.7, 1.2)
								Medication allergies	193/141	1.4 (1.1, 1.8)
								Any allergy	343/298	0.9 (0.7, 1.2)
								Any non- medication allergy	252/210	0.7 (0.6, 1.0)
								Asthma	60/48	1.0 (0.7, 1.5)
								Eczema	52/35	1.2 (0.8, 1.9)
								Hay fever	46/155	0.6 (0.4, 0.9)
							Diffuse large B cell (n = 417)	Food allergies	29/82	0.8 (0.5, 1.4)
								Animal allergies	21/63	0.7 (0.4, 1.2)
								Insect allergies	13/42	0.7 (0.3, 1.3)
								Dust	36/105	0.7 (0.5, 1.1)
								allergies	69/141	1.6 (1.1, 2.4)
								Medication allergies	116/298	0.9 (0.6, 1.3)

Table 5.4 (continued)

Author	Design	Sample size cases/controls	Response rate	Source of subjects/criteria	Disease validation	Exposure validation	NHL subtype	Exposure	Exposed cases/ exposed controls	Odds ratio (95% CI ^a)
Grulich et al. [29]	Frequency matched	Cases: 704 Controls: 694	Cases: 60% Controls: 41%	Cases: New South Wales Cancer Registry, age 20–74 Control: randomly selected from electoral rolls	Not mentioned	Questionnaire	NHL (excluding leukemia)	Allergic to: Food or medicine	222/228	1.0 (0.8, 1.2)
								One or more foods	41/119	0.3 (0.2, 0.4)
								Seafood	8/30	0.3 (0.1, 0.6)
								Preservatives	6/6	1.0 (0.3, 3.3)
								Dairy foods	11/22	0.5 (0.2, 1.0)
								One or more medicines	184/178	1.0 (0.8, 1.3)
								Antibiotics	117/110	1.1 (0.8, 1.5)
								Penicillin	65/62	1.1 (0.7, 1.5)
								Sulfa drugs	39/34	1.1 (0.7, 1.8)
								Analgesics	46/36	1.3 (0.8, 2.0)
Holly et al. [35]	Frequency matched	Cases: 1,304 Controls: 2,515	Cases: 57% (including deceased) 72% exclud- ing deceased Controls: 78%	Cases: population-based cancer registry Controls: RDD ^c and HCFA ^d (for > 65)	Cancer registry validation and pathology review	Questionnaire	Small lym- phocytic (<i>n</i> = 152)	Animal dander	5/179	0.6 (0.2, 1.4)
								Plants	23/691	0.5 (0.3, 0.8)
								Hives	9/62	2.2 (1.1, 4.5)
								Childhood eczema	–	–
								Animal dander	18/179	0.7 (0.4, 1.2)
								Plants	87/691	0.9 (0.7, 1.1)
								Hives	20/62	2.1 (1.3, 3.6)
								Childhood eczema	12/81	0.9 (0.5, 1.8)
								Animal dander	2/179	0.5 (0.1, 2.2)
								Plants	18/691	1.0 (0.6, 1.8)
							(n = 68)	Hives	3/62	1.7 (0.5, 5.5)
								Childhood eczema	2/81	1.0 (0.2, 4.0)

Table 5.4 (continued)

Author	Design	Sample size cases/controls	Response rate	Source of subjects/criteria	Disease validation	Exposure validation	NHL subtype	Exposure	Exposed cases/ exposed controls	Odds ratio (95% CI*)
								Food allergies	80/149	1.0 (0.7, 1.3)
								Allergies to plants, pollen	70/211	0.6 (0.4, 0.8)
								Allergies to dust, chalk, mold	41/91	0.9 (0.6, 1.3)
								Allergy to animals	19/76	0.5 (0.3, 0.8)
								Hives	105/161	1.2 (0.9, 1.5)
								Asthma	50/104	0.9 (0.6, 1.2)
								Allergy shots	25/84	0.5 (0.3, 0.9)
							NHL (excluding leukemia)	Medication	176/307	0.8 (0.6, 0.9)
							Women	Any non- medication	271/452	0.8 (0.6, 0.9)
							(cases = 580; controls = 838)	Food allergies	96/169	0.8 (0.6, 1.0)
								Allergies to plants, pollen	65/159	0.5 (0.4, 0.7)
								Allergies to dust, chalk, mold	43/91	0.7 (0.5, 1.0)
								Allergy to animals	32/64	0.7 (0.5, 1.1)
								Hives	110/221	0.7 (0.5, 0.8)
								Asthma	39/68	0.8 (0.5, 1.2)

Table 5.4 (continued)

Author	Design	Sample size cases/controls	Response rate	Source of subjects/criteria	Disease validation	Exposure validation	NHL subtype	Exposure	Exposed cases/ exposed controls	Odds ratio (95% CI ^a)
Melbye et al. [20]	Frequency matched	Cases: 3,055 Controls: 3,187	Eligible cases: 81% Eligible Controls: 71% Controls: population registers	Cases: hospitals and cancer registry Controls: population registers	Histopathology review and/or pathology report	Questionnaire	NHL	Allergy shots	22/54	0.6 (0.3, 1.0)
								Allergic rhinitis or hay fever	658/815	0.9 (0.77, 0.98)
								Childhood eczema	151/202	0.9 (0.7, 1.2)
								Asthma	251/275	1.0 (0.8, 1.2)
								Elevated IgE	341/372	0.7 (0.6, 0.8)
								Allergic rhinitis or hay fever	170/815	0.8 (0.7, 1.0)
								Childhood eczema	39/202	0.9 (0.6, 1.3)
								Asthma	68/275	1.0 (0.8, 1.3)
								Elevated IgE	110/372	0.8 (0.7, 1.1)
								Allergic rhinitis or hay fever	142/815	1.0 (0.8, 1.2)
								Childhood eczema	32/202	1.0 (0.7, 1.5)
								Asthma	39/275	0.8 (0.6, 1.1)
								Elevated IgE	76/372	0.8 (0.6, 1.0)
								Allergic rhinitis or hay fever	42/815	0.8 (0.5, 1.1)
								Childhood eczema	14/202	1.0 (0.6, 1.8)
								Asthma	22/275	1.3 (0.7, 1.6)
								Elevated IgE	37/372	1.1 (0.7, 1.6)

Table 5.4 (continued)

Author	Design	Sample size cases/controls	Response rate	Source of subjects/criteria	Disease validation	Exposure validation	NHL subtype	Exposure	Exposed cases/ exposed controls	Odds ratio (95% CI) ^a
Melbye et al. [20]	Nested individually matched	Cases: 198 Controls: 594	98% for the cohort	Finnish Pregnancy Cohort	Cancer registry linkage	Serum antigen- specific IgE	NHL*	Allergic rhinitis or hay fever	22/815	0.6 (0.4, 1.0)
								Childhood eczema	5/202	0.8 (0.3, 2.1)
								Asthma	11/275	0.9 (0.5, 1.7)
								Elevated IgE	19/372	0.8 (0.5, 1.4)
								Allergic rhinitis or hay fever	22/815	0.7 (0.4, 1.2)
								Childhood eczema	3/202	0.5 (0.2, 1.7)
								Asthma	10/275	0.9 (0.5, 1.9)
								Elevated IgE	8/372	0.5 (0.2, 1.0)
								Allergic rhinitis or hay fever	18/815	0.8 (0.3, 2.3)
								Childhood eczema	4/202	0.8 (0.3, 2.1)
								Asthma	14/275	1.3 (0.7, 2.4)
								Elevated IgE	11/372	0.6 (0.3, 1.2)
								Detectable versus non- detectable serum IgE	34/127	0.7 (0.5, 1.2)
								< 1-year before dx	1/10	0.3 (0.0, 2.3)
								1–4 years before dx	4/27	0.3 (0.1, 1.0)
								5–9 years before dx	15/49	1.0 (0.5, 1.8)
								≥10 years before dx	14/41	1.0 (0.5, 2.1)

Table 5.4 (continued)

Author	Design	Sample size cases/controls	Response rate	Source of subjects/criteria	Disease validation	Exposure validation	NHL subtype	Exposure	Exposed cases/ exposed controls	Odds ratio (95% CI ^a)
Soderberg et al. [11]	Swedish National Database Study; randomly selected controls	Cases: 18,186 Controls: 149,344	NA	Swedish Cancer Registry, Population Registry and Hospital Discharge Database	Cancer registry	National Hospital Discharge Database	NHL (excluding leukemia)	Asthma Asthma > 10 years prior to dx	254/2,176 101/not given	0.9 (0.8, 1.0) 0.9 (0.7, 1.1)
Vineis et al. [16]	Population based cases; randomly selected controls	Cases: 1,388 Controls: 1,718	Cases: 90% Controls: 81%	Cases: all hospitals in catchment area Controls: randomly selected from census data	Hospital record	Questionnaire	NHL (excluding leukemia)	Eczema Allergies Hay fever	Not given Not given Not given	1.2 (0.9, 1.6) 0.9 (0.7, 1.3) 0.7 (0.5, 1.0)

Table 5.4 (continued)

Author	Design	Sample size cases/controls	Response rate	Source of subjects/criteria	Disease validation	Exposure validation	NHL subtype	Exposure	Exposed cases/ exposed controls	Odds ratio (95% CI) ^a
Whittemore et al. [36]	Frequency matched	Cases: 174 Controls: 294	Cases: 60% Controls: 76%	Cases: population-based cancer registries and hospitals Controls: RDD ^c	Pathology report and slide review (subset)	Questionnaire	Mycosis fungoides	Type of skin allergy:	100/203 64/132 10/36 30/63 8/2	0.7 (0.5, 1.0) 0.7 (0.4, 1.1) 0.3 (0.1, 0.8) 0.7 (0.4, 1.2) 5.9 (1.2, 29.9)
								Any		
								Plants		
								Metals		
								Cosmetics		
								Skin medications (men)		
								Skin medications (women)		
								Other drugs		
								Hair dyes (women)		
								Food (men)		
								Food (women)		
								Insect bites (men)		
								Insect bites (women)		
								Skin allergy prick test positive		
								Skin allergy patch test positive		

Table 5.4 (continued)

Author	Design	Sample size cases/controls	Response rate	Source of subjects/criteria	Disease validation	Exposure validation	NHL subtype	Exposure	Exposed cases/ exposed controls	Odds ratio (95% CI ^a)
Zhang et al. [21]	Frequency matched; females only	Cases: 601 Controls: 717	Cases: 54% of total cases; 72% of eligible cases Controls: 69% 47% for Medicare/ Medicaid	Cases: Connecticut Tumor Registry (population-based) Controls: RDD ^c and Medicare/ Medicaid	Histologically confirmed by registry	Questionnaire	NHL	Eczema	60/62	1.3 (0.9, 1.9)
								Hay fever	96/112	1.0 (0.8, 1.4)
								Asthma	57/66	1.0 (0.7, 1.4)
							B cell	Eczema	40/62	1.1 (0.7, 1.6)
								Eczema	8/62	2.5 (1.1, 5.7)
								Eczema	12/62	2.0 (1.0, 3.9)
							Other	Eczema	10/62	0.9 (0.4, 1.7)
							Follicular	Eczema	3/62	1.1 (0.3, 3.9)
							Marginal zone	Eczema		
							Diffuse large B cell	Eczema	19/62	1.3 (0.7, 2.3)

^a95% confidence interval; ^bchronic lymphocytic leukemia (CLL) included in total NHL, but CLL results shown separately in Table 5.3; ^crandom digit dial; ^dHealth care financing administration.

association between high IgE levels and NHL was most evident within a year of diagnosis, but became progressively weaker as the time prior to diagnosis increased, leading the authors to suggest that the apparent protective effect of atopy was, in fact, due to downregulation of IgE production caused by the developing NHL [20]. This is a plausible interpretation since B-cell lymphoma might interfere with the ability to produce antibodies, including IgE. However this substudy was based on a relatively small sample of subjects, none of the CIs excluded 1.0 and no *p*-value was given, so the results need to be replicated before the conclusion can be generally accepted. De Sanjose and colleagues found that Ig levels of all classes were lower in patients with advanced relative to early stage NHL [4], evidence supporting a pathogenic effect of the cancer on IgE levels.

Overall, the cumulative data suggest a modest protective effect of atopic conditions, especially allergy and hay fever, on NHL risk. This conclusion is strengthened by a recent pooled analysis of 13 case–control studies (13,535 NHL cases, 16,388 controls) [37]. (Only some of the case–control studies incorporated into this pooled analysis are included in this review; the excluded studies did not meet the criteria to be considered individually). The pooled data showed that hay fever and food allergies were associated with a 30% statistically significant decreased risk of several NHL subtypes and of NHL overall. The protective effect persisted when allergies were reported 10 years or more prior to diagnosis, supporting an etiological effect. Thus there appears to be some evidence that decreased IgE levels and/or allergy could be both etiologically and pathogenically linked to NHL. Further cohort studies with objective measures of atopy and allergy are needed to clarify this issue. We (Martínez-Maza and colleagues) are currently conducting a nested case–control study among a cohort of US military recruits to measure indicators of atopy and B-cell activation years prior to diagnosis.

5.5 Hodgkin Lymphoma

Hodgkin lymphoma consists of several etiologically distinct entities defined by age at diagnosis, histologic subtype, and presence or absence of EBV in the genome of the neoplastic cells [38, 39], which have in common the neoplastic giant cell (Hodgkin–Reed Sternberg cell). Childhood (usually defined as diagnosed under 15 years of age) and older age Hodgkin lymphoma (usually defined as diagnosed over 44 years of age) occurs more often in males, is associated inversely with socioeconomic status, is usually EBV positive and consists mainly of the mixed cellularity subtype [38, 39]. Adolescent/young adult Hodgkin lymphoma, diagnosed from 15 to 44 years old, occurs equally among males and females, is more common among those of higher socioeconomic status and in developed countries, and consists primarily of the EBV-negative, nodular sclerosis subtype [38–40]. These etiologically distinct subtypes are often presented singly as one disease, which can obscure true associations if there is heterogeneity between subtypes.

Hodgkin lymphoma, especially adolescent/young adult nodular sclerosis, is associated with clinical and immunological abnormalities suggestive of a T-helper type 2 (Th2)-skewed pro-allergic immune response [41]. About 30–50% of untreated patients have elevated IgE levels [42, 43], and IgE bound to cell-associated CD23 is detected on the neoplastic Hodgkin–Reed Sternberg cells in most Hodgkin lymphoma tumors [44]. Amlot and colleagues reported similar increases in IgE level among both atopic and non-atopic Hodgkin lymphoma patients and in patients with both nodular sclerosis and mixed cellularity subtypes [45]. This group also suggested that clinical atopy was a favorable prognostic factor for Hodgkin lymphoma [45]. A corresponding decrease in T-helper type 1 (Th1) responses, including anergy to skin tests and decreased numbers of cytotoxic T cells and natural killer cells in lymph nodes, is a common occurrence in Hodgkin lymphoma patients [46]. Cohort studies have generally not considered Hodgkin lymphoma separately from NHL, and those that did identified only a small number of cases and thus had insufficient power with unstable risk estimates. Investigators using the Swedish National Death index (death certificates) to identify cases and the Swedish National Hospital Discharge Database to identify asthma hospitalizations found no effect of severe asthma (Table 5.2) [11, 31]; when incident cases were substituted for deaths, a decreased risk of any Hodgkin lymphoma was seen [11].

Null results for atopic conditions were reported from the majority of questionnaire-based studies (Table 5.5) [16, 19, 47–49]) with a few exceptions. Food allergy [19] and hay fever [16] were linked to a decreased risk in two studies. A roughly threefold increased risk associated with childhood eczema was found in twins discordant for adolescent/young adult Hodgkin lymphoma [51]. In a case–case study, asthma, but not allergy, was linked to a borderline increased risk of EBV-positive compared to EBV-negative Hodgkin lymphoma [48].

Thus, overall there has been no consistent evidence to support a relationship between Hodgkin lymphoma and atopy, although the number of studies is small and most combined all Hodgkin lymphoma etiological subtypes together, making interpretation difficult. The high IgE levels and atopic response observed clinically may be a result of pathogenesis; however, more well-designed studies are needed to rule out an etiological effect.

5.6 Plasma Cell Malignancies

Plasma cell malignancies consist of neoplastic monoclonal plasma cells, the highly differentiated B cells that have undergone antigenic stimulation, have passed through germinal centers, and have differentiated further to become antibody-secreting cells. This is the last, or terminal, stage in B-cell differentiation. It is not entirely clear at which stage in B-cell development and differentiation the seminal molecular lesions occur that are necessary for the development of neoplasia. It is possible that some of the initial lesions that lead to the development of plasma cell tumors occur in the germinal centers and result from the same DNA-modifying

Table 5.5 Case-control studies examining atopic conditions and risk of Hodgkin lymphoma

Author	Design	Sample size cases/controls	Response rate	Source of subjects/ criteria	Disease validation	Exposure validation	Hodgkin subtype	Exposure	Exposed cases/ exposed controls	Odds ratio (95% CI ^a)
Becker et al. [19]	Frequency matched	Cases: 340 Controls: 2,458	Cases: 87.7% Controls: 68.5%	Cases: hospitals/ offices Controls: population- based, clinic- based and hospital-based	Pathology reports	Questionnaire	All	Respiratory allergy Food allergy Medicine allergy	49/375 15/171 51/322	0.8 (0.6, 1.1) 0.6 (0.3, 1.0) 1.2 (0.8, 1.7)
		Cases: 565 Controls: 679	Cases: 77% Eligible controls: 58%	Cases: cancer registries and hospitals Controls: RDD ^b and Boston “Town Books” resource	Pathology reports and slide review	Questionnaire	Young adult (15–54)	Childhood allergy Childhood asthma	Not given Not given	0.9 (0.7, 1.2) 0.9 (0.6, 1.3)
Cozen [51]	Individually matched twins	Cases: 188 Controls: 188	Cases: 70% Controls: 70%	Volunteer Twin Registry	Pathology reports and slide review	Questionnaire	Young adult (13–50)	Older age (55–79) Childhood allergy Childhood asthma Eczema Hay fever Allergic reaction Drug allergy Animal or egg allergy	Not given Not given 14/5 21/18 22/19 16/21 10/12	0.6 (0.4, 1.2) 0.5 (0.2, 1.3) 2.8 (1.01, 7.77) 1.2 (0.6, 2.2) 1.2 (0.5, 2.1) 0.8 (0.4, 1.5) 0.8 (0.4, 1.5)

Table 5.5 (continued)

Author	Design	Sample size cases/ controls	Response rate	Source of subjects/ criteria	Disease validation	Exposure validation	Hodgkin subtype	Exposure	Exposed cases/ exposed controls	Odds ratio (95% CI ^a)
Pahwa et al. [49]	Frequency- matched male farm workers and residents	Cases: 119 Controls: 673	Not given	Cases: provincial cancer and hospital registries Controls: provincial health insurance records, voters and telephone lists	Cancer registry or hospital record	Questionnaire	All (males only)	Allergy shots	7/44	0.7 (0.3, 1.7)
Soderberg et al. [11]	Population- based database linkage	Cases 2,394 Controls: 149,344	NA	Swedish Cancer Registry, Population Registry and Hospital Discharge Database	Cancer registry	National Hospital Discharge Database	All	Asthma Asthma > 10 years prior to diagnosis	18/2,176 8/not given	0.6 (0.4, 0.9) 0.7 (0.4, 1.5)
Vineis et al. [16]	Population- based cases; randomly selected controls	Cases: 354 Controls: 1,718	Cases: 90% Controls: 81%	Cases: all hospitals in catchment area Controls: randomly selected from census data and National Health Service	Hospital record	Questionnaire	All	Eczema Allergies Hay fever	Not given Not given Not given	0.7 (0.4, 1.3) 0.9 (0.5, 1.4) 0.5 (0.3, 0.8)

^a95% confidence interval; ^brandom digit dial.

processes that lead to the development of several forms of B-cell lymphoma. What is clear is that these tumors accrue additional molecular lesions during the course of their development. It is also clear that there is a necessary precursor condition consisting of a non-neoplastic clone of identical plasma cells, termed monoclonal gammopathy of undetermined significance (MGUS), which increases with age to a prevalence of about 5% overall in the United States by age 70 [51], with a 1% per risk of transformation by unknown mechanisms to multiple myeloma [52].

Malignant plasma cell tumors consist of solitary (plasmacytoma) or metastatic (multiple myeloma) malignant clones of plasma cells that produce aberrant antibodies or components of antibodies. Plasma cell leukemia is a rarely seen manifestation of plasma cell myeloma [18]. Of all of the isotypes, IgE-producing plasma cell tumors are the least common; only a handful have been reported [53]. Little is known about risk factors for plasma cell tumors other than older age, African-American race, male sex, and obesity [27, 54–57]. Multiple myeloma has a higher mortality rate than other broadly defined types of hematopoietic cancers affecting adults and it is less common; the result is smaller studies with poorer response rates.

Six cohort studies and 10 case–control studies meeting our criteria have had sufficient numbers to evaluate atopy and risk of plasma cell tumors (Table 5.2) [22, 31–33, 58, 59]; (Table 5.6) [11, 16, 17, 19, 49, 60–64]. Questionnaire-based studies have examined the effect of atopic conditions on multiple myeloma risk but definitions range from “respiratory allergy” [19] to “severe allergic reactions” [63], making cross-study comparison sometimes difficult.

None of six studies based on self-report [32, 59, 62–64] or medical record [22] found a link between asthma and multiple myeloma. However, two cohort studies based on separate Swedish national pharmacy–death index record-based linkages found that asthmatics had a decreased risk of multiple myeloma [31, 33], limited to women only in one study [33]. Hay fever has not been linked to multiple myeloma whether measured by self-report [16, 22, 32, 59, 63, 64] or medical record [17].

Results for other atopic conditions have also been inconsistent. A medical record-based study conducted in a large health maintenance organization (HMO) [17] found that multiple myeloma was positively linked to eczema, but four questionnaire-based studies failed to confirm this finding [22, 59, 62, 63]. When stratified by isotype, eczema was associated with an increased risk of IgG, but not IgA, multiple myeloma subtype [63].

An inverse association between allergies and multiple myeloma was reported in one study [62] and in another associated with the IgG subtype only [63]; however, three other studies found no association [16, 49, 60]. Becker et al. reported an inverse link to respiratory allergy but not food or medicine allergy [19]. No association with specific allergies was found in African-Americans [63] or Seventh-Day Adventists [59]. Inoculations with allergen for purposes of desensitization (allergy shots) were not associated with multiple myeloma risk [61, 63], unless over 100 allergy shots were received [61].

Table 5.6 Case-control studies examining atopic conditions and risk of multiple myeloma

Author	Design	Sample size cases/controls	Response rate	Source of subjects/criteria	Disease validation	Exposure assessment	Exposure	Exposed cases/ exposed controls	Odds ratio (95% CI ^a)
Becker et al. [19]	Frequency matched	Cases: 278 Controls: 2,458	Cases: 87.7% Controls: 68.5%	Cases: hospitals/clinics Controls: population-based, clinic-based, and hospital-based frequency matched to all 2,480 NHL ^b cases	Pathology reports	Questionnaire	Respiratory allergy Food allergy Medicine allergy	28/375	0.7 (0.4, 0.98)
								9/171 35/322	0.5 (0.3, 1.0) 1.0 (0.7, 1.4)
Boffetta et al. [64]	Individually matched (4 controls per case)	Cases: 282 Controls: 1,128	NA	American Cancer Society volunteer cohort	Death certificate	Questionnaire	Hay fever Asthma	14/38 5/21	1.6 (0.8, 2.9) 1.0 (0.3, 2.7)
Doody et al. [17]	Individually matched (2 controls per case)	Cases: 175 Controls: 787	NA	Kaiser Permanente (HMO)	Medical record	Medical record	Hay fever Eczema	18/18 25/15	1.3 (0.6, 2.7) 2.0 (1.1, 4.0)
Eriksson [60]	Individually matched	Cases: 239 Controls: 220	NA	Cases: Swedish Cancer Registry Controls: Swedish National Population Register	Cancer registry and medical records validation	Questionnaire	Allergy	20/16	1.1 (0.5, 2.3)

Table 5.6 (continued)

Author	Design	Sample size cases/controls	Response rate	Source of subjects/criteria	Disease validation	Exposure assessment	Exposure	Exposed cases/ exposed controls	Odds ratio (95% CI ^a)
Koepsell et al. [61]	Unmatched	Cases: 698 Controls: 1,683	89% in cases (32% by proxy); 83% of controls	Cases: SEER ^c Cancer registries in Utah, Washington and Atlanta Controls: mixture of RDD ^d , and neighborhood controls	Cancer registry	Questionnaire	Allergy shots (desensitiza- tion) Number of allergy shots 1–99 100+	43/121	0.8 (0.5, 1.2)
Landgren et al. [62]	Frequency matched (females only)	Cases: 183 Controls: 717	57% in cases; 69% in RDD ^d controls; 47% in Medicare/ Medicaid controls	Cases: Connecticut Tumor Registry Controls: RDD ^d and Medicare/ Medicaid for 65+	Connecticut Tumor Registry	Questionnaire	Allergy Asthma Eczema	18/142 15/58 10/58	0.4 (0.3, 0.7) 1.1 (0.6, 2.0) 0.8 (0.4, 1.7)

Table 5.6 (continued)

Author	Design	Sample size cases/controls	Response rate	Source of subjects/criteria	Disease validation	Exposure assessment	Exposure	Exposed cases/ exposed controls	Odds ratio (95% CI) ^a
Lewis et al. [63]	Frequency matched	Cases : 573 Controls : 2,131	63% white cases 67% African- American cases 67% all RDD ^d controls; 73% whites and 78% African- Americans for HCFA ^e controls	Cases: Cancer registries Controls: RDD ^d from 4 centers for 4 cancers	Population-based cancer registry; Ig isotype from medical record (SPEP ^f)	Questionnaire	All cases (<i>n</i> = 573 cases; 2,131 controls)	26/126 38/116 94/411 25/82 53/181	0.8 (0.5, 1.2) 1.2 (0.8, 1.8) 1.0 (0.8, 1.3) 1.1 (0.7, 1.8) 1.1 (0.8, 1.5)
							Allergy shots		
							Asthma		
							Hay fever		
							Eczema		
							Severe allergic reactions		
							Allergies to :		
							Drugs		
							Dust		
							Household products Whites		
							(<i>n</i> = 367 cases; 1,164 controls)	18/89 22/63 54/240 19/57 35/124	0.7 (0.4, 1.3) 1.3 (0.7, 2.1) 0.9 (0.7, 1.3) 1.2 (0.7, 2.0) 0.9 (0.6, 1.4)
							Allergy shots		
							Asthma		
							Hay fever		
							Eczema Severe allergic reactions		

Table 5.6 (continued)

Author	Design	Sample size cases/controls	Response rate	Source of subjects/criteria	Disease validation	Exposure assessment	Exposure	Exposed cases/ exposed controls	Odds ratio (95% CI ^a)
							Allergies to: Drugs Dust Household products African- Americans (<i>n</i> = 206 cases; 967 controls)	53/161 13/61 6/21	1.1 (0.8, 1.5) 0.8 (0.4, 1.5) 0.9 (0.3, 2.4)
							Allergy shots Asthma Hay fever Eczema Severe allergic reactions	8/37 16/53 40/171 6/25 18/57	0.9 (0.4, 2.0) 1.2 (0.7, 2.3) 1.1 (0.7, 1.6) 1.1 (0.4, 2.7) 1.4 (0.9, 2.6)
							Allergies to: Drugs Dust Household products	19/52 9/32 6/15	1.5 (0.9, 2.9) 1.2 (0.5, 2.5) 1.6 (0.4, 1.3)

Table 5.6 (continued)

Author	Design	Sample size cases/controls	Response rate	Source of subjects/criteria	Disease validation	Exposure assessment	Exposure	Exposed cases/ exposed controls	Odds ratio (95% CI) ^a
Pahwa et al. [49]	Frequency- matched case-control study of male farm workers and residents	Cases: 178 Controls: 673	NA	Cases: provincial cancer and hospital registries Controls: provincial health insurance records, voters' lists, or telephone lists	Cancer registry or hospital record	Questionnaire	Allergies Skin prick allergy test	34/166 22/71	0.8 (0.5, 1.2) 1.5 (0.9, 2.7)
Soderberg et al. [11]	Swedish National Database Study; randomly selected controls	Cases: 7,189 Controls: 149,344	NA	Swedish Cancer Registry, Population Registry and Hospital Discharge Database	Cancer registry	National Hospital Discharge Database	Asthma	125/2,176	1.0 (0.9, 1.2)
Vineis et al. [16]	Population- based cases; randomly selected controls	Cases: 263 Controls: 1,718	Cases: 90% Controls: 81%	Cases: all hospitals in catchment area Controls: Census and National Health Service	Hospital record	Questionnaire	Eczema Allergies Hay fever	Not given Not given Not given	0.6 (0.3, 1.3) 1.1 (0.6, 1.8) 0.8 (0.4, 1.5)

^a95% confidence interval; ^bnon-Hodgkin lymphoma; ^csurveillance epidemiology and end results; ^drandom digit dial; ^ehealth care financing administration; ^fserum protein electrophoresis.

In summary, there is no consistent evidence for an etiological connection between atopic conditions and multiple myeloma. Studies with larger numbers using validated exposure history may help us to clarify this relationship.

5.7 Conclusion – Epidemiologic Findings

Epidemiologic studies of atopic conditions and risk of hematopoietic neoplasms are often limited by lack of power, since these cancers are much less common than solid tumors, and also by the relatively subjective measures of atopy and allergy, usually by self-report and not always reliable [65, 66]. In addition, the etiologic heterogeneity of the various subtypes of hematopoietic neoplasms is not fully understood; to the extent that etiologically distinct subtypes are presented as combined entities, subtype-specific relationships with atopy may be obscured.

In spite of these caveats, some patterns emerged. Atopic conditions have been most strongly and consistently inversely linked to childhood leukemia, especially ALL. The cumulative evidence for an inverse association between atopy and NHL is suggestive, but the inconsistency of the associations with specific types of atopy and allergy, the lack of any associations observed in most cohort studies, and the correlation between timing of diagnosis and decreasing IgE levels warrant further clarification. We found no consistent evidence to support an association between atopy and plasma cell neoplasms or Hodgkin lymphoma based on currently available data.

5.8 Potential Mechanistic Interactions between Allergy/Atopy Associated with the Development of Hematopoietic Cancers

There are several potential mechanisms by which atopy/allergy could contribute to the risk for the development of hematopoietic cancers. There may be trivial reasons for any atopy-lymphoid malignancy associations. For example, the presence of a lymphoid malignancy may dampen allergic responses, leading to an apparent protective association of allergy, while, in fact, this association would be a result of the disease process rather than an etiologic factor [20]. However, as indicated above, there is suggestive evidence for an etiologic effect, especially for childhood lymphocytic leukemia and possibly NHL.

Certainly, there are features of allergic responses (IgE, mast cell products) that have the potential to directly inhibit tumor cell growth, including the growth of hematopoietic cancers, by mediating enhanced antitumor immune responses. However, there is little evidence to directly support such a role for IgE and allergy-associated antitumor immune responses in humans, so such mechanisms remain speculative, with further studies needed to elucidate any contribution of antitumor cell IgE-mediated immune responses to protection from these cancers. There are other potential mechanisms that may provide an explanation for decreased risk of

lymphoid malignancies in atopic people, including a relative dampening of B-cell responses mediated by the interaction between IgE and CD23, and/or by a relative decrease in T-helper type 17 (Th17) cytokine responses in atopic individuals (see below). Decreased B-cell activation would be expected to result in a lower frequency of activation-associated oncogenic molecular lesions, which lead to the generation of neoplasms of mature lymphoid origin, such as NHL and multiple myeloma. Additionally, both soluble CD23 (sCD23) and Th17 cytokines have the potential to enhance the viability and growth of B-cell ALL, based on laboratory studies [67, 68].

5.8.1 IgE/Allergy-Mediated Enhancement of Antitumor Immunity

Several recent studies have pointed to a key role for IgE and other allergy-associated forms of immune function in the protection from cancer. In fact, the other chapters in this volume present many specific examples of the important role that IgE and allergy-associated immune responses can play in mediating protection from cancer. These allergy-associated and/or IgE-mediated antitumor immune responses may potentially play a role in protection from the growth of hematopoietic cancers. It is clear that IgE can mediate antitumor responses via the enhancement of various immune effector mechanisms, including antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), antigen presentation, and mast cell responses [69–73].

The especially high affinity of both the high- and low-affinity Fc receptors for IgE are thought to make antitumor antibodies of the IgE subclass more effective at mediating ADCC and additionally may allow enhanced antigen presentation of antigens bound by IgE, resulting in enhancement of the generation of antitumor cytotoxic T cells (CTLs) [69, 70, 74–77]. The two IgE Fc receptors, FcεRI (the so-called high-affinity IgE Fc receptor) and FcεRII (CD23, the “low-affinity” IgE Fc receptor) are actually both relatively high-affinity receptors ($K_d \sim 10^{-10}$ M and $K_d \sim 10^{-7}$ M, respectively) [78, 79], binding affinities that are higher than those seen for most IgG or IgA Fc receptors. This feature of the IgE Fc receptors, combined with their widespread expression on immune system effector cells, including antigen-presenting cells, mast cells, basophiles, eosinophils, Langerhan’s cells, macrophages, dendritic cells, and B cells [73, 80–83], and their signal-transducing properties [83–86], allows the potential for the effective IgE-mediated enhancement of ADCC and CTL directed to tumor cells. Additionally, IgE has been seen to bind to murine NK cells via FcRIII (CD16), the Fc receptor that is responsible for IgG-mediated cellular cytotoxicity, with IgE bound to NK cells in this manner being effective in ADCC and in the induction of cytokine production by NK cells [76].

Other antitumor mechanisms that may enhance in IgE-mediated responses are eosinophil and/or mast cell-mediated antitumor immune responses [82]. Eosinophils have been seen to mediate cellular cytotoxicity directly, providing a potential means

by which these cells, which are stimulated in allergic immune responses, could help control the growth of hematopoietic tumors [70, 87]. Similarly, activated mast cells, which are degranulated on exposure to IgE + antigen, produce a wide range of mediators that can exert direct cytotoxic and growth-inhibitory effects on tumor cells [70, 88].

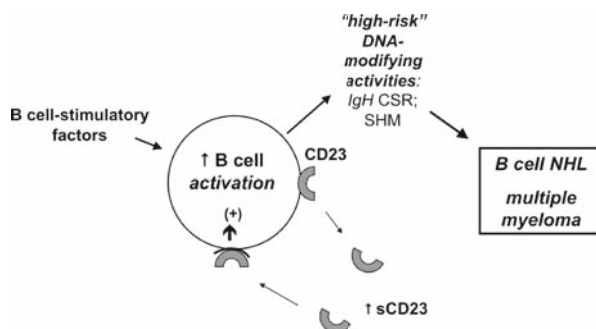
Therefore, the most direct and obvious mechanisms that might link allergic/IgE-mediated responses to protection from hematopoietic cancers involve direct enhancement of antitumor cell effector mechanisms and, in this way, are not fundamentally different from how allergy/IgE may protect from other cancers. It was recently observed that treatment of allergic patients with an anti-IgE antibody (omalizumab, Xolair), which is used for the treatment of asthma [89], results in a doubling of cancer risk, with cancer seen in 20/4127 (0.5%) of omalizumab-treated patients (including one NHL) versus 5/2236 (0.2%) placebo recipients in unpublished studies sponsored by the manufacturer of this agent (Food and Drug Administration, Center for Biologics Evaluation and Research. BLA STN 103976/0, review of clinical safety data: original BLS submitted on June 2, 2000, and response to complete review letter submitted on December 18, 2002). [89]. Another study found that multiple myeloma patients with relatively higher IgE levels had a better survival than patients with lower levels of IgE [90]. These findings are consistent with the view that IgE, or allergic responses associated with the presence of IgE, may participate in anti-myeloma immune responses, even if a history of allergy is not associated with a decreased risk for the development of multiple myeloma (see Section 5.6).

5.8.2 Stabilization of CD23 Expression by IgE

Stabilization of cell-surface CD23 by IgE binding, preventing the release of sCD23, has the potential to dampen activities that may lead to the development of hematopoietic cancers. As noted earlier, errors in DNA-modifying activities that are associated with B-cell activation, including *IgH* CSR and SHM of Ig genes, can contribute to the development of mature B-cell malignancies, such as many subtypes of B-cell NHL. In fact, there is ample evidence that chronic stimulation of B cells, by infection (e.g., HIV, *H. pylori*, EBV, hepatitis C virus (HCV)) or forms of autoimmunity that involve autoantibody production (e.g., Sjogren's), is associated with an increased risk for the development of B-cell NHL [91]. Therefore, activities that generically promote B-cell activation should increase risk for the development of these mature B-cell cancers, while dampening of B-cell activation should, conversely, decrease risk for these cancers. Similarly, factors that promote the growth and viability of pre-B cells and immature B cells may be expected to support the emergence and growth of leukemic cells.

At first glance, it might be expected that IgE-mediated allergies would be associated with an overall enhancement of B-cell stimulation. However, IgE has the potential to dampen the stimulation of mature or immature B cells. One way by

A. non-allergic individuals (low IgE levels):



B. Allergic individuals (High IgE levels):

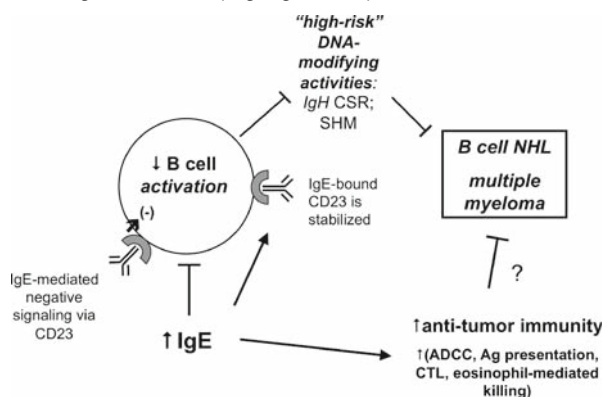


Fig. 5.1 Potential mechanisms by which IgE may prevent the development and/or growth of B-cell tumors. IgE has the potential to dampen B-cell stimulation, and thereby prevents the occurrence of DNA-modifying oncogenic events associated with the genesis of many B-cell cancers, by stabilizing cell-surface CD23, preventing the release of sCD23, which can act as a potent B-cell-stimulatory factor. In addition to this, IgE binding to CD23 also can result in negative signaling. Finally, IgE has the potential to function in antitumor immunity

which IgE could potentially inhibit the stimulation of B cells is by decreasing the release of sCD23 by stabilizing the cell-surface form of this molecule (Fig. 5.1). CD23 is a B-cell surface molecule that is induced on B-cell activation and which also is an Fc receptor for IgE (FcεRII) [78, 79]. Other types of hematopoietic cells also can express CD23. CD23 can be proteolytically cleaved to generate sCD23, a molecule that has cytokine-like B-cell-stimulatory activity, such as the enhancement of CSR [92]. In fact, elevated serum levels of sCD23 have been seen to precede the clinical diagnosis of NHL [93–96]. Additionally, sCD23 has been seen to prevent the apoptosis of ALL cells [67]. Therefore, sCD23 has B- and pre-B-cell-stimulatory properties that are consistent with a role in promoting lymphomagenesis and the viability of leukemic cells.

Binding of IgE to CD23, as would occur in atopic individuals who have elevated IgE levels, can result in the downregulation of sCD23-mediated B-cell activation in several ways: IgE binding to cell-surface FcεRII (CD23) can stabilize this molecule and prevent its cleavage and secretion, thereby preventing sCD23-mediated stimulation of B and pre-B cells [97, 98]. Additionally, IgE binding to FcεRII (CD23) on activated mature B cells may result in negative signaling, again resulting in the downregulation of B-cell activation [99, 100]. There is experimental and observational evidence that supports such a role for IgE: (1) people who have elevated serum levels of IgE have relatively decreased serum sCD23 levels and increased cellular expression of CD23 [101, 102], suggesting that IgE is able to stabilize cell-surface expressed CD23. Additionally, IgE can dampen switching to Ig isotypes other than IgE (inhibits *IgH* CSR), which would be consistent with a role for IgE in downregulating sCD23-mediated activities [101].

Therefore, IgE can interact with FcεRII (CD23) in ways that result in the inhibition of sCD23 release and activity, potentially dampening B-cell stimulation, and thereby decreasing the risk for oncogenic events that can result in the genesis of cancers of mature B cells and/or preventing the enhanced viability of pre-B cell cancer cells by sCD23. This is consistent with a protective role for IgE and atopy for the development of NHL and/or ALL. Interestingly, these interactions do not involve a role for IgE in mediating a direct antitumor cell immune response and would be expected to be unique to the pathogenesis hematopoietic cancers such as NHL or ALL. Future studies, including prospective cohort-based studies, should examine the association of sCD23 levels with the development of hematopoietic cancers of B-cell origin, including ALL and NHL, to more definitively define the association of allergy, sCD23 levels, and the development of these cancers.

5.8.3 The Immune Regulatory Milieu Associated with Allergy may be Less Supportive for the Stimulation of B-Cell Activation and/or Resistance to Apoptosis

People who have IgE-mediated allergies appear to be at decreased risk for the development of ALL and possibly some forms of NHL, while those who have autoimmune diseases that are mediated by aberrant B-cell anti-self responses have a marked increase in risk for the development of B-cell NHL [91, 103]. Both allergy and autoimmunity are characterized by changes in the host immunoregulatory environment, with IgE-mediated responses involving enhanced Th2 activity and autoimmune responses involving the enhanced activity of a recently recognized T-cell subset, Th17 cells [104–106] (Fig. 5.2). These epidemiologic observations suggest, then, that enhanced Th17 activity may be associated with the development of B-cell NHL, a hypothesis that can be addressed in future studies, especially in prospective cohort studies.

Are the properties of Th17 cells consistent with a role in the pathogenesis of these hematopoietic cancers? Th17 cells are a distinct, proinflammatory T-cell subset,

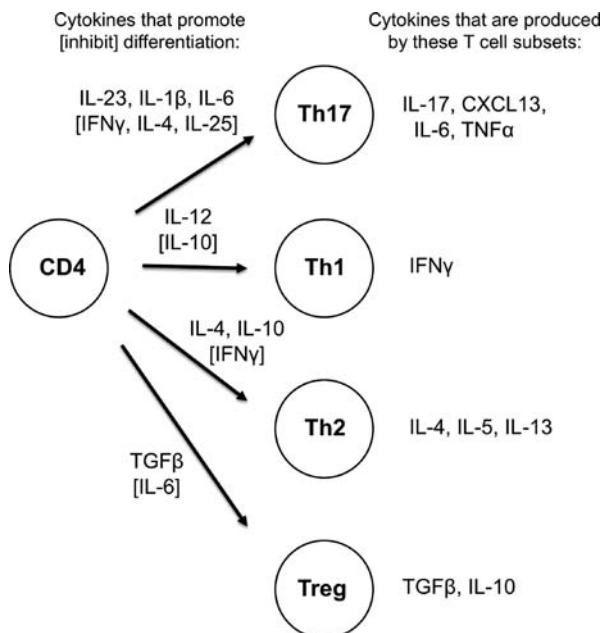


Fig. 5.2 T-cell subsets: distinct patterns of cytokine production. Several subsets of CD4-positive helper T cells have been defined, based on the pattern of cytokines produced by these different subsets, as well as by their unique biological activities

which is functionally characterized by mediating protection against extracellular bacteria and by its pathogenic role in autoimmune disorders [104, 107–110]. Th17 cells characteristically produce interleukin- (IL-) 17, CXCL13 (a B-cell stimulatory chemokine), IL-6, and tumor necrosis factor alpha (TNF α), in contrast to Th2 cells, which characteristically produce IL-4, IL-5, IL-9, and IL-13, or Th1 cells, which produce interferon gamma (IFN γ) [104, 111] (Fig. 5.2).

Extensive evidence links elevated IL-6 and TNF α production with B-cell NHL, including functional, observational, and genetic studies [112–136]. IL-6, another Th17 cytokine, has long been known to induce *IgH* CSR, with IL-6 transgenic mice developing lymphoma-like tumors (plasmacytomas) that are characterized by *myc:Ig* translocations [137]. In prior work, we also saw that elevated levels of serum IL-6 were seen to precede the development of AIDS-associated Burkitt lymphoma [114]. Therefore, Th17 cells produce several B-cell-stimulatory molecules, including several that are known to be associated with an increased risk for the development of B-cell NHL and/or which exert biological effects that can promote the development and/or growth of NHL. Additionally, CXCL13, a Th17 cytokine, has been seen to prevent apoptosis in B-cell lineage ALL [68], suggesting that this Th17 cytokine has the potential to promote the growth of these cancers.

The generation of Th17 cells from naïve Th precursor cells is stimulated by exposure of these cells to IL-23 (an IL-12-like cytokine), IL-1 β , and IL-6 (in mice),

while the generation of Th17 cells is inhibited by IL-4 and IL-25 [104]. Given this, it is unlikely that Th17 cells contribute to the pathogenesis of allergic IgE-mediated disorders, because IL-4 and IL-25 (a powerful inducer of IL-4) are both potent inhibitors of Th17 cell development [104]. Interestingly, Th17 cells appear to be virtually missing in people who have autosomal dominant hyper-IgE syndrome (HIES, “Job’s syndrome”) and decreased in people who have severe allergies [138, 139], suggesting that lack of Th17 activity may be associated with enhanced IgE production.

Several animal studies have examined IL-17 levels in asthma, finding that IL-17 acts as a potent negative regulator of established allergic asthma [140]. Paradoxically, IL-17 also appears to play a role in the initiation of asthma-associated inflammation [141]. Therefore, IL-17 and TH17 cells appear to play complex roles in asthma and may contribute to inflammation associated with asthma but also downregulate the allergic response in sensitized animals [140]. However, little is known about the role of IL-17 or Th17 cells in allergic conditions not associated with asthma.

Although the known properties of Th17 cells are consistent with the activity of this Th subset being decreased in allergy and increased in autoimmunity, therefore, having a potential role in the pathogenesis of NHL and/or ALL, much additional work needs to be done to elucidate the role of Th17 cells and cytokines in allergy and in the etiology of hematopoietic cancers. Are levels of Th17 activity decreased in allergic individuals? Is Th17 activity (elevated levels of Th17-produced cytokines) associated with the subsequent development of NHL or ALL? These hypotheses can be tested, in both epidemiologic and functional studies, especially in prospective cohort studies that have access to precancer diagnosis materials and accurate clinical information on allergic and autoimmune diseases.

5.8.4 Conclusion – Potential Interactions between Allergy/Atopy Associated with the Development of Hematopoietic Cancers

There are several potential mechanisms by which atopy/allergy could contribute to the risk for the development of hematopoietic cancers, including but not limited to: (1) features of allergic responses (IgE, mast cell products) that may mediate enhanced antitumor immune responses and/or (2) a dampening of B-cell responses, mediated by the interaction between IgE and CD23 and/or by allergy-associated changes in the relative levels of B-cell-activating cytokines, resulting in less exposure to DNA mutating and potentially oncogenic activities associated with B-cell activation. Additionally, it remains a possibility that the presence of a lymphoid malignancy may dampen allergic responses, leading to an apparent etiological protective association of allergy. Studies to better define the associations between allergy and the development of hematopoietic cancers, including both laboratory-focused studies and epidemiologic studies, are needed.

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Chapter 6

Mast Cells in Allergy and Tumor Disease

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Abstract In allergy, mast cells (MCs) are known for the notoriously detrimental role they play. MC mediators like histamine, prostaglandins, leukotrienes and thromboxane are released during the early phase of an allergic reaction and produce inflammation and local tissue responses. One pervasive roadblock in the effective development of therapeutic immunity to cancer is the fact that tumors create immune privileged sites that are resistant to the development of effective inflammation and effective anti-tumor responses. In this chapter we will present the current knowledge of the inflammatory responses elicited in allergies by IgE and MCs as well as the current knowledge on how the responses of MCs may regulate tumor growth and the immune response to tumors.

6.1 Introduction

There are a few, if any, inflammatory responses that are more rapid or intense than those mediated by IgE. IgE exerts its massive and sometimes deadly pro-inflammatory effects by triggering the release of a vast array of pro-inflammatory mediators from mast cells. MCs, first termed by Paul Ehrlich in his graduate thesis, are best known for their protective role in helminth infections and allergy [1]. During helminth infections, MCs accumulate in numbers (mastocytosis) at the site of infection (skin, intestines and lung), eosinophils are recruited, there is an induction of goblet cell hyperplasia, high levels of IgE are elicited and a Th2 inflammatory environment is created, leading to the development of protective immunity. In allergy, MCs are known for the notoriously detrimental role they play. MC mediators like histamine, prostaglandins, leukotrienes and thromboxane during the early phase of an allergic reaction are released and produce local tissue responses. In the respiratory tract, the classic symptoms of sneezing, edema and mucus secretion

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are evident, with vasodilatation in the nose and bronchoconstriction in the lung. As allergies proceed in the lung, there is accumulating cellular infiltration, fibrin deposition and tissue destruction as a result of a sustained allergic response leading to edema and further inflammatory cell recruitment. Immunologically, helminth infections and allergies show striking similarities such as induction of Th2 responses, eosinophilia, goblet cell hyperplasia, mastocytosis and high levels of IgE [2]. However, helminth infection and allergy seem to be mutually exclusive as has been shown by several epidemiological studies [3–5].

One pervasive roadblock in the effective development of therapeutic immunity to cancer is the fact that tumors create immune privileged sites that are resistant to the development of effective inflammation. Given this fact and the extraordinary capacity of IgE to mediate intense inflammatory responses a series of studies starting in the 1950s were undertaken to induce allergic reactions against tumors [6]. This novel approach in the context of the newly emerging field of AllergoOncology, the use of “allergy” as anti-tumor therapy, was later strengthened by epidemiological studies that show a protective effect of the presence of IgE on the development of malignancies [7–10]. Given the state of advanced antibody engineering, the widespread use of therapeutic antibodies and the ease with which one can produce fully human tumor-specific antibodies, IgE monoclonal antibodies directed at tumor antigens can be vigorously tested for their therapeutic value. In this chapter we will present the current knowledge of the inflammatory responses elicited in allergies by IgE and MCs as well as the current knowledge on how the responses of MCs may regulate tumor growth and the immune response to tumors.

6.2 Mast Cells in Allergy

6.2.1 *Allergies and Mast Cell Subsets*

In non-atopic individuals, B cells are skewed toward the production of allergen-specific IgG4 or IgA and to lesser extent IgG1, whereas in atopic patients B cells are driven to switch to IgE production [11]. However, IgE production is not a prerequisite for the development of disease, as allergy can be either IgE or cell (non-IgE) mediated or a combination of both. Although allergies are multifaceted, the involvement of MCs is considered one of the hallmarks in both mouse models and humans, and depending on the model, MCs have been proven to be the critical effector cells in allergy [11, 12]. MCs are divided into two subsets based on location and content: mucosal (MMC) and connective tissue (CTMC) MCs. These subsets can be distinguished based upon the presence of proteolytic enzymes in the granules. MMCs contain both chymases, chymotryptic peptidases, and tryptases, serine-class peptidases, whereas CTMCs only contain chymases [12]. Activation of MCs after allergen encounter leads to the release of chymases among many other mediators and does not seem to be dependent on subset. Chymases are mainly involved in

tissue remodeling [13], and thus MCs degranulation could exert a profound impact on the tumor microenvironment.

MMC and CTMC may be involved in Th2 versus Th1 responses, respectively. MMC and CTMC differ in the expression of STAT4, in that the latter express STAT4 and may play a role in driving Th1 responses. These findings suggest that CTMCs might not be prominently involved in Th2-driven allergies [14]. STAT4 seems to be dispensable for Th1 skewing in T cells, but the presence of STAT4 in CTMCs leads to the production of interferon gamma (INF- γ) and nitric oxide (NO) and is involved in the response to interleukin-18 (IL-18), IL-4, and IL-13. Additionally, Th1 skewing by CTMCs shows increased tumor cell lytic activity [14, 15]. The contrast of CTMCs with MMCs is even more striking when both subsets get triggered by IL-18, a cytokine that synergizes with IL-12 to induce cell-mediated immunity. CTMCs produce INF- γ when stimulated with IL-12 and IL-18, the same treatment elicits the production of IL-4 and IL-13 in MMCs, hallmark cytokines of IgE-mediated allergy [14, 16]. However, a strict demarcation of MC subsets into mediating Th1 versus Th2 responses cannot be delineated based on the current definitions of CTMCs versus MMCs. Allergens can enter the body through ingestion (food allergies are mainly MMCs mediated), inhalation (allergic rhinitis and asthma are mediated by both CTMCs and MMCs) or via the skin (atopic dermatitis is mainly CTMCs mediated) suggesting that both subsets play a role in allergy. It is likely that there are additional phenotypic distinctions among MC subsets that would further delineate them into MCs that play a role in Th1 versus Th2 responses. No doubt, it is critical to develop a more comprehensive understanding of the functional and transcriptional profile of subsets of MCs if they are going to be exploited to mediate anti-tumor immunity.

6.2.2 Mast Cells and Dendritic Cells

There is little doubt that the presentation and cross-presentation of tumor antigens by dendritic cells (DCs) is essential for the development of tumor-specific CD4⁺ and CD8⁺ T cells and protective anti-tumor immunity. Emerging data illustrate that MCs play an early role in DC migration and function, and it is in this context that MCs may contribute to the development of tumor immunity. Contact hypersensitivity (CHS) was regarded a Th1 cell-mediated allergy and has been studied extensively [17]. However, Th2-dependent CHS has been reported and seems to be dependent on allergen [18, 19]. One of the important early events in the development of CHS is the degranulation of MCs leading to the release of vasoactive and inflammatory mediators, like IL- α/β , histamine, tumor necrosis factor (TNF)- α , leukotrienes and granulocyte-macrophage colony-stimulating factor (GM-CSF) [20–22]. It has been shown that these mediators can dramatically modulate DC maturation and induce DC migration. Especially in Th2-driven CHS, TNF- α plays a crucial role in controlling DC migration [23]. Late phase disease is characterized by the infiltration of the skin by MCs, local swelling and recruitment of antigen-specific T cells, and

other leukocytes, to the site where the hapten is applied [24–26]. Additionally, the role of MCs in CHS has been refined very recently and even though the disease still develops in the absence of MCs, this study showed that MCs are regulators of the magnitude and the cytokine microenvironment [27]. Many insights on the interplay between MCs and DCs/Langerhans cells (LCs) come from CHS studies.

As mentioned earlier, TNF- α plays an important role in the development of CHS and can be produced by MCs in soluble and membrane-bound “cell-associated” form [28–30]. TNF- α is necessary for optimal response at the site where the hapten is applied during challenge [28]. Additionally, MC-deficient mice show impaired migration of LCs to the draining lymph nodes (dLN), a delay of approximately 18 h was observed while activation of MCs by IgE and allergen showed enhanced migration of LCs from the epidermis [31, 32]. The two observations were elegantly fused to show that cell-membrane-associated TNF- α produced by MCs regulating DCs/LCs migration, both in the skin and in the lung [33]. This implies that under atopic conditions, MCs modulate the adaptive immune responses by triggering increased antigen presentation in the dLN through enhanced DC migration.

There are TNF- α -independent ways MCs promote DC/LC migration. Patients with atopic dermatitis (AD) and CHS show a diminished defense against pathogens by reduced amounts of antimicrobial peptides (AMPs) and increased permeability of the skin by delayed repair [34–36]. Therefore, the lesions formed during encounter with the allergen are confronted with a wide array of Toll-like receptor (TLR) activation signals. It is known from studies with TLR2 agonist *Staphylococcus aureus* peptidoglycan (PGN) that MCs can induce LC migration equally well in wild type (WT) or TNF- $^{-/-}$ mice [22]. The authors concluded that this was TLR2/MyD88 independent but complement dependent, which is plausible since PGN can activate complement efficiently. This observation could implicate that PGN leads to IgE-independent complement-dependent degranulation since IgE/antigen-mediated degranulation yielded the same results although the latter is dependent on TNF- α [22, 37]. Another study shows that MC cells up-regulate IL-1 β when activated with imiquimod, a TLR7 agonist. Even though there was a trend suggesting involvement of TNF- α , there was a significant reduction in LC migration in IL-1 α/β - $^{-/-}$ mice [38]. MCs also express a functional TLR4 receptor [39, 40]. Upon infection of gram-negative bacteria MCs respond by the release of TNF- α as the main pro-inflammatory cytokine, thereby promoting DC maturation and migration [39]. Additionally IL-1 β , IL-6 and IL-13 are produced and in the presence of IgE-mediated degranulation as seen in allergies IL-5 and IL-10 are released as well, thereby creating a Th2 cytokine environment [41]. These studies clearly show that multiple different responses to infection during CHS or AD can lead to aggravation of disease that is independent of the allergen but dependent on MCs which are abundantly present.

Another mechanism by which MCs influence DC function is through indirectly modulating the allergic microenvironment. It has been shown that MCs regulate thymic stromal lymphopoietin (TSLP) expressed by epithelial cells. TSLP activates DCs and lead to the induction of Th2 cells from naive CD4 $^{+}$ T cells [42, 43]. It has been shown in a murine allergic rhinitis model that allergen challenge leads to

upregulation of TSLP in a MC- and IgE-dependent manner, thereby contributing to the Th2 environment and enhanced DC migration and maturation [44].

These modulatory properties imprinted by MCs on DCs are reflected in the development of the subsequent T-cell responses. In allergy models, it has been shown that MCs induce a Th2 profile by the production of PGE2 and histamine. These mediators induce DCs to produce CCL17/22, which are Th2-cell recruitment factors and also suppress the frequency of Th1 allergen-specific cells both in vivo and in vitro [45–47]. This further emphasizes the important role of MCs during allergic responses. Altogether, the current understanding is that there is an intimate and complex network of communication between MCs and DCs, which can exert profound effects on the development of acquired immunity.

6.2.3 Mast Cells and T-Cells

Although MCs indirectly influence T-cell responses via DCs, there is a body of information that shows MCs may also directly impact the T-cell compartment. During allergen encounter, IgE-sensitized MCs degranulate and release a wide array of mediators in the environment. Many of these factors affect the migration, development, proliferation and phenotype of T cells as has been reviewed extensively. As such, this represents another way in which MCs can orchestrate the development of the adaptive immune response [48–52]. The observation that MCs and T cells can be found in close proximity to each other suggests that cell-to-cell contact and/or regional cytokine production play key roles in this interaction [53]. Although MCs are not present in high numbers in secondary lymphoid organs, under inflammatory conditions, like during allergic reactions, MCs migrate from the periphery to the regional node [54, 55]. This suggests that MCs can interact with T cells both in tissue and in lymphoid organs. Additionally, since MCs express MHC-I and MHC-II, a role as antigen-presenting cells has been suggested and in fact demonstrated in vitro [56–58].

The idea of MCs acting as antigen-presenting cells (APCs) is not a novel concept. In 1993, Frandji et al. observed that MCs can activate T cell in vitro in an antigen-dependent manner leading to T-cell proliferation [56]. Yet even 10 years earlier, it was reported that MC progenitors could up-regulate MHC-II in the presence of IFN- γ [59]. Paradoxically, IFN- γ leads to the down-regulation of co-stimulatory molecules CD80 and CD86 on MCs [60]. More recently, MCs have been shown to express other co-stimulatory molecules of both the TNF-super family (OX40L, CD30L, Fas and GITR) as well as the B7 family CD80, CD86, programmed death ligand (PD-L)1 and PD-L2 [61]. IL-4 up-regulates MHC-II, albeit less than with IFN- γ but in combination with the up-regulation of co-stimulatory molecules [62]. Additionally, IL-4-cultured MCs but not IFN- γ -cultured MCs induce T-cell proliferation in vitro [52, 60]. These results suggest that MCs can enhance Th2 responses by positive feedback of IL-4 but dampen Th1 by negative regulation of IFN- γ .

Engagement of TLR4 on MCs induces MHC-II expression, and MCs can phagocytose, process and present bacterial antigens in the context of MHC-I [58]. All

these studies clearly show that MCs can be functional APCs; however, whether the same occurs in vivo is not yet clear. Since it appears that MCs undergo a cytokine-dependent up-regulation of MHC molecules it is feasible that the antigen-presenting function of MCs is to activate T cells locally in the peripheral tissues, where pro-inflammatory cytokines are released.

Although the role of MCs as *bone fide* APCs in vivo is debatable, antigen-independent regulation of T-cell response has been widely studied and recognized. The release of a wide array of immune-modulatory mediators induces recruitment, activation, skewing and proliferation of T cells. When activated, MCs release their granular content with the first effects being thought to involve recruitment of T cells and other leukocytes from the blood. The release of soluble TNF- α leads to up-regulation of several adhesion molecules, such as ICAM1, VCAM1 and VAP1, on endothelial cells needed for extravasation of T cells from the blood to the site of inflammation [63, 64]. Although seemingly contradictory, TNF- α also induces T-cell migration to the dLN during infection [65]. In this context, we speculate that TNF- α may function as a gatekeeper leading the T cells through the site of inflammation by recruiting them from the blood and directing them toward the dLN. Another factor known to promote extravasation is histamine. During allergies, the release of histamine increases the permeability of the vasculature, thereby increasing the efflux of T-effectors among other leukocytes [66].

A long list of chemokines can be released to fine tune the inflammatory response locally [51, 67, 68]. Once the T cells reach the location of allergen challenge, the release of IL-4, IL-13 and IL-10 by MCs sets the stage for a Th2 response [68]. Although not always the case, it seems that MCs preferentially skew toward a Th2 response. For instance histamine can both promote Th1 responses or suppress Th2 and Th1 T-cell activation depending on the receptor engaged [69].

In addition to the role of MCs during allergic inflammation by their influence on the adaptive immune response MCs have a well-established innate immune function and play an important role in wound healing and in the eradication of nematodes. These innate functions are also revealed during adaptive immune responses as tissue remodeling and destruction.

6.2.4 Mast Cells and Airway Tissue Remodeling

MCs products can directly affect airway epithelium and airway smooth muscle (ASM) function to enhance airway inflammation and hyperresponsiveness. Persistent MC activation during asthma can also lead to airway remodeling. This process is associated with damage and aberrant repair to lung tissue, including epithelial damage both at the surface of the lung and in the underlying basal lamina, increased development of myofibroblasts from fibroblasts, increased size and number of smooth muscle cells, and increased numbers of goblet cells (which produce mucus) [70]. One of the principle MC mediators of this process is β -tryptase, which enhances the proliferation of fibroblasts, airway epithelial and endothelial cells,

and smooth muscle cells, leading to global airway thickening [71–73]. Other products that contribute to airway remodeling are transforming growth factor (TGF)- β , which drives the differentiation of fibroblasts and ASM cells, and amphiregulin, which causes the proliferation of lung fibroblasts as well up-regulates mucin gene expression [74–76]. In addition, the MC products histamine, prostaglandin D₂ and leukotriene C₄ can cause ASM contraction [77, 78].

There is increasing evidence that angiogenesis plays an important role during tissue remodeling to provide oxygen and nutrients to the inflamed tissue as well as to allow the influx of inflammatory cells to the site. The most compelling evidence for this is that increased vascularity of the airways is observed in patients with both severe and mild asthma in comparison to healthy controls [79–82]. MCs derived IL-4, IL-5, IL-13 and TGF- β that can induce the production of vascular endothelial growth factor (VEGF) by fibroblasts and ASM cells contributing strongly to neo-vascularization [83, 84]. In addition, VEGF can to some extent regulate immune responses by inhibiting DC maturation and serve as a chemoattractant for other lymphocytes [85, 86].

6.3 Mast Cells in Cancer

6.3.1 Introduction

It has been proposed that the infiltration of MCs at the tumor site can greatly enhance tumorigenesis; MC accumulation has been confirmed in both experimental models in rodents and in primary human tumor biopsies; and MC accumulation in tumors is often associated with a poor prognosis [87, 88]. It has been suggested that MCs contribute to enhanced tumor growth by enhancing tumor-associated angiogenesis and by facilitating tissue remodeling. As noted above, MC production and induction of pro-angiogenic factors, as observed in asthma, may support the creation of a supportive environment for the growth of tumor cells. Likewise, the same mediators responsible for tissue remodeling in asthma may help to degrade the extracellular matrix and facilitate tumor growth and metastasis. Furthermore, MCs may enhance tumor growth through the release of tumor growth factors and by means of suppressing the development of protective anti-tumor immunity.

6.3.2 Mast Cells and Angiogenesis

Several factors underscore the importance of vasculature and angiogenesis for neoplastic growth and spread [89]. The original observations that tumor-associated vasculature plays an important role in the growth of neoplasms were made by Goldmann in 1907 [90]. Half a decade later (1970), a positive correlation of tumor growth and induced vasculature became evident. During the early stages of tumor development, tumors need oxygen and nutrients for continued growth and differentiation. Excessive tumor growth creates a hypoxic environment which in turn

promotes new blood vasculature through the up-regulation of angiogenic mediators released by tumor as well as non-tumor cells.

The impact of MCs on tumor angiogenesis was initially described in 1988 when it was shown that the angiogenic response was impaired in mast cell-deficient (W/W^v) mice in comparison to WT mice. In these studies, fewer W/W^v mice than WT mice developed spontaneous lung metastases and W/W^v mice exhibited fewer lung metastases per mouse. Bone-marrow reconstitution of W/W^v mice restored the angiogenic responses and also the incidence of hematogenous metastases to that of WT mice [91]. These studies implicated a role of MCs *in vivo* for tumor angiogenesis. Later Coussens and co-workers demonstrated that early angiogenic activity was greatly reduced in W/W^v mice using the HPV16 transgenic mouse model of squamous cell carcinoma, showing that up-regulated expression of the matrix metalloproteinase (MMP)-9 coincided with the angiogenic switch in early tumor lesions [92].

Recent studies confirmed these earlier observations establishing that MCs are an essential hematopoietic component for pre-neoplastic polyp development. It was seen that from the onset, adenomatous polyps are infiltrated with MCs and their precursors. Depletion of MCs either pharmacologically or by using MC-deficient animals resulted in a profound remission of existing polyps [93]. In addition, MCs have been shown to be necessary for the angiogenesis and macroscopic expansion of c-Myc-induced pancreatic β -cell tumors [94].

MCs have been shown to be selectively recruited to the site of tumor growth by means of tumor-derived peptides, RANTES and MCP-1 [95, 96], and several important angiogenic factors are expressed and secreted by MCs, such as IL-8, IL-1, VEGF, fibroblast growth factor-2 (FGF-2), TGF- β , TGF- α , MMP-2, MMP-9, nerve growth factor (NGF), platelet-derived growth factor (PDGF), leptin, tryptase, chymase, histamine and heparin [97].

IL-8 acts both as an angiogenic factor as well as a tumor cell chemotactic factor and tumor mitogen [97]. Inhibition of IL-8 by neutralizing antibodies reduces human non-small cell lung carcinoma progression in mice [98].

IL-1 secreted by MCs contributes to neovascularization by stimulating the secretion of VEGF from stroma surrounding tumor as well as directly acting on tumor growth [99]. In addition, IL-1 can induce selective secretion of IL-6 without overt degranulation in cultures of human MCs. High levels of circulating IL-6 are observed in almost every type of tumor studied and predict a poor patient outcome [100].

MC MMPs contribute to tumor metastases and invasiveness due to their proteolytic activity [101]. MC MMP products, for instance, have been seen to contribute to brain tumor metastases by regulating the permeability of the blood-brain barrier [102].

Currently MC involvement in tumor growth by means of neovascularization is relatively well accepted due to studies in murine tumor models. Below we will review observations linking MCs and tumor growth from human studies and will further elaborate on potential roles of MCs on tumor growth.

6.3.3 Mast Cells in Human Tumors

Some of the most aggressive human tumors, including breast carcinoma, malignant melanoma and colorectal adenocarcinoma, are notably populated with MCs at their periphery [87, 88, 103]. MCs have been found in the connective tissue surrounding tumor and in the environment of lymphatic and blood vessels. In mammary tumors, MCs are found on the invading, growing front of the tumor, and they contain elevated tryptase expression as compared to MCs in benign lesions.

Similar observations have been made about MCs in renal tumor tissue, and increased MC density correlates with poor prognosis in non-small lung carcinoma and increased VEGF expression [104]. Increased VEGF expression found in clinical samples may be the basis for the studies reported by Norrby and Wooley in 1993 [105], demonstrating a positive correlation of MCs with increased tumor angiogenesis. In the 1990s, a closer correlation was seen between angiogenesis and MCs in several different tumor models, such as angioma, lymphoma and multiple myeloma [106]. In Table 6.1 we summarize several important mouse and human studies investigating the connection between MCs and tumor and list the major outcomes of the studies.

MCs are represented throughout the literature as contributing to tumorigenesis and poor prognosis. In tumor specimens from Hodgkin's lymphoma (HL), where sections were stained with MC-specific antibody that detects tryptase, MCs were seen in virtually every case. Furthermore, higher MC infiltration correlated with increased tumor aggressiveness [107]. If there is a case to be made for MCs contributing to enhanced tumor growth and metastasis, then lessening the infiltration or function of MCs in tumors might be an attractive therapeutic approach. In a study of advanced human breast carcinoma it was seen that recombinant human stem cell factor (SCF) (c-kit ligand) promoted human MC activation *in vivo*. As such, neutralization of c-kit ligand on MCs may represent a potential therapeutic target [108].

6.3.4 Mast Cells Mediators of Tumor Growth or Rejection

The link between inflammation and cancer has long been recognized and has been recently reviewed by Coussens et al. [109]. The inflammatory nature of the MCs may allow them to act as both beneficiaries of tumor growth and contributors of tumor rejection.

With the wealth of correlative clinical data, the early infiltration of MCs into human tumors appears to correlate with a poor prognosis. Given this evidence, animal cancer models have been used to try to understand whether MCs contribute to tumor growth and demise of the host, or in fact protect the host from cancer. Contrary to many human clinical observations, studies in one murine model have shown that MCs can exert a protective role for the host in a genetic model of intestinal tumorigenesis. This model of spontaneous intestinal adenomas in the

Table 6.1 Mast cell infiltration and role in tumor progression in human and murine tumor models

Tumor	Mouse model	Outcome of study	References
Murine, pro-tumor Mouse plasmacytoma	BALB/c nu/nu mice	Primary mast cells accelerate tumor growth through Ang-1 and VEGF-A	Nakayama et al. [127]
K14-HPV16 spontaneous murine model (models human cervical cancer)	(KitW/KitWW ^v) HPV16 transgenic mice	Mast cell infiltration and activation of MMP-9 coincides with the angiogenic switch in premalignant lesions. Premalignant angiogenesis is diminished in MC-deficient HPV16 transgenic mice	Coussens et al. [128]
b-cell carcinoma	plns-mycERTAM;RIP7- bcl-xL;KitW-sh;KitW-sh	Mast cells are necessary for angiogenesis and macroscopic expansion of Myc-induced pancreatic islet tumors	Soucek et al. [93]
Murine, anti-tumor Murine mammary adenocarcinoma cell line (4T1)	BALB/c mice	Imatinib mesylate (Gleevec) accelerated the growth and peri-tumoral blood clotting of mammary adenocarcinoma in mice by inhibiting the survival and function of MCs	Samoszuk et al. [129]
Human, pro-tumor MM	–	Angiogenesis parallels progression of MM and MC participate in the induction by release of angiogenic factors. Poor prognosis in MM correlates directly with increased MC number	Ribatti et al. [130]
Rectal cancer	–	In a study of 331 rectal cancer samples a correlation was found of worsened overall survival rate with increase in MC present around tumor	Fisher et al. [131]
Melanoma	–	A high correlation was demonstrated between microvessel counts and total metachromatic and tryptase-reactive MCs. Suggesting that tryptase secreted by MCs cooperate in angiogenesis induction in human melanoma and as such increasing tumor progression	Ribatti et al. [132]
Oral SCC	–	MCs in all lesions and normal mucosa express tryptase. Density of MCs and microvessels increase with disease progression	Iamaroon et al. [133]
Lung adenocarcinoma	–	MCs correlate with angiogenesis and poor outcome in stage I lung adenocarcinoma	Imada et al. [134]
HL	–	MCs were detected in all tumor samples and increased number of MCs correlated with nodular sclerosis histology and a worse relapse-free survival	Molin et al. [106]

Table 6.1 (continued)

Tumor	Mouse model	Outcome of study	References
Pterygium	–	Angiogenesis was highly correlated with MC tryptase-positive cell count in pterygium tissues. Neovascularization in pterygium may be sustained by MC angiogenic mediators	Ribatti et al. [135]
Human, anti-tumor Breast, head and neck, lung, ovarian, NHL, HD	–	MCs are present in fibrotic regions of cancer and MC heparin is a inhibitor of tumor growth	Samoszuk et al. [136]
Breast carcinoma	–	A high number of MCs in tumor stroma were related to low S phase fraction, DNA diploidy, high proportion of intraductal growth and a high degree of tubule formation. A high MC count was related to a favorable prognosis	Aaltomaa et al. [137]
Breast carcinoma	–	Tissue microarrays of invasive breast carcinoma demonstrated the presence of MCs in the stroma correlating with a good prognosis. The presence of MCs showed an inverse correlation with CD68 positive macrophages and no correlation with the presence of B cells or T cells	Dabiri et al. [138]
Ovarian cancer	–	There was no significant association between angiogenesis and mast cell density. Peri-tumoral MC infiltration in tumors with high MVD predicts for improved survival in women with advanced epithelial ovarian cancer	Chan et al. [139]
Human, neutral RCC	–	The correlation between microvessels and MC counts was established and compared with tumor stage, grade and additional clinicopathologic parameters. No correlation was found between MC number and tumor size, stage, grade or patient survival	Tuna et al. [140]
Breast cancer	–	No difference in 10-year disease-free survival was established in patients with and without MC infiltration suggesting no correlation between MC numbers and prognosis	Fisher et al. [141]
NSCLC	–	Macrophage and MC infiltration of tumor cell islets confers a marked survival advantage in NSCLC	Welsh et al. [142]

Abbreviations: MM, multiple myeloma; SCC, small cell carcinoma; HL, Hodgkin's lymphoma; NHL, non-Hodgkin's lymphoma; HD, Hodgkin's disease; RCC, renal cell carcinoma; NSCLC, non-small cell lung carcinoma; VEGF, vascular endothelial growth factor; MVD, mean vessel density

multiple intestinal neoplasia (Min, APC^{Min/+}) mouse was seen to have an increased number of MCs and increased abundance of MC-associated proteinases. The MC-deficient Min (Min-Sash) mice developed 50% more adenomas than littermate controls, and the tumors were 33% larger in Min-Sash mice. These results suggest a protective, anti-tumor role for MCs in a genetic model of early-stage intestinal tumorigenesis [110]. Indeed, many of the cytokines produced by MCs can conceivably lead to impaired tumor growth. For example, the IL-4 receptor (IL-4R) is expressed on human breast carcinoma and signaling through the IL-4R can lead to tumor cell apoptosis, allowing for the possibility that MCs may act in a protective role to the host by directly killing tumor cells via IL-4 or other apoptosis-producing cytokines like TNF- α . Additionally, TNF- α contributes to leukocyte infiltration into the tumor which may also be protective [29, 111].

However, the overwhelming evidence in animal models suggests that MCs facilitate tumor growth and progression. In mammary tumor development MCs allow for tumor angiogenesis, contribute to tissue remodeling and metastasis through the release of MMPs and the release of growth factors such as SCF and NGF [112].

Using the mouse model of MC deficiency of the Kit^W/Kit^{Wv} mice Galli et al. conclusively demonstrated that these mice have decreased susceptibility to the development of 1,2-dimethylhydrazine-induced intestinal tumors [113]. These results were later supported, as described above, by studies in spontaneous human colorectal cancers where MCs are essential for polyp development and play a protective role to the tumor in intestinal tumorigenesis [93, 110]. Furthermore, the same studies show that TNF- α is required for the growth of adenomatous polyps, and it is proposed that MC-derived TNF- α is acting in an autocrine loop as a MC recruiting factor to increase the concentration of MCs at the site. Figure 6.1 summarizes the multiple ways by which MCs may either promote tumor growth or contribute to tumor rejection.

6.3.5 Mast Cells Regulate Adaptive Immune Responses to Tumors

In addition to the generally accepted role of MCs influencing tumor growth by means of angiogenesis and tissue remodeling, MCs may exert an immunomodulatory role in the generation of anti-tumor immune responses. Tumors utilize several mechanisms by which they evade the immune response including the induction of T-cell ignorance and anergy and by means of active suppression of effector cells [114–118]. The idea that MCs are beneficial for tumor formation together with the fact that MCs can selectively release cytokines and other mediators without degranulation raises the question whether MCs contribute in the development of an immune privilege within the tumor microenvironment. Such selective release of MC mediators is called piece meal degranulation and is a critical factor in MC versatility and their ability to release certain products without others and without degranulation [119].

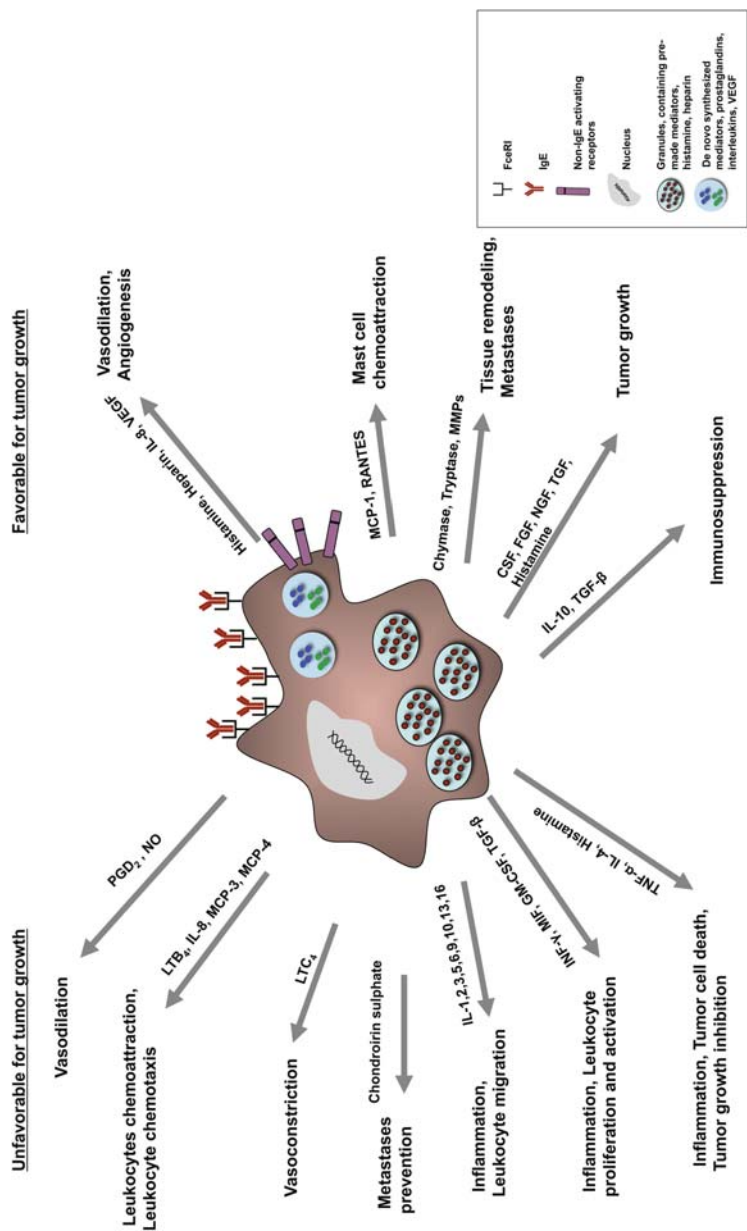


Fig. 6.1 Mast cell mediators and their effect on tumor growth. MCs carry and release multiple mediators that can both favorably and unfavorably affect tumor growth. Among the MC mediators thought to be unfavorable to tumor growth are those which cause leukocyte infiltration to the tumor site, vasoconstriction, prevent metastases, cause inflammation, cause leukocyte proliferation and activation and have direct anti-tumor effect by means of tumor cell death and tumor growth inhibition. Conversely, other factors, such as the pro-angiogenic mediators IL-8 and VEGF, are thought to contribute favorably to tumor growth by inducing angiogenesis; chymases and tryptases are known for their role in tissue remodeling and tumor metastasis and other factors such as FGF and NGF act directly on tumor growth. Factors such as IL-10 act in an immunosuppressive manner and yet others in the increased chemoattraction of additional MCs. What role MCs play during tumor progression likely varies depending on their immediate environment and the stimuli they encounter

Some of the immunoregulatory components of MC function are believed to be mediated by the release of histamine. Histamine released from MCs has been shown to play a dual role in that it can stimulate the proliferation of tumor cells through the H1 receptors (H1R) and act as a negative modulator of immune cells through H2 receptors (H2R) [112]. Results suggest that histamine in tumor tissue suppresses local tumor immunity via H2R activation that results in tumor growth promotion. In addition, and somewhat conflicting with the immune stimulatory role of signaling through H1R, histamine has been seen to enhance growth of melanoma in a H1R-dependent manner [120]. The effect of histamine directly on tumor growth is currently quite a conflicting topic as studies report differing results depending on dosage of histamine used as well as type of tumor studied. While it is likely that tumor-infiltrating MCs provide enough histamine to act as a tumor growth factor, exogenous administration of high-dose histamine seems to instead result in an anti-tumor effect of histamine in both a direct and an indirect manner [121].

MCs may contribute to tumor growth via immune modulation in the same way described above due to their interactions and influence on DC migration and functionality as well as by directly modulating T-cell responses. MCs produce IL-10, a common immunomodulatory cytokine produced by a variety of T-cells including T-regulatory cells (T_{reg}). Interestingly, MC-derived IL-10 has been shown to play a significant role in the suppression of UV-B-induced CHS [122]. MC-derived IL-10 is also seen to reduce immune responses after mosquito bites [123]. Hence, the production of IL-10 by MCs at the tumor site may contribute to the generation of an immunosuppressive microenvironment. In addition to providing immunosuppressive factors, MCs may also act in a suppressive way by removing proinflammatory mediators from the tumor microenvironment such as serotonin. Serotonin was recently shown to promote T-cell proliferation and activation, and MCs express the serotonin-specific transporter (SERT) by which they can take up and store serotonin [124–126].

As reviewed earlier, MCs can up-regulate the expression of several costimulatory molecules, and in addition it has been shown that MCs express PD-L1 and PD-L2, the ligands for the inhibitory receptor PD1 expressed on T cells [61]. These inhibitory ligands are expressed on tumors and tumor-infiltrating DCs and in doing so contribute to tumor immune evasion [127].

Further supporting a suppressive immunoregulatory role of MCs, it was recently shown that MCs are necessary intermediaries in regulatory T-cell-mediated graft tolerance. In this context, MCs were shown to prevent the rejection of haplo-mismatched skin allografts in tolerant mice. Hence, further investigation is needed to establish whether MCs and their mediators can alter the development of protective T-cell immunity to tumors [128].

6.4 Conclusion

Altogether, the role of MCs during allergic inflammation is very complex, and it is clear that MCs can influence the adaptive immune response by influencing antigen

presentation, as well as T-cell recruitment, activation, skewing and proliferation. It is perhaps easy to see then how these MC-mediated effects in the immune system may as well play an important role in tumor disease. Many studies show that MCs mediate processes that modify the tumor microenvironment in terms of angiogenesis and tissue remodeling that can help tumor grow and metastasize. Furthermore, MCs may also play an influential role in the development of adaptive immunity within the tumor microenvironment. It seems feasible to hypothesize that MCs may act in a tumor-friendly manner through a controlled release of certain mediators while having an anti-tumor effect upon a robust and immediate release of their granular content. As such MCs may play a dual role in propagating the development of cancer. Whether MCs truly regulate the development of anti-tumor immunity, how they do it and the identity of the molecular mediators are currently being studied.

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Chapter 7

The IgE Antibody and Its Use in Cancer Immunotherapy

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Abstract The immunoglobulin E (IgE) is a class of antibody that is capable of mounting a robust immune response resulting in anaphylaxis, which plays a central role in allergic reactions against environmental agents and immunity against parasites. Multiple studies also suggest that IgE plays a role in cancer immunosurveillance, which implies that this class of antibody can be exploited for the treatment of cancer. While most therapeutic antibodies developed for cancer therapy are of the IgG class, IgE has several advantages including the exceptionally high affinity for its Fc receptors (FcεRs) and its low serum concentration that provides less competition for Fc receptor occupancy. In addition, FcεRs are expressed on relevant effector cells involved in antibody-dependent cell-mediated cytotoxicity (ADCC) and/or antibody-dependent cell-mediated phagocytosis (ADCP) including antigen-presenting cells (APC). In fact, multiple IgEs targeting different tumor-associated antigens (TAA) have been developed and shown to be effective against cancer cells in vitro and in animal models. Further advances of current technologies and experimental models, coupled with a better understanding of IgE-mediated immune effector mechanisms against targeted tumors, are expected to help elucidate the full potential of tumor-specific IgE antibodies for cancer immunotherapy.

7.1 IgE and Its Relevance in Cancer Therapy

7.1.1 Immunoglobulins

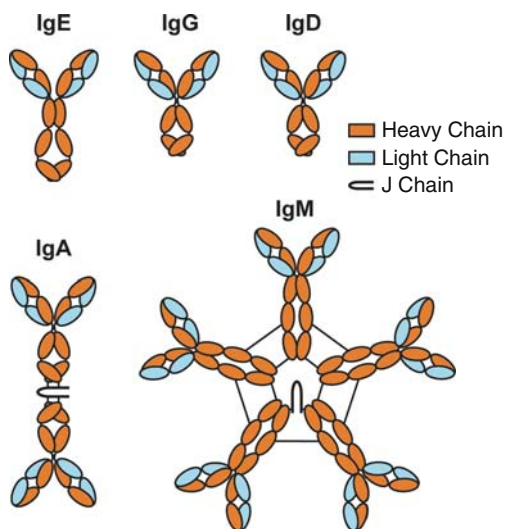
Immunoglobulins (Ig, also known as antibodies) are glycoproteins and are central effector molecules of the humoral immune response [1–3]. They are highly selective for a broad spectrum of antigens including pathogens as well as tumor-associated

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antigens (TAA) and potentiate the immune response by activating host effector systems against the targeted antigen. Antibodies are composed of heavy (H) and light (L) chains and exhibit a H_2L_2 heterotetramer configuration. In humans there are five classes of antibodies that are distinguished by their heavy chain structure designated by lower-case Greek letters: α (IgA), δ (IgD), ϵ (IgE), γ (IgG), and μ (IgM) [1–3]. There are two subclasses of IgA (IgA1 and IgA2) and four subclasses of IgG (IgG1, IgG2, IgG3, and IgG4). The light chains are designated κ and λ and are shared by all heavy chain classes. IgD, IgE and IgG are monomeric, while IgA can also be dimeric and IgM is usually pentameric (Fig. 7.1). Multimeric antibodies contain a joining (J) chain, which is a 15-kilodalton (kDa) polypeptide that promotes polymerization [1]. The IgM molecule exists as a pentamer with the J chain or occasionally as a hexamer without the J chain. In addition, polymeric antibodies contain an 18-amino acid extension, known as the “tailpiece,” in the carboxy-terminus that contains a cysteine residue that is also required for polymerization. The approximate molecular weights (m.w.) of the different classes are as follows: 180 kDa for IgD; 190 kDa for IgE; 150 kDa for IgG1, IgG2 and IgG4; 170 kDa for IgG3 due to an extended hinge region; 160 kDa for both subclasses of monomeric IgA in serum and 300–360 kDa for secretory dimeric IgA and 900–970 kDa for IgM [1]. Similar to humans, mice also have five classes of antibodies (IgA, IgD, IgE, IgG and IgM) [4]. However, there is only one murine subclass of IgA that is approximately 160 kDa as a monomer and 350–400 kDa as a secretory dimer. The murine IgG subclasses are also different when compared to the human IgG subclasses. The murine IgG subclasses are IgG1, IgG2a, IgG2b and IgG3 (which are all approximately 160 kDa). Murine IgM is about 900 kDa, while the IgD and the IgE are comparable in size to their human counterparts (180 and 190 kDa, respectively) [4].

Fig. 7.1 Comparative structure of the human antibody classes. All five classes of antibodies share the basic H_2L_2 heterotetrameric structure that is organized into discrete domains. Antibodies of the IgE, IgG and IgD class are monomeric, while IgA (dimer) and IgM (pentamer) are polymeric. The polymeric antibodies contain a J chain, a polypeptide that promotes polymerization



7.1.2 The Structure of the IgE Antibody and Its Binding Properties

The heavy chains of IgE are composed of five domains: four constant domains (C ϵ 1, C ϵ 2, C ϵ 3, and C ϵ 4) and one variable domain (V_H) (Fig. 7.2) [5]. The light chains are composed of two domains: one variable (V_L) and one constant domain each (C_L) (Fig. 7.2). Like the other classes of antibodies, the variable regions are responsible for antigen binding while the constant region is responsible for effector functions. IgG, the most abundant Ig in human serum and the most widely used in clinical applications [5–7], has a structure similar to that of IgE but lacks the fourth constant domain of the heavy chain (Fig. 7.2). Thus, the IgG heavy chain contains only three constant domains (C γ 1, C γ 2, and C γ 3). Moreover, IgE has six N-linked glycosylation sites compared to only one for IgG. The additional domain of the IgE (C ϵ 4) increases the overall size of the molecule compared to that of IgG; however, the crystallographic structure of the molecule has revealed a bent conformation in solution, and when bound to its high-affinity receptor Fc epsilon receptor (Fc ϵ RI), implying that the constant heavy region of the molecule may fold on itself to reduce its overall hydrodynamic radius [8, 9]. It has been suggested that the C ϵ 2 domain of the IgE takes the place of the hinge region in IgG [8]. The effect of this domain on the structure and function of the molecule remains to be clearly established.

The binding affinity of human IgE for Fc ϵ RI ($K_a = 10^{10} \text{ M}^{-1}$) is two orders of magnitude higher than that of IgG for its high-affinity receptor Fc γ RI (CD64;

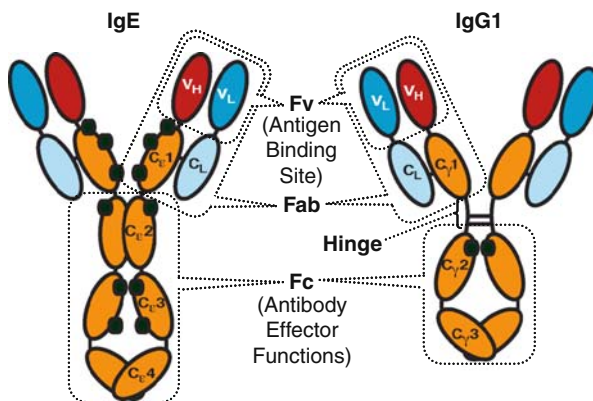


Fig. 7.2 Schematic diagram comparing the domain structures of human IgE and IgG1. Antibodies are composed of two pairs of identical heavy (H) and light (L) chain proteins linked by disulfide bonds forming H₂L₂ heterotetramers. The Fab region consists of one constant and one variable domain from each heavy and light chain. The variable region (Fv), composed of both the heavy and light chain variable domains, is responsible for antigen binding and is located at the amino-terminus of the antibody. The remaining constant region includes the Fc portion of the antibody and is responsible for its effector functions. Black circles denote N-linked glycosylation sites. Note the hinge region in the IgG1 joining the C γ 1 and C γ 2 domains. This region, which provides flexibility, is absent in the IgE

$K_a = 10^8 \text{ M}^{-1}$) [10–12]. This high-affinity interaction is attributed in part to a conformational change observed upon binding to its receptor [9, 13, 14]. In fact, K_{on} and K_{off} rates of IgE binding to its high-affinity receptor suggest that the main difference between IgE and IgG binding is in the K_{off} rate of their interactions, where the IgE Fc ϵ RI K_{off} rate is four orders of magnitude lower than that of IgG for Fc γ RIII (CD16) [15–17]. The region of the IgE associated with Fc ϵ RI binding resides within the C ϵ 2 and C ϵ 3 domains and involves two distinct sites [18]. The human IgE C ϵ 3 domain is also responsible for binding to the low-affinity IgE receptor, Fc ϵ RII (CD23). Membrane-bound CD23 has three lectin domain “heads” that are connected to the membrane via a triple α -helical coiled-coil “stalk” [19]. CD23 is known as the “low-affinity” Fc ϵ R since the affinity of human IgE for a single lectin domain “head” is about 10^6 – 10^7 M^{-1} [19–22]. However, the avidity of human IgE for the naturally occurring CD23 molecule with its three “heads” is about 10^8 – 10^9 M^{-1} , which approaches the affinity of human IgE for Fc ϵ RI [19, 21, 22]. Each C ϵ 3 region contains a glycosylated asparagine residue (N394), which allows two branched glycosylated chains to reside at the center of the IgE. The structure of the branching sugars consists of two *N*-acetyl-glucosamine and five mannose units per chain [8]. These branching chain glycosylations are not essential for the binding of IgE to its low-affinity receptor CD23. Instead a lysine residue (K352) also present in the C ϵ 3 domain facilitates the interaction to a large extent [23]. In addition to binding Fc ϵ RI and CD23, IgE antibodies can bind CD21 (a CD23 co-receptor) and galectin-3 (a carbohydrate-rich lectin also known as epsilon-binding protein) [19]. The effects of IgE interactions with these molecules are yet to be fully understood.

7.1.3 The Function of IgE and Its Relevance in Cancer Therapy

IgE plays a central role in allergic reactions against environmental agents and immunity against parasites [19]. Additionally, several studies strongly suggest a potential role of IgE in cancer surveillance. Relevant epidemiological studies on the association of allergies with cancer support a lower cancer risk among people with a history of allergies. In fact, an inverse correlation between the incidence of atopic conditions and high levels of serum IgE and the risk of some cancers has been demonstrated [24]. These epidemiological studies are discussed in Chapters 4 and 5. Furthermore, antibodies of the IgE class isolated from pancreatic cancer patients were shown to mediate cytotoxicity against targeted cancer cells [25]. Moreover, levels of polyclonal IgE are directly correlated with the overall survival in patients with multiple myeloma [26]. Taken together, these studies suggest a natural role of IgE in the immunosurveillance of cancer. Interestingly, treatment of patients affected with moderate to severe asthma with omalizumab (Xolair[®]), a recombinant humanized IgG1 specific for the constant region of human IgE, has raised some concerns regarding the safety of anti-IgE therapy. Omalizumab interacts with the IgE molecule in the region where it binds to the Fc ϵ RI and thus binds to all circulating IgE regardless of antibody specificity, leading to the downregulation of IgE

levels and FcεRI expression [27–29]. However, in patients treated with omalizumab, 0.5% (20 out of 4,127) developed cancer compared to 0.2% (5 out of 2,236) treated with placebo [29]. These results are supported by an earlier study that revealed 1 in 200 patients treated with the antibody developed breast, prostate, melanoma, non-melanoma skin, or parotid gland malignancies during the median observation period of 1 year [30]. Due to a possible increase in cancer risk, it has been suggested that omalizumab should not be used in asthma patients with a personal or family history of cancer [29]. It is tempting to speculate that the general interference of the interaction between IgE and FcεRI caused by omalizumab may be blocking the cancer immunosurveillance activity of endogenous IgE leading to an increased risk of cancer development.

The potential role of IgE in cancer immunosurveillance strongly suggests that this class of antibody should be exploited as a therapeutic for the treatment of cancer. However, regardless of whether IgE plays a role in cancer surveillance, this antibody has several intrinsic advantages that increase its therapeutic potential compared to the commonly used IgG in cancer therapy. One of the major advantages of IgE over IgG that can be exploited for the treatment of human cancers is the low serum levels of IgE. The average serum concentration of IgE is less than 0.0003 mg/ml in non-atopic adults [31]. The level of IgE in circulation is lowest of all the antibody classes, consisting of only 0.02% of total circulating Igs, whereas IgG is the most abundant at 85% [31]. The low serum levels of IgE compared to IgG provides less competition for FcR occupancy. In fact, the loss of specific IgG from effector cells due to the high levels of competing serum IgG for host FcγR-expressing cells is a limitation of IgG antibody-dependent cell-mediated cytotoxicity (ADCC) [32], a critical mechanism of anti-tumor protection. The extremely low levels of IgE in the serum have been attributed both to its low synthesis rate and to its short serum half-life (2.5 days, when not bound to FcεRs) [31]. Chapter 2 describes the tight regulatory mechanisms involved in maintaining the low serum concentrations of IgE.

Another advantage of IgE-mediated cancer therapeutics is that IgE binds its FcεRs with exceptionally high affinity. As mentioned above, FcεRI binds IgE with an affinity (K_a) of 10^{10} M^{-1} , which is two orders of magnitude higher than that of IgG for the FcγRI (CD64) [10–12]. Human FcεRI is expressed on human monocytes, macrophages, eosinophils, basophils, mast cells, Langerhans cells, dendritic cells (DC), and platelets [33]. In contrast to the human receptor, murine FcεRI is expressed only on mast cells and basophils [33]. Even though FcεRII (CD23) is the low-affinity FcεR, its affinity for IgE is as high as that of IgG for its high-affinity receptor FcγRI (CD64) [24, 33]. CD23 is expressed on eosinophils, monocytes, lymphocytes, follicular DC, Langerhans cells, and platelets [34, 35]. Additionally, there is no known inhibitory FcεR for IgE, while IgG binds to the inhibitory FcγRIIB (CD32b) that decreases ADCC and antibody-mediated antigen presentation for IgG molecules [36, 37]. The fact that IgE does not elicit complement-dependent cytotoxicity (CDC) like IgG does should not dampen its therapeutic efficacy since CDC does not seem to be a major player in the anti-tumor activity of many therapeutic antibodies targeting cancer cells, such as rituximab [38]

and trastuzumab [39, 40], and it has been reported that complement activation may interfere with ADCC activity [41].

Through the interaction with Fc ϵ RI, IgE mediates many downstream events that lead to the induction of a potent immune response characterized by immediate hypersensitivity, an acute inflammatory response followed by a late phase reaction 6–8 hours thereafter [42]. The uniqueness of the IgE-mediated allergic (anaphylactic) response is due to the presence of mast cells in the tissue that are sensitized by IgE bound to Fc ϵ RI. When these Fc ϵ Rs are crosslinked due to the binding of antigens (allergens) to multiple IgE on the surface of the mast cell, these cells are rapidly, or “explosively,” degranulated releasing a variety of factors. These factors include enzymes such as tryptase that lead to the breakdown of tissue matrix proteins, chemical mediators such as histamine that increase blood vessel permeability and blood flow to the area, and lipid mediators such as leukotrienes that stimulate smooth muscle contraction and the infiltration of eosinophils, basophils, and other lymphocytes to the tissue [42]. All of these factors together trigger tissue damage and the acute inflammatory response. If this response occurs in the tumor microenvironment due to the presence of a tumor-specific IgE, it is expected to lead to massive tumor cell death and phagocytosis of these dying cells by professional antigen-presenting cells (APC) such as DC. These activated APC will then migrate to the local lymph nodes and stimulate cognate T cells that will initiate adaptive (cellular and humoral) immune responses against the tumor. Importantly, this immune response will not only be against the IgE-targeted TAA but will expand to a broad spectrum of antigens expressed by the cancer cells, a process known as epitope spreading. In addition, tumor-specific IgE can enhance this antigen uptake and presentation of TAA via the interaction with Fc ϵ R expressed on APC such as DC, Langerhans cells, and monocytes/macrophages, which are efficient stimulators of both primary and secondary T-cell responses [33, 43–46]. Therefore, IgE is able to prime naïve T cells to IgE-reactive antigens as well as amplify established T-cell responses.

It is well known that IgE can mediate ADCC of targeted cancer cells through the interaction of its Fc region with Fc ϵ RI on the surface of effector cells [47] (Fig. 7.3). IgE can also mediate antibody-dependent cell-mediated phagocytosis (ADCP) of cancer cells through the interaction with CD23 on the surface of effector cells [47] (Fig. 7.3). Effector cells such as DC and macrophages can potentially phagocytose live cancer cells as well as apoptotic cancer cells, entirely or as apoptotic bodies. This phagocytosis can occur in the absence of IgE, but the presence of the antibody is expected to enhance the uptake of targeted cells resulting in better antigen presentation. This suggests that the IgE antibody can be used as an adjuvant of cancer vaccines, a possibility that is discussed in detail in Chapter 9. It is important to note that depending on the effector cell type, certain effectors can preferentially act through ADCC (such as basophils and mast cells), while other effectors can act preferentially through ADCP (such as DC and Langerhans cells). Moreover, other cells such as monocytes/macrophages are capable of eliciting targeted cell cytotoxicity through both mechanisms: ADCC and ADCP [47–49]. It is also expected that antibodies of the IgE class would be able to target secreted or shed TAA in the

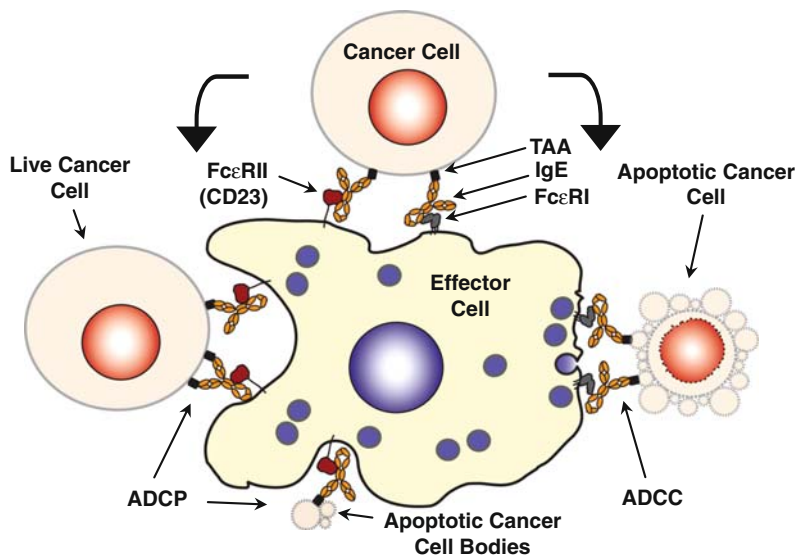


Fig. 7.3 Scheme of the possible interactions between immune effector cells and targeted tumor cells, mediated by an IgE specific for a tumor-associated antigen. The IgE specific for a TAA interacts with the FcεRI or the FcεRII expressed on the surface of the effector cell and may trigger cell killing through ADCC or ADCP. Antibodies bound to apoptotic cells or apoptotic bodies are also expected to trigger phagocytosis

circulation that will make complexes with the IgE leading to internalization of these antigens through endocytosis by professional APC expressing the FcεRs [33]. In fact, IgE-mediated soluble antigen uptake and presentation has been demonstrated [45, 46]. However, it is important to stress that the targeted soluble antigen should be preferentially monoepitope, resulting in the binding of one or two antigens to only one IgE. This is particularly relevant when the IgE is administered systemically to avoid the general crosslinking of FcεRIs on the surface of basophils and mast cells that may lead to systemic anaphylaxis. This enhanced antigen uptake and presentation is expected to activate an adaptive immune response without even targeting the tumor microenvironment. Finally, in the specific case of murine IgE, the activation of natural killer (NK) cells can be mediated through the binding of IgE to FcγRIII (CD16), the Fc receptor that is responsible for IgG-mediated cellular cytotoxicity [50]. This interaction between murine IgE and NK cells implicates IgE in ADCC and in the induction of cytokine production by NK cells [50].

In summary, there are several potential advantages of IgE over IgG that make this class of antibody an attractive anti-cancer agent. Antibodies of the IgE class, even if they are administered in a passive immunotherapy setting, are expected to be capable of a two-pronged attack against malignant cells. They are expected to directly target and eliminate cancer cells as well orchestrate a potent secondary and broad-spectrum anti-tumor immune response, thus linking passive and active immunotherapy.

7.2 Reaginic Antibodies and Local Anti-Tumor Anaphylaxis

Reaginic antibodies, namely IgE, were shown to be the primary mediators of anaphylaxis, and the term “atopic reagins” was coined by Arthur F. Coca and Ella F. Grove in 1925, referring to elements of human serum that were capable of transferring immediate hypersensitivity against a particular antigen from person to person [51, 52]. However, the acknowledgment and designation of the term “IgE” as the fifth class of antibodies did not occur until 1968 [52, 53].

Investigators seeking to understand the sensitization of mast cells involved in the adaptive immune response against tumor antigens during the early 1970s discovered that mice were capable of eliciting antibodies against these antigens that could be classified as reagins [52, 54]. Using a passive cutaneous anaphylaxis (PCA) test, the activity of these reagins (IgE) against melanoma antigens was demonstrated in vivo [54]. Sera from C57 black (C57Bl) mice bearing subcutaneous B16 murine melanoma cells were obtained 7 or 10 days after tumor challenge. In addition, the subcutaneous tumors were resected and fractionated to prepare extracts of presumed TAA. Splenocyte extracts were also prepared for use as negative controls [54]. BALB/c mice were intradermally injected with the serum samples from the B16-bearing C57Bl mice. Seventy-two hours later the tumor extracts combined with the Evans blue dye were injected intravenously. A positive PCA reaction against B16 extracts was observed as evidenced by the leakage of the dye from the blood vessels while no reaction was observed with splenocyte extracts. The existence of anti-tumor reaginic antibodies in both C57Bl and BALB/c mice used in this PCA experiment was demonstrated and served as initial proof-of-principle of the potential role of reaginic (IgE) antibodies as key effectors in local anaphylaxis against cancer [54]. These results indicate that IgE are produced in mice in response to tumor formation, leading the authors to suggest that basophils and mast cells sensitized with IgE may play a role in the host immune response against cancer cells.

It was not until half a decade later that a more detailed study established a potential connection between reaginic IgE antibodies inducing passive local anaphylaxis (PLA), also known as PCA, and a possible mechanism of anti-tumor activity in mice [55]. These studies found that if radioactively labeled splenocytes or an extracellular dye (Lissamine green) were administered intravenously, only small amounts would penetrate an established tumor. Conversely, if the labeled splenocytes or the dye were administered intratumorally, they remained trapped within the tumor for several days. These investigators concluded that C3H mice bearing syngeneic McC3 fibrosarcoma had reduced vascular permeability within established tumors. This phenomenon could be reversed by the intratumoral induction of PLA achieved by sequential injection of ovalbumin (as the antigen) and sera containing high titers of anti-ovalbumin reaginic antibodies [55]. Importantly, this PLA resulted in anti-fibrosarcoma activity. The active agent inducing these effects following the PLA event was found to be histamine-related given that administration of the histamine antagonist, cyproheptadine, into mice prior to inducing intratumoral PLA cancelled the beneficial effects of the procedure. The increased vascular permeability in the tumors that resulted from PLA induction facilitated the entry

of lymphoid cells and soluble molecules into the tumor microenvironment, a process cited as a possible “gatekeeper” mechanism of anti-tumor activity of the PLA reaction [55]. It is important to note that although these studies found no evidence of tumor-reactive IgE in the serum of the protected mice, the induction of PLA was mediated by ovalbumin antiserum with significantly high titers of IgE antibody. This study served to highlight the beneficial role of LPA within solid tumors and suggested that tumor-specific IgE may be effective for anti-cancer therapy.

7.3 Generating Tumor-Specific Monoclonal IgE

7.3.1 *Development of Monoclonal and Recombinant IgE*

Advances in the field of passive immunotherapy mediated by antibodies of the IgE class, as with other antibody classes such as IgG, have been propelled by parallel advances in the understanding of the responses elicited by these molecules and by advances in the molecular and cellular biology techniques that have facilitated the manipulation of these proteins. The production of monoclonal antibodies from rodent hybridomas in 1975 by Hans Köhler and César Milstein [56] was a significant breakthrough in the field of antibody therapy. The adaptation of these advances to the production and purification of recombinant antibodies of desired specificity [57] facilitated not only the development of novel IgE antibodies but also the manipulation of these proteins under controlled conditions to evaluate the mechanisms that underlie their physiological activity.

The first recombinant IgE was a mouse/human chimeric antibody composed of murine variable chains fused to human λ light and ϵ constant heavy chains specific for the hapten 4-hydroxy-3-nitro-phenacetyl (NP) [58]. The aim of this study was to produce large quantities of NP-specific IgE as a protective agent against severe allergies but would additionally serve as the foundation for further work describing tumor-specific recombinant IgE with human effector functions. The initial characterization of this recombinant IgE demonstrated its capacity to induce degranulation of basophils when crosslinked by the hapten (5-iodo-4-hydroxy-3-nitrophenacetyl caproate, NIP) coupled to bovine serum albumin [58]. This degranulation included histamine release and the passive sensitization of effector cells in vitro. Moreover, pre-incubation of this anti-NP IgE with basophils prevented their degranulation induced by a common house dust mite antigen combined with sera from allergic individuals with high levels of IgE specific for this antigen. This is most likely due to its effect as a competitor for Fc ϵ R occupancy. By generating the first recombinant IgE, this study proved to be an important milestone in understanding the physiological activity of IgE in the context of acute allergic reactions.

The technology for generating recombinant antibodies of different classes and subclasses with desired specificity also facilitated the first comparisons of the effector functions of human antibodies. A matched set of mouse/human chimeric antibodies specific for the hapten NP was expressed in murine myeloma cells [59].

The panel of chimeric Igs consisted of IgM, IgE, IgA2, and four IgGs (IgG1, IgG2, IgG3, and IgG4). As a measure of their activity, the panel of antibodies was tested for the capacity to bind the C1q complement protein, mediate the lysis of red blood cells derivatized with the hapten through CDC, or facilitate the cytotoxic killing (ADCC) of the hapten-derivatized human acute lymphoblastic leukemia cell line HPB-ALL using human peripheral blood mononuclear cells (PBMC) as effector cells [59]. The results indicated that the IgG subclasses IgG1 and IgG3 were far superior at eliciting both CDC and ADCC under the conditions used for this study. As expected, the recombinant chimeric IgE did not activate complement. It is important to note that the target cells used in the ADCC assay (HPB-ALL) were artificially labeled with the antigen. In addition, the studies were conducted in vitro and the repertoire of effector cells was limited to PBMC. The inability of IgE to induce complement activation along with the poor induction of ADCC compared to IgG1 and IgG3 together with the expected concern of induction of systemic anaphylaxis has discouraged the use of antibodies of the IgE class as a possible treatment for human cancer.

7.3.2 Use of Tumor-Specific IgE for the Passive Immunotherapy of Cancer

Although most antibodies in clinical use are of the IgG class, the use of IgE for the passive immunotherapy of cancer would offer some advantages over their IgG counterparts (as discussed in Section 7.1.3). These advantages include the higher affinity of IgE for its FcεRs, the low serum levels of IgE (which means less competition for binding to FcRs), the expression of the FcεRs on a broad spectrum of effector cells, and its capacity to trigger antigen processing and presentation leading to a secondary immune response. Moreover, as discussed below, several studies suggest that antibodies of the IgE class can be potent anti-cancer agents. Importantly, the use of antibodies of the IgE or IgG is not necessarily mutually exclusive since they can potentially be used in combination (simultaneously or sequentially), resulting in an additive or synergistic effect through the recruitment and engagement of different FcRs and effector cells.

7.3.2.1 Murine IgE Specific for the Glycoprotein 36 of the Mouse Mammary Tumor Virus

The use of monoclonal antibodies of the IgE class for the passive treatment of cancer was explored for the first time in the 1990s. Two murine monoclonal IgEs specific for the major envelope glycoprotein 36 (gp36) of the mouse mammary tumor virus (MMTV) were developed using the hybridoma technology [56] in which the spleens of immunized BALB/c mice producing the anti-gp36 IgE were fused with P3 × 20 myeloma cells [60]. These hybridoma cells were then injected intraperitoneally into BALB/c mice. Ten days following injection, ascites containing the anti-gp36 IgE were collected. In order to evaluate the anti-cancer potential of these IgE, syngeneic C3H/HeJ mice were subcutaneously challenged with H2712 murine mammary carcinoma (MMTV⁺) cells in combination with 20% ascites fluid containing 12.5 μg

of the anti-gp36 IgE. These mice were subsequently treated with 25 μ g of the anti-gp36 IgE via intraperitoneal injection every fourth day for 8 weeks. This treatment demonstrated a 50% protection rate from cancer development compared to control animals that received 20% normal mouse serum instead of the IgE-containing ascites fluid. Interestingly, all control mice ($n = 6$) died within 44 days, while 66% (4/6) treated with the anti-gp36 IgE survived during this time frame. One of these four mice died on day 72, but the three remaining mice were tumor-free 175 days after tumor challenge. A similar level of protection was observed when the H2712 tumor cells were injected intraperitoneally and mice were treated for 6 weeks at 4-day intervals with 25 μ g of the anti-gp36 IgE. All mice treated with normal mouse serum died within 34 days while two of six mice treated with the IgE were still alive after 139 days after tumor challenge. Importantly, the anti-gp36 IgE was unable to protect mice from tumor development in mice challenged with murine MA16/C mammary carcinoma (MMTV⁻) cells. These results demonstrate that the tumor specificity of the antibody is required for the anti-tumor effect of the anti-gp36 IgE.

7.3.2.2 Rat/Human Chimeric IgE Specific for Murine CD8

A rat/human chimeric IgE specific for the Ly-2 antigen was constructed for the initial purpose of evaluating antibody-mediated elimination of CD8-expressing target cells *in vivo* [61]. Murine CD8 is a cell surface glycoprotein that serves as a marker of cytotoxic T lymphocytes (CTL). It is a heterodimer consisting of the Ly-2 (α chain) and Ly-3 (β chain) [62]. The anti-tumor effects of this IgE were later explored [63]. One obstacle facing those evaluating the *in vivo* anti-cancer activity of recombinant IgE is the fact that human IgE does not interact with the mouse Fc ϵ RI [33, 64, 65]. In order to overcome this problem and to direct CTL to recognize TAA in a non-major histocompatibility (non-MHC)-dependent manner, a unique strategy utilizing a chimeric Fc ϵ RI was used [63]. This chimeric receptor was composed of three components: (1) the extracellular domain of human Fc ϵ RI, (2) the membrane proximal and transmembrane domains of human Fc γ RIIA, and (3) the cytoplasmic domain of human CD3 ζ . The chimeric receptor was stably expressed in the CTLL-R8 murine T-cell line under the control of the mouse CTL-specific perforin promoter. *In vitro* assays demonstrated that the binding of human IgE to this chimeric receptor resulted in target cell cytotoxicity. In this IgE-mediated ADCC assay, murine E3 thymoma cells (Ly-2⁺) pre-incubated with the chimeric rat/human anti-Ly-2 IgE were used as malignant target cells. Targeted cytotoxicity of the thymoma cells was observed when combined with CTLL-R8 cells expressing the chimeric Fc ϵ RI receptor on the cell surface (3H2 cells). The ability of 3H2 cells to lyse E3 thymoma cells *in vivo* was also explored using a Winn assay [63]. For this assay, 3H2 effector cells plus E3 target cells at an effector-to-target ratio of 10:1 in the presence or absence of the chimeric rat/human anti-Ly-2 IgE were incubated for 4 hours. E3 cells incubated in media alone (without 3H2 cells or antibody) were used as a control. The cells were then harvested, washed, and injected subcutaneously into C57BL/6 mice. E3 thymoma cells grew rapidly and caused mouse death within 5–7 days. The addition of 3H2 cells significantly prolonged survival; however, the anti-tumor effect was more prominent in mice treated with E3 and 3H2 cells pre-incubated with the chimeric

rat/human anti-Ly2 IgE. Only one of five of these mice developed a subcutaneous tumor (compared to four of five with 3H2 cells alone). In addition, the only tumor that developed in this group developed 1 week later than the tumors of the 3H2-alone treated mice. In a second experiment where the effector-to-target ratio was reduced to 3:1, all mice treated with only E3 tumor cells died in the first 15 days. Two of five mice treated with the mixture of E3 target tumor cells plus 3H2 effector cells, and a non-targeting IgE survived without the development of the tumor. Moreover, all five mice treated with the mixture of E3 target tumor cells, 3H2 effector cells plus the targeted anti-Ly2 IgE survived tumor-free. These results demonstrate that cancer-specific IgE can be used to redirect CTL that express a chimeric IgE receptor to lyse cancer cells providing further evidence for the potential use of IgE for cancer immunotherapy in humans.

The chimeric rat/human anti-Ly2 IgE used in the above studies has also been used in a similar approach using primary human T cells, genetically engineered to express a recombinant human Fc ϵ RI [66]. For this study, the chimeric receptor consisted of the extracellular domain of human Fc ϵ RI, the human Fc γ RII hinge and transmembrane regions, the cytoplasmic signaling domain of human CD28, and the cytoplasmic signaling domain of the human T cell receptor ζ chain. The anti-Ly2 IgE in combination with primary T-cells expressing the chimeric Fc ϵ RI (T-CL9) were shown to induce IgE-mediated murine E3 thymoma (Ly-2⁺) cell lysis in vitro [66]. Lysis was not observed in 3LL (Ly-2⁻) murine Lewis lung carcinoma cells. Importantly, anti-Ly2 IgE in combination with T-CL9 and E3 thymoma cells also resulted in the production of immune-stimulatory cytokine production (IFN- γ and GM-CSF) that was only observed with the combination of these three components. In addition, transfer of chimeric Fc ϵ RI-expressing T cells demonstrated in vivo anti-tumor activity [66]. E3 thymoma tumors were established via intraperitoneal injection in non-obese diabetic severe combined immunodeficient (SCID) mice. Mice were treated with T cells expressing the recombinant receptor (T-CL9) or mock transduced T cells that had been previously incubated with the anti-Ly2 IgE by intraperitoneal injection. Mice received treatment on days 0, 1, 2 and 4. T-CL9 cells significantly enhanced mouse survival from 18 to 27 days [66]. Pre-incubation of T-CL9 cells with a non-specific IgE did not enhance survival compared to control animals. Similarly enhanced survival was observed in mice with established tumors. Thus, the tumor-specific IgE is required for the in vivo ability of the anti-Ly2 IgE to redirect the cytotoxic effects of the recombinant T cells toward the targeted cancer cells.

7.3.2.3 Murine and Mouse/Human Chimeric IgE Specific for an Antigenic Determinant on the Surface of Colorectal Carcinoma Cells

In order to evaluate the cytotoxic effects of a cancer-specific IgE, a murine IgE (mIgE 30.6) was tested in parallel with a chimeric mouse/human IgE (chIgE 30.6) [67]. Both antibodies contain the variable region of the murine IgG2b antibody 30.6 and bind an antigen expressed on the surface of human colorectal carcinoma cells, such as COLO 205 cells. Neither of these antibodies demonstrated any direct in

vitro cytotoxicity against COLO 205 cells [67]. To evaluate the *in vivo* anti-tumor effects of the two IgE antibodies, BALB/c SCID mice subcutaneously xenografted with COLO 205 cells were treated intravenously with a single injection of 1 μ g of mIgE 30.6, chIgE 30.6 or buffer (PBS) 5 days after tumor challenge. Only mice treated with mIgE 30.6 significantly inhibited tumor growth; however, the effect was transient and tumor growth returned to the same rate as control mice starting 48 hours after mIgE injection. Three consecutive treatments with 1 μ g mIgE 30.6 every 2 days starting 5 days after tumor challenge did not enhance this anti-tumor effect [67]. A previous study by this group demonstrated that milligram quantities of a mouse/human chimeric IgG1 with the same variable regions (chIgG 30.6) are required to inhibit COLO 205 growth in SCID mice [68]. The superior *in vivo* activity of mIgE compared to that of the previously reported chIgG may be due to the higher affinity of IgE for its Fc ϵ R compared to the affinity of IgG to its Fc γ R, and therefore, lower amounts of IgE are required to induce a highly tumoricidal effect by immune effector cells. However, the mIgE and chIgG were not tested simultaneously. The inability of chIgE 30.6 to elicit an anti-tumor response was not attributed to a difference in the relative affinity or serum half-life compared to the mIgE 30.6. The lack of efficacy of chIgE 30.6 is not surprising since human IgE does not interact with murine Fc ϵ RI [33, 64, 65]; thus there was no activation of an anti-tumor immune response in these mice. Neither the mechanism nor the transient nature of the anti-tumor effect of mIgE is known. However, the transient effect is not due to the loss of antigen expression [67]. It has been suggested that the relevance of the cross-linking of Fc ϵ R to release cytokines, such as tumor necrosis factor (TNF) or interleukin-4 (IL-4), from mast cells may be involved in the anti-tumor effect of mIgE 30.6 [67].

7.3.2.4 Mouse/Human Chimeric IgE Specific for Human Folate Binding Protein

Another study has also aimed to compare the anti-tumor effect of cancer-specific antibodies of the IgE and IgG classes. Mouse/human chimeric IgE (MOv18-IgE) and IgG1 (MOv18-IgG1) antibodies specific for the folate binding protein (FBP) have been developed [69]. FBP is expressed at higher densities on the surface of ovarian cancer cells compared to normal cells making it an appropriate target for antibody therapy. *In vitro* studies demonstrate that MOv18-IgE is able to induce degranulation of human basophils as measured by histamine release [69]. In this assay, both a non-specific IgE and MOv18-IgE induced histamine release from basophils when crosslinked by an anti-human IgE. Importantly, only MOv18-IgE induced histamine release in the presence of IGROV1 (FBP⁺) cancer cells, demonstrating a cancer-specific response of the MOv18-IgE. In order to test the *in vivo* efficacy of the MOv18 antibodies, C.B-17 scid/scid (SCID) mice were subcutaneously challenged with IGROV1 cells followed by an intravenous injection with a mixture of human PBMC and either MOv18-IgE or MOv18-IgG1. Thirty-five days after tumor challenge control mice showed excessive tumor growth (Fig. 7.4). In contrast, both the MOv18-IgE and the MOv18-IgG1 inhibited tumor growth up

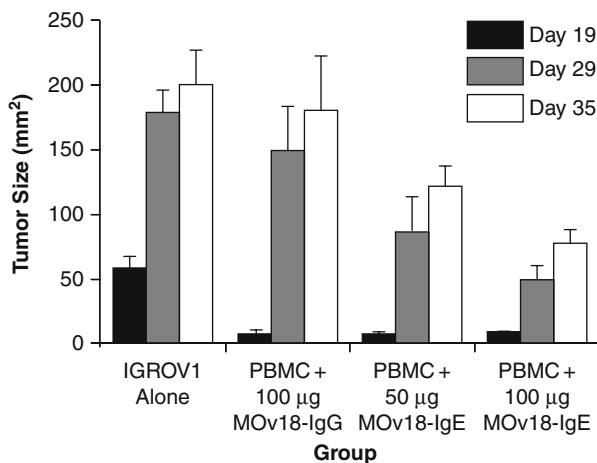


Fig. 7.4 MOv18-IgE inhibits the growth of IGROV1 human ovarian adenocarcinoma cells in vivo in the presence of human PBMC as effectors. A matched variable region set of mouse/human chimeric IgE and IgG1 antibodies specific for FBP was tested for their ability to inhibit the subcutaneous growth of cells in a SCID mouse xenograft model of human ovarian cancer. Mice were treated intraperitoneally with anti-asialo G_{M1} to eliminate host natural killer cells. One day later, mice were challenged subcutaneously with 2×10^6 IGROV1 cells followed by intravenous injection of 2.5×10^6 human PBMC combined with either 100 µg MOv18-IgG1, 100 µg MOv18-IgE or 50 µg MOv18-IgE. The mean tumor size $\pm$ the standard error of the mean (SEM) measured on days 19, 29 and 35 are shown ($n = 4$ for all groups). Reproduced with permission from Fig. 4A Gould et al. [69]. Copyright Wiley-VCH Verlag GmbH & Co. KGaA

to day 19; however, MOv18-IgG1 did not demonstrate protection after this time. Only MOv18-IgE (100 µg) was able to prevent tumor growth for an additional 16 days (to day 35) compared to mice treated with the same concentration of MOv18-IgG1, which developed tumors after 19 days. It is also important to note that mice treated with half of the concentration (50 µg) of IgE compared to IgG1 (100 µg) showed 40% inhibition of tumor growth at day 35 (Fig. 7.4). Due to the inability of human IgE to bind murine $Fc\epsilon R$, the use of human PBMC was essential for the anti-tumor effect of MOv18-IgE [69]. This demonstrates that human effector cells are required for the IgE-mediated anti-tumor response. It is important to note that the anti-tumor activity of MOv18-IgE may be underestimated in this model since the effectors are limited to human PBMC, which are exogenously administered. In contrast, in humans there would be a permanent supply of the entire effector repertoire including PBMC, and thus, the anti-tumor activity of IgE is expected to be superior compared to that observed in this mouse model. Furthermore, the capacity of the IgE to elicit an adaptive immune response could not be evaluated since immunodeficient mice were used. Despite these drawbacks, this study demonstrates that the anti-cancer effects of MOv18-IgE were superior compared to those of its IgG1 counterpart and that the Fc-mediated effector functions of the antibody are essential.

The anti-cancer effect of MOv18-IgE was confirmed using a different mouse model [70]. Nude mice (nu/nu) were challenged intraperitoneally with human HUA ovarian cancer cells expressing FBP. The following day, human PBMC were injected intraperitoneally alone or in combination with 100 μ g MOv18-IgE or MOv18-IgG1. Treatment with PBMC and MOv18-IgE significantly increased survival to 40 days compared to mice treated with the mixture of PBMC and MOv18-IgG1 (22 days) or compared to mice treated with PBS (19 days). MOv18-IgG1 in combination with PBMC in this model did not show a protective effect. Immunohistochemistry analysis of HUA xenografts demonstrated that human monocytes were localized around tumor lesions in MOv18-IgE-treated mice only, suggesting that these effector cells play an important role in the anti-tumor effect of this antibody. This was confirmed using monocyte-depleted PMBC in this tumor model [48].

Further studies have helped to identify the mechanisms of the anti-cancer effects of MOv18-IgE. In vitro assays demonstrated the ability of MOv18-IgE to lyse cancer cells via ADCC by human monocytes [70] or eosinophils [48]. ADCC is an important and relevant mechanism of action of recombinant antibodies approved for cancer immunotherapy [71]. In addition, in vitro analysis demonstrated that MOv18-IgE is able to induce ADCP through the interaction with its low-affinity receptor CD23 expressed on the surface of (IL-4)-activated monocytes [48]. IL-4 was shown to induce the expression of CD23, but not Fc ϵ RI, on the surface of monocytes isolated from human PBMC. The role of CD23 in ADCP was confirmed by using an antibody to block the binding of IgE to CD23 in which this ADCP activity was decreased. In contrast, blocking CD23 did not affect the MOv18-IgE-induced ADCC activity, indicating that ADCC is mediated by Fc ϵ RI and not by CD23. In addition, primary monocytes pre-stimulated with MOv18-IgE showed an increase of surface Fc ϵ RI expression, which correlated with the increase of MOv18-IgE-mediated ADCC, confirming that the high-affinity IgE receptor is required for IgE-mediated ADCC in vitro. These results have also been observed using U-937 (a human monocyte cell line) as effector cells [47]. Galectin-3, a beta-galactosidase-binding lectin that also binds human IgE, was shown not to be relevant for the ADCC and ADCP activities of MOv18-IgE in this model [47]. These studies have helped to define the mechanism of MOv18-IgE-mediated toxicity as well as to shed light on the basic biological function of the Fc ϵ Rs. Both IgE-mediated ADCC and ADCP are discussed in detail in Chapter 8.

7.3.2.5 Engineered IgE Specific for Human HER2/*neu*

HER2/*neu* (also known as ErbB-2 or HER-2), a member of the epidermal growth factor family, is overexpressed on approximately 25–30% of breast cancers and is associated with poor prognosis [72]. Its overexpression and association with more aggressive disease along with its extracellular accessibility make this antigen attractive for antibody therapy. In fact, the FDA-approved antibody trastuzumab (Herceptin[®]), a humanized IgG1 specific for HER2/*neu*, can lead to an objective response in some patients with tumors overexpressing HER2/*neu* when used

alone or an even higher response when combined with chemotherapy [73–78]. Unfortunately, most of the patients with advanced breast cancer who achieve an initial response to trastuzumab-based treatment acquire resistance and eventually succumb to the disease [75, 79]. Therefore, as a new treatment for HER2/*neu* expressing tumors, an anti-HER2/*neu* IgE was developed [49]. This humanized IgE was derived using DNA sequences corresponding to the published protein sequences of the heavy and light chains of the trastuzumab variable regions [49]. This IgE was expressed in HEK293 human embryonic kidney cells and its in vitro efficacy was compared to trastuzumab. Both trastuzumab and its IgE homologue exhibited similar direct anti-tumor effects on SK-BR-3 human breast cancer cells in vitro [49]. Both antibodies also induced U-937-mediated killing of a murine colon carcinoma cell line expressing human HER2/*neu* (CT26-HER2/*neu*) as well as SK-BR-3 cells. However, the mechanism of cell killing was found to be different for the two antibodies. Trastuzumab was shown to mediate ADCP of target cells, while the IgE facilitated ADCC. In addition, the IgE antibody stimulated the degranulation of mast cells when crosslinked with HER2/*neu*-positive cancer cells, indicating that an anti-tumor immune response with the characteristics of an allergic reaction can be induced by a cancer-specific IgE.

Our group has also developed a new generation of anti-HER2/*neu* IgE. This antibody is fully human in origin and contains the variable regions of the C6MH3-B1 single-chain Fv (scFv) specific for the extracellular domain of human HER2/*neu* that was originally isolated from a human phage library [80]. This scFv was also previously affinity matured in vitro by sequential mutation of the third complementary determining region (CDR) in both the variable heavy chain (V_H) and the variable light chain (V_L) regions [81]. The highest affinity mutations of both the V_H and the V_L were selected based on binding to HER2/*neu*. The affinity-matured scFv demonstrated a 1230-fold increase in affinity compared to the parental C6.5 scFv [81]. These affinity-matured V_L and V_H regions were cloned into either the human κ light chain or the human ϵ heavy chain expression vectors, respectively, to yield a fully human anti-HER2/*neu* IgE [82]. This antibody, expressed in murine myeloma cells, is properly assembled and secreted, binds antigen and binds Fc ϵ RI. Although the anti-cancer effect of this antibody is currently being explored, it is important to note that a major advantage is that this molecule is totally of human origin (not chimeric or humanized). In addition, this anti-HER2/*neu* IgE binds a different epitope compared to the FDA-approved anti-HER2/*neu* IgG1 trastuzumab (Herceptin[®]) [83]. Thus, this IgE antibody could be used in combination (simultaneous or sequential) with trastuzumab to activate a diverse and more potent immune response targeting breast cancer.

7.3.2.6 Chimeric IgE Targeting Human CD20

The anti-CD20 IgG1 antibody rituximab (Rituxan[®]) is currently being used in the clinic for the treatment of non-Hodgkin's lymphoma (NHL). However, resistance to the antibody occurs in most patients [84, 85] and alternative strategies are still needed. We have developed a novel anti-CD20 antibody composed of the variable

region of rituximab, the human constant κ light chain and the human Fc ϵ constant region (anti-CD20 IgE). This antibody expressed in murine myeloma cells is properly assembled and secreted and exhibits specific binding to the antigen and to the Fc ϵ RI. Preliminary studies show that anti-CD20 IgE elicited significant direct cytotoxicity and ADCC activity against human CD20⁺ NHL cell lines [82, 86]. Moreover, crosslinking of anti-CD20 IgE with lymphoma cells expressing CD20 triggered local cutaneous anaphylaxis in transgenic mice expressing human Fc ϵ RI α [86]. We expect that the anti-CD20 IgE, like other antibodies of the IgE class targeting TAA, can be used as a direct anti-tumor agent for passive immunotherapy as well as an adjuvant of cancer vaccines, which may potentially have a significant impact on the treatment of NHL.

7.3.3 Induction of an Endogenous IgE Response via Mimotope Vaccination

The above studies are focused on the development of tumor-specific IgE antibodies and their use for passive immunotherapy of cancer. An alternative strategy is the induction of an endogenous IgE response that targets a TAA [87]. For this purpose, a novel approach was taken to establish a vaccination protocol that would result in the induction of an anti-cancer-specific IgE response in vivo. Using the epitope recognized by trastuzumab (humanized anti-HER2/*neu* IgG1), epitope mimics (mimotopes) were generated [88]. This mimotope consisted of the 1, 12-cyclic peptide C-QMWAPQWGPD-C coupled via its carboxy terminus to a succinimidyl-4-(*N*-maleinimidomethyl) cyclohexane-1-carboxylate-activated immunogenic carrier, keyhole limpet hemocyanin (KLH), through a linker (GPGPG) by *S*-acetyl-thio-acetate ("QMW-KLH"). A non-targeted peptide 1, 12-cyclic C-DGGWLSKGSW-C coupled to KLH in the same manner was used as a negative control ("KLH"). Vaccination using these mimotopes yields the generation of endogenous "trastuzumab-like" antibodies in vivo [87, 88]. Moreover, it has been shown that a specific IgE response can be induced by the combination of oral immunization with the mimotope and antacid treatment that is known to induce food allergy [89, 90].

The above vaccination strategies were combined in BALB/c mice to elicit an endogenous anti-HER2/*neu* IgE response [87] (Fig. 7.5). For the antacid treatment, mice were given omeprazole, a proton pump inhibitor, orally once daily for 2 days prior to vaccination. Mice were also given omeprazole twice on the day of immunization. The mimotopes were administered orally in the protective ulcer medication sucralfate as described previously for the induction of food allergy [89, 90]. This strategy resulted in the induction of a high-titer endogenous IgE response (Fig. 7.6). In addition, the induced IgE recognized the mimicked oral antigen (HER2/*neu*) on the surface of human SK-BR-3 breast cancer cells and resulted in both the degranulation and cytotoxicity of RBL-2H3 rat basophilic leukemia cells expressing the rodent Fc ϵ RI [87]. Taken together, these studies show that this unique vaccination strategy can lead to the induction of an endogenous anti-cancer IgE response.

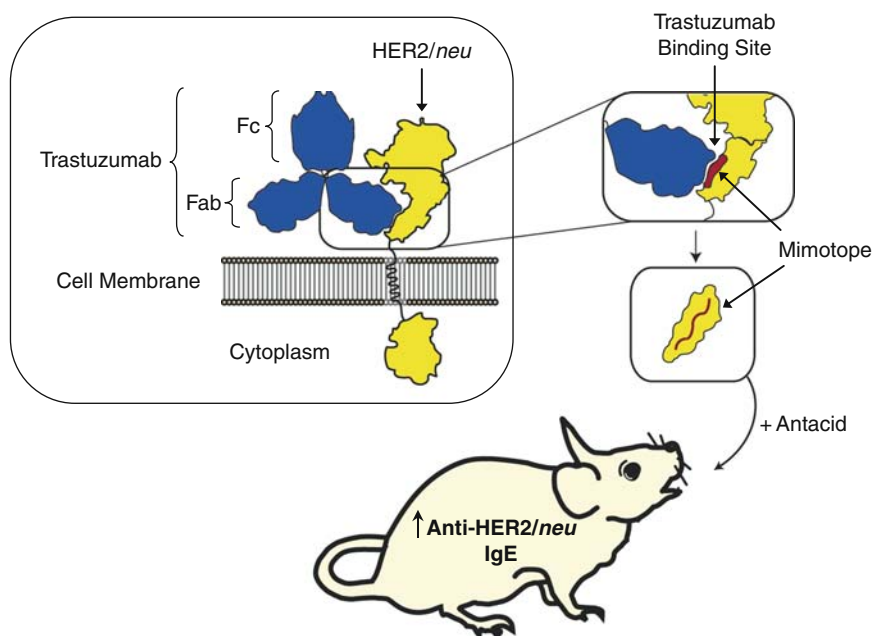


Fig. 7.5 Schematic diagram outlining the strategy for the generation of mimotope peptides and their administration during oral vaccination. HER2/*neu* is a transmembrane receptor consisting of three domains: extracellular, transmembrane and cytoplasmic. The extracellular domain of HER2/*neu* is recognized by the FDA-approved therapeutic antibody trastuzumab (Herceptin®). The region of HER2/*neu* that is recognized by trastuzumab was used to generate a mimotope that when used in combination with an antacid in an oral vaccination setting would lead to the generation of an endogenous anti-HER2/*neu* IgE antibody response in the mouse

7.4 Conclusions

Since the early 1990s the number of antibodies of the IgE class developed for passive immunotherapy of cancer has rapidly increased, and it is expected that this growth will continue. In order to target both epithelial and hematopoietic malignancies, IgE antibodies targeting different TAA such as FBP, HER2/*neu*, and CD20 have been developed. Importantly, these antibodies have demonstrated significant anti-tumor activity in vitro and in some cases in animal models. Moreover, an endogenous IgE response that targets a TAA can be induced by a vaccination strategy. Studies of IgE-mediated anti-tumor immunity have advanced the understanding of IgE biology and uncovered specific roles for its different receptors, FcεRI and FcεRII (CD23). Armed with this knowledge and the tools required to develop tumor-specific IgE antibodies, investigators are focused on addressing the effectiveness of these approaches in light of the success and limitations of IgG-based immunotherapy. These efforts are expected to result in the continued generation of IgE antibodies that can harness the allergic reaction and redirect it to fight the

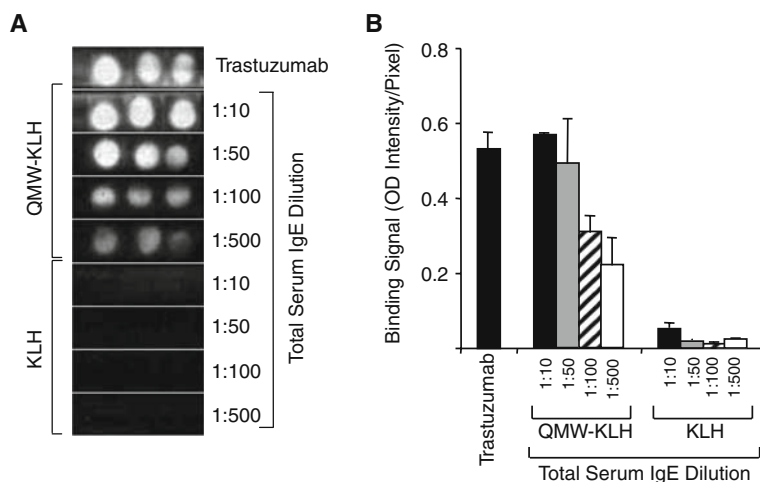


Fig. 7.6 Induction of endogenous antigen-specific IgE is achieved by oral vaccination using a mimotope peptide. BALB/c mice (seven per group) were immunized with the mimotope C-QMWAPQWGPDC (“QMW-KLH”) or the carrier molecule KLH by itself as described in Fig. 7.5. Each animal received 11.6 μ g of the proton pump inhibitor omeprazole intravenously on days 1, 2 and 3 of each immunization cycle. Mice were immunized on days 3, 17, 45, 59 and 87. **Panel A:** QMW-specific serum IgE titers determined by DotBlot assay. **Panel B:** Optical density of the DotBlot spots determined by densitometry analysis of a 20×20 pixel area from three independent spots along with standard deviations per condition and dilution. Signals from these spots are shown as total optical density per square pixel area. Panel B of this figure was created using the data shown in panel A, which was adapted from Fig. 2 in Riemer et al. [87] with permission from The American Association for Cancer Research

tumor leading to the induction of a potent anti-cancer response capable of reducing tumor burden or even facilitating full tumor rejection with long-term anti-tumor immune memory. However, a limitation in the basic and translational studies of IgE antibodies that have human constant regions has been the lack of appropriate animal models to properly and fully evaluate their efficacy and toxicity. Given the involvement of IgE in anaphylaxis, toxicological studies are critical for the preclinical analysis of these IgE molecules. Advances in the model systems for the study of human IgE are expected to overcome this limitation and expedite the evaluation of IgE immunological functions *in vivo*. Such advances include the development of Fc ϵ RI transgenic animals such as mice expressing the human Fc ϵ RI α chain [64, 65]. Transgene expression in these mice parallels the effector cell-specific expression of Fc ϵ RI in humans, making this an attractive model to evaluate both efficacy and toxicity. In fact, these “humanized Fc ϵ RI” transgenic mice have already been utilized to evaluate PCA *in vivo* induced by IgE with human Fc fragments [64, 86], and they open the possibility to further evaluate the potential use of human IgE for the treatment of human cancers. Advances in the understanding of IgE-mediated anti-tumor immune responses, protein design, and experimental models help us to better

understand the benefits and potential drawbacks of IgE-mediated immunotherapy of cancer paving the way to clinical trials.

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Chapter 8

IgE Interacts with Potent Effector Cells Against Tumors: ADCC and ADCP

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Abstract We examine the properties of IgE antibodies which may render them effective against tumors. This may be due to the uniquely high affinity of IgE antibodies for their Fc epsilon (Fcε) receptors and the expression of IgE antibodies on potent effector cells. Owing to these properties, IgE antibodies activate effector cell mechanisms that may be different from and stronger than those mediated by antibodies of other classes, thereby inducing superior anti-tumor responses. In examining the potential of IgE–FcεRI complexes on effector cells to activate effector cells (and describe evidence to-date) we suggest that the interactions of IgE antibodies with tumor-associated antigens on tumor cells and Fc receptors on IgE receptor-bearing cells trigger the association of tumor and effector cells. The outcome of these interactions is effector cell activation and effective tumor cell death by a number of mechanisms including antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP).

8.1 IgE Operates Through Powerful Fc Epsilon Receptors

IgE has two known receptors: the high-affinity receptor FcεRI and the low-affinity receptor CD23 (FcεRII) [1, 2]. FcεRI and CD23 are specific for the Fc epsilon (Fcε) region of the antibody. The IgE-binding molecule galectin-3 binds to oligosaccharides of IgE [3].

8.1.1 The High-Affinity IgE Receptor FcεRI

In humans, FcεRI is found on a variety of immune cells, namely, mast cells, basophils, eosinophils, monocytes, Langerhans cells, dendritic cells (DC) and platelets. There are two forms of FcεRI: the tetrameric form ($\alpha\beta\gamma_2$) is expressed

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on mast cells and basophils, and the trimeric form ($\alpha\gamma 2$) is found on monocytes [4], eosinophils [5–8] and antigen-presenting cells (APC) [9–12]. The IgE α subunit with two immunoglobulin extracellular domains contains the IgE-binding site with a ligand:receptor stoichiometry of 1:1, while the β and γ subunits have no role in binding IgE. The γ -chain, a ubiquitous signal transduction subunit, is shared by a number of immunoreceptors, and notably IgG receptors Fc γ R [13] and the IgA receptor Fc α RI [14] on a range of hematopoietic cells. It is essential for cell surface expression of the receptor complexes and is known to enhance intracellular signaling [15, 16]. The presence of the β -chain serves as an amplifier of IgE-mediated functions both *in vitro* and *in vivo* [17, 18], is known to enhance expression of unoccupied receptor on the cell surface [19] and is therefore, at least in part, responsible for the observed high levels of Fc ϵ RI expression in mast cells and basophils [20]. Ligation of the receptor by IgE and cross-linking by antigen results in the activation of multiple signaling pathways, leading to a variety of effector cell responses (Fig. 8.1). Unlike humans, mice express only the tetrameric form ($\alpha\beta\gamma 2$), and the distribution of Fc ϵ RI is restricted to mast cells and basophils [1, 15].

The affinity of IgE for Fc ϵ RI ($K_a = 10^8 - 10^{11} \text{ M}^{-1}$) is 2–5 orders of magnitude higher than that of IgGs for their receptors [4, 21] (Table 8.1, Fig. 8.1). The high affinity of the receptor–antibody interaction may have profound implications for both the potency of IgE-mediated effector functions and the longevity of IgE-mediated immune responses. Due to the high affinity of the IgE–Fc ϵ RI complex,

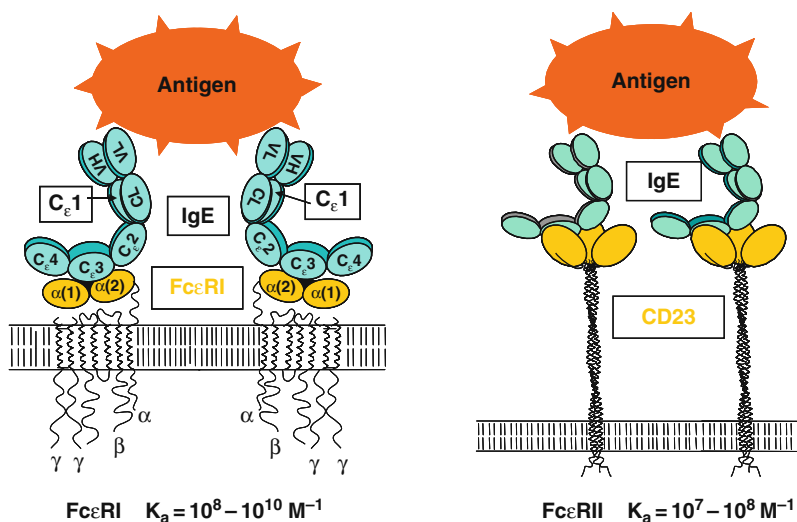


Fig. 8.1 Schematic representation of IgE antibody binding to the extracellular domains of the Fc ϵ RI α -chain (*left*) and to the lectin domains of CD23 (*right*) and reported binding affinities for each interaction. Cross-linking of surface-bound IgE–Fc ϵ receptor complexes by antigens triggers effector cell activation

Table 8.1 Binding affinities of Immunoglobulins for their Fc receptors

Fc receptor	Ig ligand	Affinity (K_a)	References
FcαR	IgA	10^6 M^{-1}	Wines et al. [130]
FcεRI	IgE	$10^8\text{--}10^{11}\text{ M}^{-1}$	Gould et al. [1]
FcεRII (CD23)	IgE	$10^7\text{--}10^8\text{ M}^{-1}$	Gould et al. [1]
FcγRI (CD64)	IgG	$10^8\text{--}10^9\text{ M}^{-1}$	Ravetch and Kinet [4]
FcγRII (CD32)	IgG	$<10^7\text{ M}^{-1}$	Maenaka et al. [21]
FcγRIII (CD16)	IgG	10^5 M^{-1}	Maenaka et al. [21]

IgE is the only antibody class that can be strongly retained by effector cells in the absence of antigen. The high affinity of IgE for FcεRI may account for the long half-life of IgE in tissues (around 2 weeks measured in skin), which is longer than that measured for IgG (2–3 days). The relative retentions of each antibody class in tissues are proportional to the affinity constants [1, 4]. The high affinity of the IgE–FcεRI complex may suggest the potential of IgE to engage FcεRI in superior anti-tumor cell responses in tissues compared to those mediated by IgG and its receptors. This may be because FcεRI on the surface of effector cells recruited into tissues may sequester IgE.

A number of studies on the emerging protective role of IgE in parasitic infections implicate the requirement for FcεRI expressed on the surface of mast cells to mediate the inflammatory cascades that confer protection against parasites [22–24]. The roles FcεRI play in allergic responses, both in the “early phase,” involving mast cell activation and degranulation, and the “late phase,” involving recruitment and activation of inflammatory cells, have been extensively studied [25]. FcεRI-expressing tissue mast cells and blood basophils bind antigen-specific IgE and on cross-linking by multivalent antigen release potent inflammatory mediators, such as histamine, leukotrienes, proteases and a variety of Th2-type cytokines, initiating and enhancing the recruitment and activation of T cells, monocytes, eosinophils, basophils and APC to the site of antigen challenge (Fig. 8.1). The resulting inflammatory response may also serve to activate and perpetrate immune effector functions in situ. In addition, local or recruited IgE-sensitized APC promote IgE production by B cells, thus maintaining mast-cell and APC sensitization and long-term immunologic memory and immune surveillance both in situ and systemically.

IgG receptors on the surface of effector cells are capable of mediating anti-tumor responses. FcγRIII (CD16), which is the nearest IgG receptor homologue to FcεRI, containing the ligand-binding α-chain with the closest homology to the α-chain of FcεRI, and which has a very low affinity for IgG ($K_a = 10^5\text{ M}^{-1}$) [21] (Table 8.1), has been shown to participate in IgG antibody-dependent T-cell-, natural killer (NK) cell- and macrophage-mediated tumor cell cytotoxicity [antibody-dependent cellular cytotoxicity (ADCC)] [4, 26–28]. FcγRIII has also been reported to engender macrophage-mediated tumor cell phagocytosis [antibody-dependent cellular phagocytosis (ADCP)] [29]. The high-affinity IgG receptor, FcγRI or CD64, with affinity for IgG ($K_a = 10^8\text{ M}^{-1}$) two orders of magnitude lower than that measured for

the IgE–FcεRI complex [4], can mediate both ADCC and ADCP of tumor cells [30–33]. Since FcγRs confer anti-tumor responses, it is likely that the potency of engaging IgE with FcεRI in parasitic infections and allergies may also be harnessed to combat cancer cells. The high-affinity of IgE–FcεRI complexes implies that strategies aimed at engaging IgE–FcεRI against tumor cells may confer superior anti-tumor cell responses in tissues compared to those mediated by IgG and its receptors.

8.1.2 The Low-Affinity IgE Receptor (CD23)

The human low-affinity IgE receptor, CD23 a type II integral membrane protein, is different from all other immunoglobulin receptors in that it belongs to the C-type lectin superfamily. Its two splice variants, CD23a and CD23b, differ in their first six (CD23b) or seven (CD23a) amino acids at the cytoplasmic N-terminus and contain different signaling motifs that determine their functions [34]. Human CD23a is expressed only on antigen-activated B cells prior to differentiation into antibody-secreting plasma cells and is involved in IgE antibody-dependent antigen presentation and regulation of IgE synthesis. The expression of human CD23b is induced by IL-4 on various inflammatory cells, for example, T cells, eosinophils, platelets, monocytes, B cells, DC and epithelial cells [1, 35]. Engagement of CD23b by IgE–antigen complexes promotes monocyte/macrophage activation, induces nitric oxide synthase (iNOS) and generates pro-inflammatory cytokines [36, 37] (Fig. 8.1). These processes are thought to play a role in clearance of parasitic infections through the release of TNF-α and nitric oxide (NO) [38, 39]. A natural function for human CD23b is IgE antibody-dependent cellular ADCP of hapten-coated red cells by monocytes/macrophages [35, 40], suggesting that CD23 may mediate this function against tumor cells.

In humans, IgE binds to CD23 with relatively high affinity ($K_a = 10^7 - 10^8 \text{ M}^{-1}$), as high as that of IgG for its high-affinity receptor FcγRI [1, 4] (Table 8.1, Fig. 8.1). Numerous studies now describe the functions of CD23 inducing inflammatory and memory responses in response to allergens and parasitic infections. There are also reports that tumor-associated macrophages (TAMs) express CD23 in a number of human cancers [41]. Furthermore, the soluble fragments of CD23 (sCD23) have also been associated with blood-borne cancers. Increased sCD23 levels are associated with poorer patient survival in chronic lymphocytic leukemia [42]. It is conceivable that sCD23 may be elevated in certain malignancies, resulting in blocking the formation of IgE–FcεRII complexes on the surface of effector cells, thereby interfering with IgE-mediated anti-tumoral responses. However, a potential role for CD23 in tumor killing is only starting to emerge [43, 44]. The effector cell functions mediated by CD23 in the context of allergies and parasitic infections, coupled with its comparatively high affinity for IgE, may make it a potentially effective IgE receptor for mediating tumor cell targeting and killing.

8.1.3 *Galectin-3*

Galectin-3, also known as the beta-galactoside-binding lectin galectin-3, epsilon-binding protein (e-BP), IgE-binding protein or Mac-2, binds specifically to oligosaccharide structures with a terminal β -galactose through a lectin–carbohydrate interaction [45]. It recognizes both IgE and Fc ϵ RI and can activate immune responses [3, 46]. Due to its ability to form pentameric complexes with oligosaccharides, it can crosslink Fc ϵ RI or receptor-bound IgE on the surface of mast cells and basophils [47, 48]. Despite its potential to enhance mast cell activation and mediate the phagocytotic properties of monocytes, increased expression of galectin-3 has been associated with poor prognosis in thyroid and pancreatic cancers, whereas reduced levels of the protein were detected in colon, ovarian, breast, endometrium and skin cancers, compared to normal tissues [49]. Galectin-3 expression in malignancies may have a bearing on the immunogenic nature of the tumor, but this has not yet been fully explored. Although expressed in monocytic cells, at least in one in vitro study, galectin-3 did not appear to mediate tumor cell death [43]. To-date, there is no evidence linking galectin-3 to IgE antibody-dependent tumor cell killing although further research may shed more light on potential mechanisms that may link galectin-3 to IgE-mediated immune cell activation signals against cancer cells.

8.2 What is Known About IgE Effector Cells in Cancer? Missing Activation Signals May Tip the Balance in Favor of Tumor Growth

The relationship between tumor cells and the immune system is vastly complex. The dialog between tumor cells and the immune system in cancer results in inflammation and tumor regression but has also been associated with tumor growth, angiogenesis, invasion and metastasis. Patient prognoses for some cancers also appear to be dependent on the nature of the immune responses elicited in the tumor microenvironment; positive and negative correlations between immune infiltrate and clinical outcome have been reported. It is well established that solid tumors are associated with inflammatory responses resulting in infiltration of mast cells, B and T lymphocytes, neutrophils, NK cells, DC, macrophages and eosinophils [50]. These cells are recruited by a variety of cytokines and chemokines expressed by local inflammatory cells that bind to upregulated adhesion molecules and cell surface receptors in situ. Tumor cells also secrete cytokines and chemokines that contribute to this process; often, this promotes angiogenesis, tumor growth and metastasis [51].

Most of the inflammatory cell infiltrates are IgE effector cells. These cells have the potential to direct strong anti-tumor activities in situ that may tip the balance

against tumor growth and invasion. Herewith, we describe the relationship between these effector cells and cancer tissues and how effector cell activities are influenced by tumor cells.

8.2.1 Mast Cells and Basophils

Tryptase-positive mast cells infiltrate the invasive fronts in greater numbers than in any other area of malignant tumor lesions, but their contribution to cancer growth and spread has been controversial [52]. In breast cancer the presence of mast cells in the peritumoral areas has correlated with a favorable clinical prognosis [53, 54]. Hodgkin's lymphoma, endometrial cancer and melanoma patients with higher numbers of mast cells in tumor lesions have a worse prognosis, whereas mast cell numbers in lymph nodes do not correlate with clinical outcomes in Hodgkin's lymphoma [55–57]. Increased mast cell numbers in breast cancer lesions and lymph nodes with metastases have been reported compared to those from benign lesions and from lymph nodes clear of metastatic disease [58–60].

Mast cell activation and degranulation promotes the destruction of local tissue architecture, tissue remodeling and angiogenesis, and this is thought to assist tumor cell growth and metastasis [61]. A number of factors may also contribute to the inability of resident mast cells to mount effective immune responses against tumors. One reason may be the location of mast cells in the invasive fronts, away from the core of solid tumors, suggesting that mast cells may respond to tumor cell stimuli and may be recruited to assist tumor growth and invasion. Another factor may be the production of tryptase, this may result in the loss of local tissue architecture that promotes tumor invasion but also in stimulating proliferation of vascular endothelial cells, promoting tumor-associated neovascularization. The production of angiogenic factors and cytokines by mast cells in response to tumors (VEGF, TGF- β , FGF-2, IL-8, TNF- α , MMP-9) appears to tip the balance in favor of tumor growth and metastasis [62–66]. It has been suggested that the intensity of mast cell activation/response in the absence of antigenic stimuli may be a contributing factor in this process [61, 67]. Since mast cells are potent IgE effector cells, it is therefore tempting to consider that treatment with tumor antigen-specific IgE antibodies may stimulate mast cells against cancer cells and help redress the balance in favor of immune activation and IgE-mediated anti-tumoral responses.

The role of basophils in cancer has not been extensively elucidated. One notable exception is perhaps blood malignancies such as chronic myeloid leukemia, where basophil numbers are increased in later stages of the disease and basophil markers are used for diagnostic but also prognostic evaluations [68]. Basophils have also been observed in the inflammatory infiltrate in experimental tumors. They may be recruited into tumor areas by in situ expression of cytokines such as IL-4 and in response to chemoattractant signals like VCAM-1 and MCP-1 [69] and are thought to be associated with tumor regression. Their potential IgE-mediated anti-tumor functions are yet to be explored.

8.2.2 *Monocytes/Macrophages*

TAMs are found in virtually all types of tumors and can comprise more than 50% of the total tumor mass in both primary and metastatic lesions [70]. Blood monocytes are constantly recruited to the tumor sites by chemokines and cytokines released by tumor cells (CSF-1, CC chemokines) and neighboring endothelial cells (VEGF). Evidence suggests that TAMs switch off anti-tumor immunity and are associated with poor clinical outcomes [71]. Once in tumor sites, TAMs can function by promoting tumor growth and metastasis, producing angiogenic factors and metalloproteinases [72, 73]. Macrophage infiltration of various tumors such as melanoma, stomach and colorectal cancers has been correlated with immunogenicity but also inversely correlated with metastatic potential, while also the opposite has been reported for cancers of the breast, prostate and endometrium.

Macrophages from normal and infected tissues have been shown to lyse tumor cells, present tumor-associated antigens to T cells and express co-stimulatory factors that enhance T cell and NK cell anti-tumor functions [74]. On the contrary, TAMs are generally thought to be immature in their phenotype and this may be partly due to their in situ exposure to tumor cell and immune cell-secreted cytokines such as IL-4, IL-10 TGF- β 1 and prostaglandin E2. It has been proposed that these signals polarize macrophages into the alternatively activated type II or M2 macrophage phenotype. M2 TAMs constitutively express high levels of IL-1 β and IL-6 and low levels of TNF- α . They are thought to have impaired antigen-presenting capacity, can suppress activation of T cells, induce T cell apoptosis, switch off DC maturation through secretion of IL-10 and engage in tissue repair and remodeling in favor of tumor growth, angiogenesis and invasion [73, 75].

TAMs accumulate in hypoxic tumor regions where they produce iNOS-derived NO, which can induce tumor cell apoptosis. However, it has been suggested that cytotoxic mediators, such as NOs that are produced by monocytes/macrophages in cancer tissues, are modulated by tumor cells. Arginase expression by TAM is thought to be a tumor-induced mechanism preventing formation of sufficient NO concentrations that are cytotoxic to tumors [76]. This may ensure that iNOS expression and/or activity are reduced, leading to lower levels of NO production than required for tumor cell cytotoxicity. Tumor-induced modulation of NO production is also potentially anti-apoptotic and/or pro-angiogenic. Thus, in tumors the balance of NO production appears tipped in favor of tumor growth [77, 78].

Although TAMs are known to be “conditioned” by tumor cells in situ to tumor growth, they also harbor the potential to become activated to kill tumor cells and release tumorigenic, pro-apoptotic and angiostatic compounds [79–82]. There are on-going studies evaluating targeted therapies aimed at exploring the plastic nature of these cells to this effect [83, 84].

So could TAMs be primed by IgE antibodies to turn against tumor cells? Immunohistochemical studies in breast cancer confirm the presence of CD23 on the surface of TAMs and the ability of this receptor to mediate expression of iNOS in human macrophages [36, 41, 85, 86]. Engaging CD23 by IgE and antigen on the surface of monocytic cells induces production of inflammatory mediators such

as TNF- α and iNOS in inflammatory responses in allergies and parasitic infections [85–88]. It is therefore conceivable that tumor antigen-specific IgE-mediated signals could target circulating monocytes and TAMs, enhance their tumor killing mechanisms and induce an allergy-like anti-tumor response in situ. Numerous findings now suggest that macrophages associated with tumors may be activated by IgE antibodies to kill tumors and that the pro-tumor growth signals may not be an inevitable fate of monocytes/macrophages. Future studies may help elucidate the potential of IgE antibodies in providing crucial signals that recondition TAMs to target immune responses against tumor cells.

8.2.3 *Eosinophils*

Infiltrating eosinophils are associated with a significantly better prognosis in some malignancies (esophageal squamous cell carcinoma, gastric cancer, head and neck cancer and colorectal carcinoma) [89–92]. Eosinophils contain cytotoxic granule proteins, such as major basic protein (MBP), eosinophil peroxidase, eosinophil cationic protein and eosinophil-derived neurotoxin, which are capable of inducing tissue damage and dysfunction [93], enhancing local inflammation and immune responses. Some, like eosinophil-derived neurotoxin, can induce tumor cell apoptosis and have been considered as potential treatments for cancer [94]. Eosinophil-derived cytotoxic mediators could therefore be harnessed to kill tumor cells. Several studies indicate that cytokines such as IL-4 or chemokines such as eotaxin-1 are associated with the inhibition of tumor cell growth and tumor cell killing [95, 96].

A number of experimental models have demonstrated that eosinophil infiltration in solid tumors is correlated with tumor eradication and evidence suggests that eosinophils participate in tumor cell killing in vitro and also in anti-tumor immune surveillance in vivo [97, 98]. It is also of great importance that eosinophils can exert potent anti-tumor activities in Th2 cytokine-rich environments, normally associated with immune tolerance in cancer. In a recent study of mouse melanoma, eosinophils were seen to be concentrated in necrotic areas and capsule regions of the tumors, where evidence of eosinophil MBP suggested extensive degranulation and eosinophil participation in tumor necrosis [99]. Eosinophil recruitment was a dynamic and an on-going process that occurred independently of Th2-dependent immune responses, implying that these cells can perform potent anti-tumoral functions in situ. In a separate study of CTL-resistant mouse melanoma, regression of tumor metastases was directly associated with tumor-associated eosinophil activation via STAT6, degranulation and secretion of eotaxin-1 and clearance of lung and visceral metastases [100].

Although eosinophils express exceedingly small numbers of IgE receptors on their cell surfaces [7, 101, 102], engagement of receptors with IgE has been shown to activate these cells to kill tumor cells [44]. The role of these cells in protecting against parasitic infections by ADCC mechanisms has also been reported

[103]. Eosinophils may also co-operate with other IgE effector cells such as monocytes/macrophages in tumor clearance, and this has been demonstrated in a number of in vivo studies [97, 104, 105]. The anti-tumoral potential of these potent IgE effector cells is yet to be fully realized. It certainly appears that the cells possess tumor-killing potential in situ, which may further be enhanced by implementation of IgE antibody therapies for cancer.

8.2.4 Natural Killer Cells

As essential effector cells of the innate response, NK cells have been associated with successful defenses in vivo, tumor cell killing capacity ex vivo and better patient prognosis in a number of cancers such as multiple myelomas, breast and non-small cell lung cancers [106–108]. NK cell anti-tumoral activities are also thought to form part of the clinical benefits conferred by therapeutic antibodies such as trastuzumab in breast cancer patients [109]. Fc ϵ RI expression has not been detected in NK cells, and IgE-mediated anti-tumoral activities may not be mediated via this receptor. However, NK cells are able to mediate ADCC through engagement of Fc receptors by antibodies; indeed, it has been demonstrated that FcR gamma subunit-deficient mice exhibit loss of phagocytic and ADCC functions, suggesting the potential for antibodies to activate NK cells against cancer cells [110]. NK cells in breast cancer tissues can secrete enhanced levels of IL-4, compared to those secreted by NK cells from patient blood. Increased levels of tumor-associated IL-4 would upregulate CD23b on resident and tumor-infiltrating monocytes/macrophages [111], possibly enhancing their tumor-targeting and killing potential. The potential effects of tumor antigen-specific IgE antibodies on NK cell anti-tumor activities still remain to be elucidated.

8.2.5 Antigen-Presenting Cells

APC such as B lymphocytes, monocytes/macrophages, Langerhans cells and DC in tumor infiltrates express CD23 and/or Fc ϵ RI. It has been proposed that one of the immune escape mechanisms of tumor cells is the pathway inducing defective differentiation and maturation of APC, thereby reducing adaptive immune responses against tumor antigens. IL-6 secreted by tumor cells and resident TAMs in tumor microenvironments is thought to inhibit the differentiation of CD34+ cells into mature DC and thus reduce their antigen-presenting capacity. It has also been demonstrated that immature DC can be activated by locally secreted lymphokines in the tumor microenvironments, such as IL-4, IL-13 or IFN- γ ; these have been shown to rescue maturation of DCs known to be blocked by tumors [112, 113].

Engagement of IgE with the high-affinity IgE receptor Fc ϵ RI on the surface of DC increases the efficiency of antigen uptake and presentation by 100- to 1,000-fold [114]. This may be significant in the context of IgE therapy against tumors,

since even small amounts of IgE on the surface of DCs may be sufficient to lead to an efficient activation of autologous T-cells and thus provoke powerful anti-tumoral adaptive immunity. Furthermore, expression of CD23 has been detected at different stages of DC maturation and CD23 expression has also been detected, albeit at lower levels, under immunosuppressive conditions induced by tumor cells [113]. This suggests that DCs harbor the potential to engage IgE via CD23, leading to antigen presentation. Although the outcome of the antigen presentation depends on the local environment, tumor antigen-specific IgE antibodies may contribute to enhance maturation of DC as well as stimulate effective adaptive immune responses against cancers [115–118].

The role of B lymphocytes in cancer has been controversial. In some in vivo models of colon carcinoma and melanoma, B cells possess tumor growth-promoting functions, possibly through secretion of immunomodulatory cytokines such as IL-6 and IL-10 [119–121]. It is conceivable however, that even within the immunosuppressive environments of tumors, tumor antigen-specific IgE antibodies may tip the balance against tumor growth by exploiting the potent mechanisms of B-cell activation, antigen presentation and induction of adaptive immunity, associated thus far with IgE in allergic diseases and immune responses against parasites.

8.3 Evidence for IgE and Effector Cell-Mediated Anti-Tumoral Responses

Is IgE a possible missing link with potential to “re-educate” immune effector cells against cancer? The benefits of IgE-mediated effector functions against tumor cells have been emerging in a number of in vivo and in vitro systems. Mounting evidence also points at the importance and pivotal role of IgE effector cells in mediating IgE inflammatory responses against tumors and a possible role for IgE in promoting adaptive immunity. Exciting insights into mechanisms of effector cell activation and tumor cell targeting and killing are also being elucidated.

8.3.1 Evidence for PBMC and Monocyte Activities in IgE-Mediated Anti-Tumoral Responses

Tumor killing effector functions mediated by antibodies have been previously evaluated in ADCC assays using human peripheral blood mononuclear cells (PBMC) to target tumor cells. IgE-mediated anti-tumoral effector cell functions have also been studied in similar assays and in some cases in direct comparison to corresponding IgG antibodies. Evidence now suggests that primary monocytes/macrophages and monocytic cells engaged with tumor antigen-specific IgE have the potential to kill tumor cells. We describe what has been learned about PBMC and monocyte/macrophage anti-tumoral activities mediated by IgE.

8.3.2 Chimeric Ovarian Carcinoma Antigen-Specific MOv18 IgE – Efficacy and Effector Cells

The potency of effector functions activated by tumor antigen-specific IgE has been explored in a series of studies on chimeric antibodies (with human constant regions and mouse variable regions) MOv18 IgE and MOv18 IgG1. These antibodies were engineered from the original MOv18 clone against a tumor-specific antigen, folate-binding protein (FBP), expressed in 80% of ovarian cancers. IgE and IgG were compared in two disparate human xenograft models of ovarian carcinoma grown in immunodeficient mice. Treatment with IgE afforded superior protection than the equivalent IgG1 antibodies in both systems [122, 123].

In one human ovarian carcinoma xenograft model, grown subcutaneously in severe combined immunodeficiency (SCID) mice, intravenous treatment of the mice with MOv18 IgE combined with human PBMC, added as effector cells, had a superior and longer-lasting effect in restricting tumor growth than the same treatment with IgG1 [122]. IgE-mediated effects on tumor growth were only observed when antibodies were combined with human PBMC, and as the human Fc ϵ region of the chimeric IgE is not recognized by mouse Fc ϵ receptors, it became apparent that the human IgE effector cells were necessary for the anti-tumoral functions of IgE. Histological examination of tumor sections from mice treated with MOv18 IgE exhibited larger areas of tumor necrosis, compared to controls, further suggesting that anti-tumoral effector cell functions were activated in response to IgE treatment. This study clearly pointed at the potentially superior effector functions of IgE as a cancer therapeutic.

Subsequent studies in a patient-derived human ovarian carcinoma model, grown orthotopically (intraperitoneally) in nude mice, demonstrated that intraperitoneal administration with MOv18 IgE and human PBMC gave significantly greater protection than PBMC alone, while MOv18 IgG1 with human PBMC offered no survival advantage (Fig. 8.2A) [123]. Immunohistochemical studies of tumor sections showed infiltration of human monocytes/macrophages (CD68+) into tumor lesions, while no human macrophage infiltrates were found in tumors from mice treated with human PBMC alone (Fig. 8.3). Monocyte/macrophage infiltration correlated with significantly enhanced survival of mice given tumor antigen-specific IgE. Immunohistochemical studies examining the presence of other human PBMC effector cells in xenograft tumor areas failed to identify any infiltrating (CD3+) T cell, (CD19+ and CD20+) B cell or (CD56+) NK cell populations associated with tumors. These observations suggested that human macrophages may be important IgE effector cells that are recruited by tumor antigen-specific IgE in tumor areas and their presence may contribute to tumor growth arrest in this *in vivo* model.

More recent studies utilizing the ovarian carcinoma xenograft model confirm a vital role of monocytes as IgE effector cells in anti-folate receptor IgE antibody responses *in vivo* [43, 44]. Depletion of monocytes from PBMC abrogated the survival advantage conferred by MOv18 IgE, while reconstitution of monocytes in the PBMC treatments restored IgE-mediated enhanced survival of mice (Fig. 8.2B,

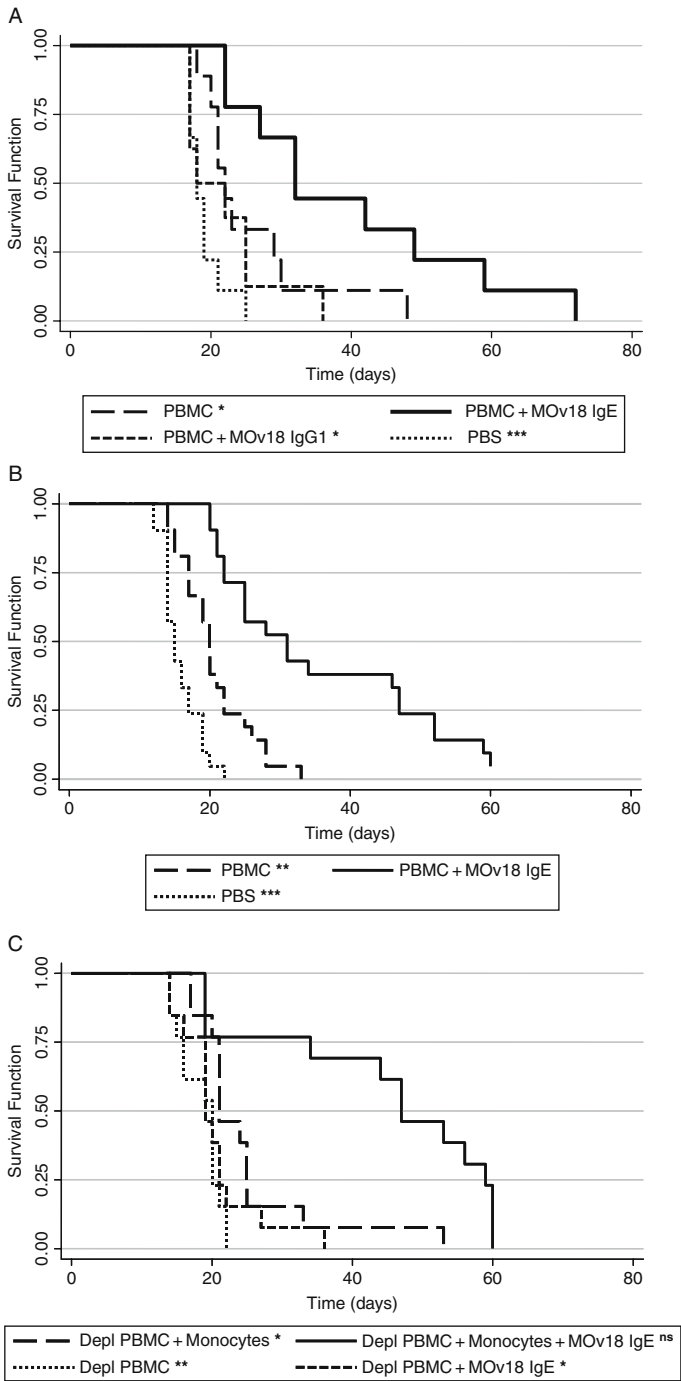


Fig. 8.2 (continued)

8.2C) [44]. In a separate study, U937 monocytic cells, which express Fc ϵ RI and low levels of CD23, when administered in combination with MOv18 IgE prolonged the survival of ovarian carcinoma-bearing mice compared to controls [43]. These findings confirmed that monocytes are important effector cells in tumor antigen-specific IgE-mediated responses *in vivo*.

8.3.3 Lessons on the MOv18 IgE-Mediated Tumor Killing Mechanisms – ADCC

The above data demonstrating tumor infiltration by monocytes and their significant involvement in prolonging survival of tumor-bearing mice were complemented by *in vitro* assays examining the interactions between monocytes and tumor cells mediated by IgE. A number of these studies not only implicated monocytes as IgE effector cells but also gave valuable insights into the mechanisms of IgE-mediated tumor targeting and killing.

In preliminary *in vitro* ^{51}Cr -release (ADCC) assays examining MOv18 IgE-mediated effector functions, MOv18 IgG and human PBMC conferred superior ADCC of FBP-expressing IGROV1 ovarian tumor cells compared to those of MOv18 IgE and PBMC [122]. In a subsequent study, using two ADCC assay systems, levels of IgE-mediated ADCC of tumor cells by PBMC were 50% lower compared to those measured with the corresponding IgG1 [123]. These data were in stark contrast to the clear superiority of MOv18 IgE over IgG1 in two human ovarian carcinoma xenograft models *in vivo*. A number of important findings and insights arose from these observations:

- a) Long retention of IgE–Fc ϵ RI complexes in tissues: The intrinsic affinity of IgE–Fc ϵ RI is at least 10^2 higher than that of IgG–Fc γ R complexes, reflected in prolonged half-life of IgE (~14 days) in tissues compared to IgG (~2–3 days). This may be translated to recruitment and retention of antibody in tumor areas and may contribute to the superior efficacy of IgE *in vivo*. The opposite is true



Fig. 8.2 (A) Survival of ovarian carcinoma human xenograft-bearing nude mice: survival (days) of nude mice injected with a patient-derived intraperitoneal xenograft is enhanced following treatment with chimeric folate receptor-specific antibody MOv18 IgE together with human PBMC (solid line). Survival is plotted for each of nine animals treated as shown. Data from Karagiannis et al. [123]. Copyright Wiley-VCH Verlag GmbH, and Co. KGaA. Reproduced with permission. (B) Effect of monocytes and MOv18 IgE antibody on survival of human ovarian carcinoma xenograft-bearing mice. Survival is superior in animals treated with the combination of MOv18 IgE and human PBMC. Survival is plotted for each of 13 animals treated as shown. (C) Impaired efficacy using PBMC depleted of monocytes can be salvaged by reconstituting PBMC with monocytes, implying that monocytes are necessary and sufficient for therapeutic IgE-mediated folate receptor targeting. Survival is plotted for each of 21 animals in each group, studied in the same experiment shown in (B). Data reproduced with permission from Karagiannis et al. [44]. Significance of values compared with samples given PBMC + MOv18 IgE by the Student's *t* test. n/s, $p > 0.05$; * $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$

- for IgE in circulation (half-life ~2 days) compared to IgG (~20 days) and may be reflected in the lower tumor killing capacity of IgE compared to IgG in vitro.
- b) Effects of Fc ϵ R1 receptor density on tumor killing by monocytes: The nature and density of Fc receptors expressed on the surface of human peripheral blood monocytes were examined. No CD23 expression was detected and the abundance of Fc ϵ R1 on monocytes (~2,900 molecules/cell) is about 100 times lower than that on mast cells or basophils and 50–100 times lower than that of Fc γ R2 receptors on monocytes (~200,000 molecules/cell). Fc γ R2 is the IgG receptor with the closest homology to Fc ϵ R1 and is also the Fc γ receptor implicated in IgG ADCC. Despite the 50-fold reduction in abundance of Fc ϵ R1 than Fc γ R2 on monocytes, MOv18 IgE was half as active in ADCC as MOv18 IgG1 in vitro [123], and this is most likely due to the high affinity of the IgE–Fc ϵ R1 interaction.
 - c) Effect of Fc ϵ R1 receptor occupancy on tumor killing by monocytes: Another significant observation was that human PBMC from allergic donors were less effective than those from non-allergic individuals in mediating IgE ADCC. Studies on occupancy of IgE receptors on monocytes demonstrated that levels of ADCC were proportional to the numbers of free IgE receptors on the surface of monocytes. These findings implicated Fc ϵ R1 in the mechanism of IgE-mediated tumor killing by ADCC.
 - d) Role of Fc ϵ R1 in tumor cell ADCC by monocytes: Purified human monocytes were able to kill tumor cells by ADCC in vitro. Since freshly purified blood monocytes were found to express low levels of Fc ϵ R1, but no CD23, this further implicated the high-affinity receptor in IgE tumor death by ADCC.

8.3.4 MOv18 IgE-Mediated Tumor Killing Mechanisms – ADCP

Early microscopical observations of effector–tumor cell interactions gave the first clue of another potential tumor killing mechanism not detected by ADCC assays [123]. Time course examinations of IgE-mediated effects of PBMC–tumor cell contact revealed that tumor cells and monocytes, but not T cells, B cells or NK cells

Fig. 8.3 (continued) sections of nude mice following i.p. tumor transplant and treatment with MOv18 IgE and human PBMC. Horizontal bars indicate the mean values. **(B)** Immunohistological examination of human and mouse macrophage infiltrates in sections of ovarian carcinoma xenografts grown in nude mice treated with MOv18 IgE and human PBMC. Human macrophages were rarely observed in ovarian carcinoma xenografts from mice treated with PBMC alone **(a)** compared with those treated with PBMC and MOv18 IgE **(b)**. Human macrophages are mostly seen in stromal areas adjacent to tumor islands **(b)**. Mouse macrophages are abundant in stromal areas surrounding tumor as well as in tumor islands whether mice were treated with PBMC alone **(c)** or PBMC and MOv18 IgE **(d)**. Figure from Karagiannis et al. [123], copyright Wiley-VCH Verlag GmbH, and Co. KGaA. Reproduced with permission

from PBMC, engaged in physical contact in the presence of MOv18 IgE. Monocyte–tumor cell contact and tumor cell death were observed as early as 20 min in culture and contact was maintained up to 3 h. ADCP of tumor cells by monocytes was evident from 90 min in cultures and always associated with loss of viability and/or membrane integrity of tumor cells.

These observations prompted a series of experiments aimed at measuring ADCP as well as elucidating the mechanisms underlying IgE-mediated ADCP of tumor cells. A new three-color flow cytometric (ADCC/ADCP) assay was developed to simultaneously measure cytotoxicity and phagocytosis of tumor cells (Fig. 8.4) [124]. There were already indications that the high-affinity IgE receptor, FcεRI, mediated ADCC of tumor cells, and further experiments confirmed this function (Fig. 8.5A). There were also enough data to suggest that the low-affinity IgE receptor, CD23b, which can be expressed in response to IL-4 on the surface of monocytes/macrophages, is capable of mediating ADCP and that it mediates IgE effector functions in allergic diseases and parasitic infections. Stimulation of monocytes by IL-4 induces expression of CD23. Expression of CD23 by monocytes enhanced their tumor killing capacity (from 25 to 30% without CD23 to up to 70% tumor cell death in vitro), and this increase in tumor cell death was attributed to tumor cell ADCP (Fig. 8.5B). Microscopical observations confirmed these findings and demonstrated that induction of CD23 expression resulted in enhanced monocyte–tumor cell contact and extensive phagocytosis of tumor cell material, which validates the findings of the ADCC/ADCP assays and the role of CD23 in tumor cell killing by ADCP [44]. A further hint of an anti-tumoral role for CD23 in vivo was provided by evidence that enhanced CD23 expression on the surface of monocytic cells in combination with MOv18 IgE was associated with increased

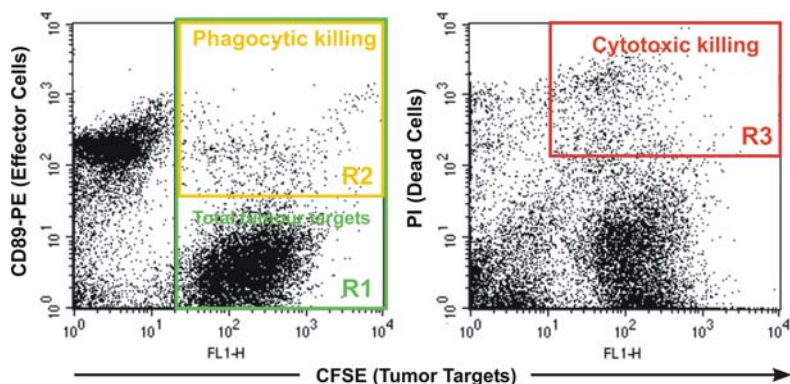


Fig. 8.4 A three-color flow cytometric assay can simultaneously measure ADCC and ADCP of tumor cells by effector cells. Dot plots of mixed monocyte effector and IGROV1 ovarian tumor target cells from which calculations were made. Region 1 (R1) represents total CFSE⁺ tumor cell targets. Region 2 (R2) depicts the CFSE⁺ tumor cells present within PE-stained monocytes (CFSE⁺/PE⁺), depicting ADCP. Region 3 (R3) contains tumor cells killed externally by effector cells (ADCC) (CFSE⁺/PI⁺). Data reproduced with permission from Karagiannis et al. [44].

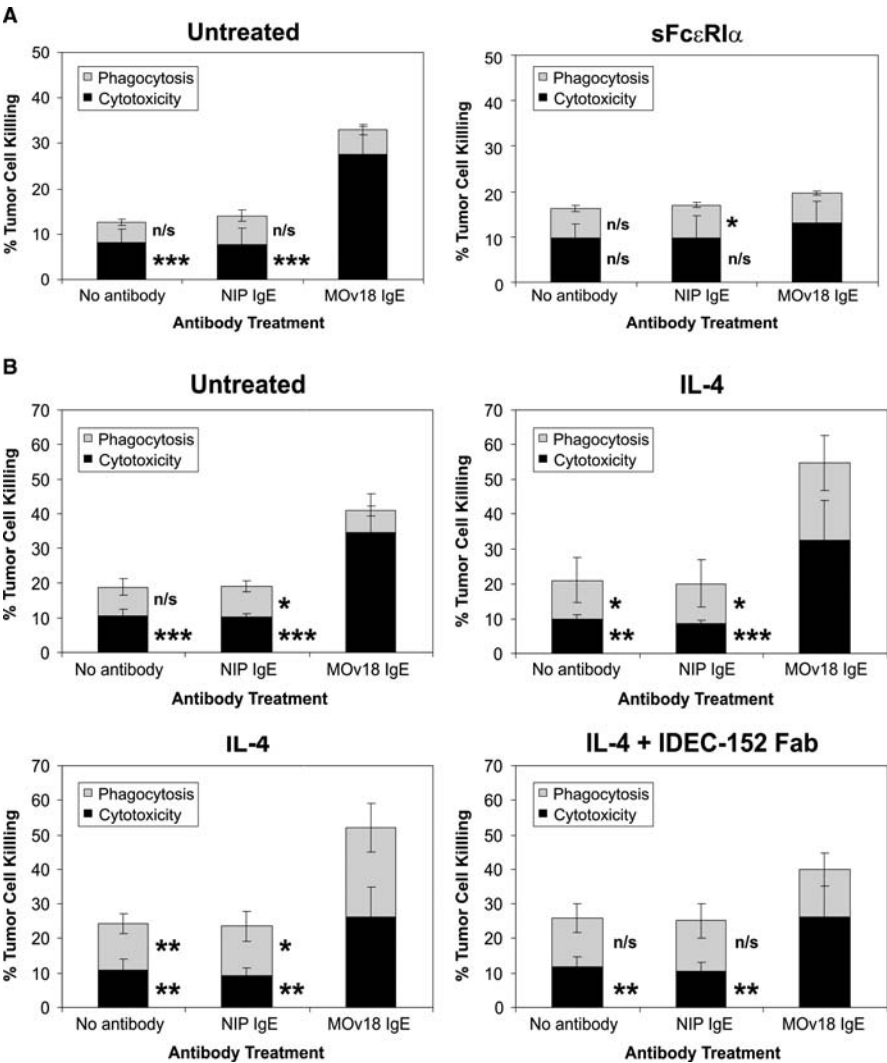


Fig. 8.5 (A) Monocytes can be activated by MOv18 IgE to kill tumor cells in vitro. The addition of MOv18 IgE to monocytes and IGROV1 tumor cells results in increased ADCC, compared to background levels measured with hapten-specific antibody (NIP IgE) or with no antibody controls (*left*). Blocking of MOv18 IgE binding to the IgE receptor, FcεRI, with soluble IgE receptor, correlated with loss of IgE-mediated killing of tumor cells (*right*). (B) CD23 role in MOv18 IgE-mediated ADCC of ovarian tumor cells. MOv18 IgE-mediated killing of IGROV1 tumor cells by untreated (*top left*) monocytes results in cytotoxic killing of tumor cells. Stimulation of monocytes with IL-4 enhanced the expression of CD23 (*top right*) and resulted in enhanced tumor killing capacity and killing by ADCC. ADCC killing of tumor cells by IL-4-stimulated primary monocytes (*bottom left*) was blocked by an anti-CD23 antibody Fab (*bottom right*), confirming a role for CD23 in tumor cell killing by ADCC. ADCC, black bars; ADCC, gray bars. Results shown are means $\pm$ SD of six independent experiments. Significance of values compared with samples given MOv18 IgE by the Student's *t* test. n/s, $p > 0.05$; * $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$. Reproduced with permission from Karagiannis et al. [44].

survival of human ovarian carcinoma xenograft-bearing mice compared to treatment with CD23-dim monocytic cells and MOv18 IgE .

From the volume of data showing that CD23-expressing monocytic cells accumulate in tumors where IL-4 is expressed, it is tempting to speculate that the “missing signal” in the form of a tumor antigen-specific IgE antibody may activate these cells to kill tumor cells and help reverse the tumor growth-promoting role of TAMs.

8.3.5 In Vitro Evidence from a Trastuzumab IgE Study

Further evidence that IgE antibodies are capable of activating monocytic cells to target and kill tumor cells was presented in a recent study examining the functional properties of an engineered IgE homologue to trastuzumab (trastuzumab-like IgE) against the human epidermal growth factor receptor 2 (HER2/neu), a tumor-associated antigen expressed in approximately 30% of breast carcinomas [125]. Trastuzumab (Herceptin[®]), a humanized IgG1, is the main antibody in clinical use for the treatment of early and metastatic breast cancers. Based on previous research on the effects of the chimeric MOv18 IgE in activating effector cells to kill tumors, and the clinical efficacy of trastuzumab, the potential anti-tumoral properties of a corresponding trastuzumab IgE merited examination. Trastuzumab (IgG1) and trastuzumab IgE were therefore compared in functional assays.

Trastuzumab-like IgE retained the well-characterized tumor cell growth arrest properties of trastuzumab (IgG1), independently of effector cells. Both trastuzumab and trastuzumab-like IgE could activate monocytic cells to kill tumor cells, and the levels of tumor death mediated by each antibody were comparable. However, they operated by different mechanisms: trastuzumab mediated tumor cell ADCP, whereas trastuzumab IgE killed tumor cells by ADCC after 2.5 h in culture. These distinct mechanisms of killing were confirmed by microscopic observations of monocytic cell–tumor cell interactions. Addition of trastuzumab and trastuzumab IgE induced enhanced monocytic-tumor cell contact, and ADCP of tumor cells was evident with trastuzumab. The antibody concentrations required to achieve maximum tumor cell killing by ADCC/ADCP were the same for trastuzumab (IgG1) and trastuzumab-like IgE in these assays. Trastuzumab-like IgE concentrations required for killing of breast cancer cells by effector cells were also 10-fold lower than the optimum found for MOv18 IgE killing of ovarian tumor cells in the same in vitro assay system. This implies that lower levels of trastuzumab-like IgE, compared to MOv18 IgE, may be required to achieve in vivo tumor growth arrest. In vitro tumor cell viability assays confirmed that like trastuzumab, trastuzumab-like IgE without effector cells can also mediate tumor growth arrest after 24 and 48 h in culture, suggesting that trastuzumab-like IgE retains the ability to block vital tumor cell growth signals through engagement of the HER2/neu receptor.

As trastuzumab-like IgE functioned with similar potency, but through mechanisms different from those of trastuzumab (IgG1), in vivo studies in clinically

relevant tumor models may help further elucidate efficacy and effector cell functions of IgE therapies against breast cancer. Furthermore, in light of different effector cell mechanisms potentiated by each antibody, examining whether a combination of trastuzumab and trastuzumab IgE could have potential synergistic anti-tumor effects is warranted.

8.3.6 Experimental Evidence Supporting Stimulation of Mast Cells, Basophils and Eosinophils by IgE

Mast cells are well-known activators and perpetrators of IgE responses in allergic diseases. Emerging evidence suggests that these powerful immunostimulatory effector cells can also become activated in response to tumor antigen-specific IgE. Human peripheral blood basophils release histamine in response to engagement of surface FcεRI with a chimeric antibody MOv18 IgE against the ovarian carcinoma antigen FBP when cross-linked by either polyclonal antibodies or FBP-expressing tumor cells [122]. In vitro microscopical studies of human PBMC incubated with FBP-expressing ovarian carcinoma cells and MOv18 IgE demonstrated basophils in frequent contact with tumor cells, engaging in targeted exocytosis of basophil granule contents [123]. This observation was significant as basophils are rare (less than 2%) in PBMC populations and the expected ratio of basophil effector: tumor target cell would be less than 1:1. This suggested that tumor antigen-specific IgE can recruit and activate basophils to contact and target antigen-expressing tumor cells. Recent studies of an engineered trastuzumab-like IgE antibody against the human breast cancer antigen HER2/neu demonstrated that trastuzumab IgE–FcεRI complexes on the surface of mast cells can be cross-linked by HER2/neu-expressing tumor cells to trigger mast cell degranulation in an antigen-specific manner [125].

These data are in agreement with growing evidence in favor of possible anti-tumoral functions of mast cells and basophils in vivo [126, 127] (see Noelle R., Chapter 6). It is tempting to speculate that these powerful IgE effector cells can be primed by tumor antigen-specific IgE antibodies to activate downstream immune responses against cancer cells; these responses may resemble inflammatory mechanisms well known in allergic diseases and parasitic infections.

A role for eosinophils as effector cells in IgE-mediated tumor targeting and killing has also been demonstrated [44, 128] (see Parish C., Chapter 11). In an in vitro assay examining the functions of the chimeric MOv18 IgE antibody specific for the ovarian carcinoma antigen FBP (MOv18 IgE), it was demonstrated that IgE could activate blood eosinophils to specifically kill tumor cells by ADCC in vitro; light and fluorescent microscopical observations revealed degranulating eosinophils associated with tumor cells in response to treatment with MOv18 IgE leading to tumor cell death (Fig. 8.6) [44]. In agreement, evidence by ADCC and proliferation studies using spleen-derived T lymphocytes from IgE-inoculated mice as effector cells confirmed the tumor cell killing (ADCC) capacity of cytotoxic T cells and

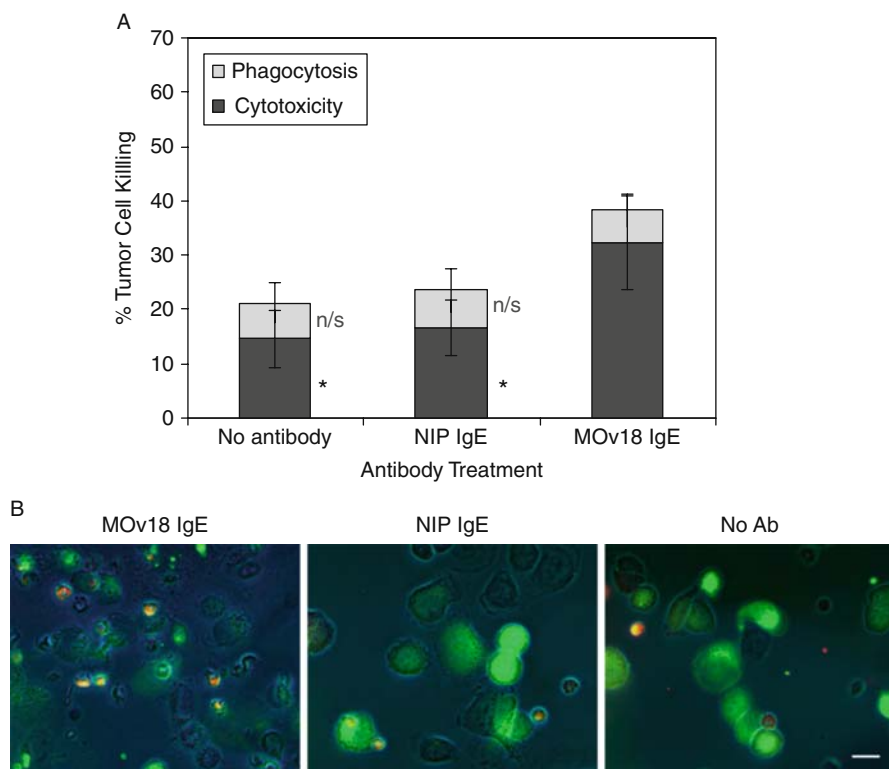


Fig. 8.6 Anti-folate-receptor antibody MOv18 IgE can activate human eosinophils to kill tumor cells. **(A)** MOv18 IgE-mediated ovarian carcinoma IGROV1 cell killing of tumor cells by primary eosinophils. ADCC, black bars; ADCP, gray bars. Results are means $\pm$ SD of four independent experiments. Significance was compared with samples given MOv18 IgE by the Student's *t* test. n/s, $p > 0.05$; * $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$. **(B)** Superimposed bright-field and fluorescent images of IGROV1–eosinophil interactions. CFSE-stained IGROV1 tumor cells (green) and CD49d-PE-labeled eosinophils (red) were incubated with IgE antibodies for 2.5 h. Treatment with MOv18 IgE resulted in frequent contact between eosinophils and tumor cells; tumor cell destruction was evident (*left*). Eosinophil-IGROV1 cell contact was observed less frequently in samples given control NIP IgE (*middle*) or no antibody (*right*). Original magnification, $\times 400$. Scale bars, 20 μ m. Reproduced with permission from Karagiannis et al. [44]

proliferative capacity of spleen-derived CD4⁺ T cells following antigenic tumor peptide stimuli. Eosinophil depletion eliminated IgE-mediated tumor growth arrest, suggesting induction of IgE-induced anti-tumoral immunological and inflammatory responses [128] (see Vangelista L. and Siccardi A., Chapter 9).

It appears, therefore, that the ingredients for the induction of an IgE-mediated response characteristic of allergic or inflammatory diseases are in place in human malignant diseases. These come in the form of resident and infiltrating mast cells and eosinophils in tumor regions and their capacity to induce potent inflammatory responses by IgE-mediated engagement of tumor cells *in situ*.

8.3.7 IgEs Against Pancreatic Cancer – A Case for Patient-Derived IgE Antibodies and Their Effector Functions

The presence of IgE antibodies in patients with pancreatic cancer and their tumor cell killing potential was investigated in a recent report, giving value to IgE as a potential therapeutic approach and confirming that naturally expressed IgE antibodies possess the ability to stimulate effector cells to kill cancer cells [129]. Fu et al. demonstrated that IgE and levels of soluble CD23 were uniquely elevated in pancreatic cancer patient sera, while levels of other immunoglobulin classes did not differ between patient and control groups. Pancreatic patient serum IgEs, but not IgEs from normal blood sera, could recognize a 50-kDa unidentified tumor antigen in pancreatic tumor cell lysates. Importantly, IgEs purified from patient sera killed pancreatic tumor cells only when combined with human PBMC in ADCC assays.

The presence of sCD23 in patient sera was not further examined in this report and levels of IgE and sCD23 were not correlated to clinical outcomes for the patients, but further examination of the role of sCD23 in IgE-mediated tumor cell killing may provide vital clues of how IgE effector functions are modulated in cancer patients. Finally, this study raises the potential that patient-derived tumor antigen-specific IgEs can direct inflammatory and immune cells against tumor cells and may be considered as future cancer therapeutics.

8.4 Conclusion

The mechanisms by which IgE antibodies exert their anti-tumor effects against cancer cells are now starting to emerge. IgE antibodies can engage both cell surface IgE receptors, FcεRI and CD23, and activate several lines of effector cells against tumor cells in vitro and in vivo employing a range of anti-tumoral activities. Research into the anti-tumoral functions of IgE antibodies demonstrate that the tumor-promoting roles of inflammatory cells in cancer may not be their only anticipated fate and that the fine balance between tumor growth-inducing inflammation and tumor targeting may be redressed in cancer therapy by IgE antibodies. Monocytes/macrophages, eosinophils, mast cells and basophils, either resident in tumor areas or recruited from the circulation, may be directed by tumor antigen-specific IgE antibodies to contribute to potent anti-tumoral responses. IgE may also activate APC to stimulate adaptive immune responses; these mechanisms still remain to be elucidated.

A number of biological/immunological questions facing the research community in the cancer immunology arena remain: (a) What are the immunological signals that down-regulate effective IgE immune responses against tumor cells in cancer and which parts of these networks may be amenable to therapeutic interventions? (b) Could IgE antibodies, and their Fcε receptors, either in their membrane-bound or soluble forms (such as various fragments of sCD23) be used as biomarkers in malignancies and could their presence have diagnostic or prognostic value? (c) What

are the effector cell mechanisms/pathways that will determine/predict the efficacy of IgE antibodies as cancer therapeutics? (d) What are the pivotal model systems that will predict the safety of cancer-specific IgE antibodies for clinical applications? (e) IgEs against tumor antigens have shown considerable promise for cancer therapy in various in vitro and in vivo systems. The question still remains whether antibodies of the IgE class can fulfill their potential in translational medicine and help us win the battle against cancer.

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Chapter 9

IgE as Adjuvant in Tumor Vaccination

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Abstract Activation of the antigen-IgE-FcεRI axis results in a potent inflammatory state. The redirection of this IgE-mediated activation of the immune system from allergic reactions toward tumors is the main theme of the new AllergoOncology field. Our particular approach has been to employ IgE as an adjuvant in anti-tumor vaccination. IgE-coated tumor cells can protect against tumor challenge, an observation that supports the involvement of IgE in anti-tumor immunity. The adjuvant effect of IgE was shown to result from eosinophil-dependent priming of the T-cell-mediated adaptive immune response. Moreover, the role of FcεRI in IgE anti-tumor adjuvant activity has been recently demonstrated. The interaction of tumor cell-bound IgE with receptors triggers the release of mediators with following recruitment of effector cells, cell killing and tumor antigen cross-priming. Starting from these evidences, several improvements toward a simple and universal use of IgE in anti-tumor cellular vaccines have been accomplished. In view of narrowing the gap between experimental models and therapeutic applications, the field is now shifting toward a humanized systems, employing human IgE and human FcεRIα transgenic mice.

9.1 Introduction

The powerful immunological IgE role exerted physiologically against intestinal parasites and pathologically during allergic reactions is determined by its dual capacity to cause acute and to promote late inflammatory reactions. The molecular basis of IgE strength is provided by its high binding affinity to FcεRI. The activation of the antigen-IgE-FcεRI axis profoundly affects the immune system both in its cellular orchestration and programming, resulting in a potent inflammatory state [1]. Redirection of the IgE-mediated inflammatory state toward tumors is part of the new field of AllergoOncology [2]. With this view in mind, an attempt on using IgE as a potent adjuvant in anti-tumor vaccination has been undertaken.

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9.2 Mouse IgE is Responsible for Decreased Tumor Growth and Improved Mice Survival

IgE activates innate immunity by inducing mast-cell and basophil degranulation with release of cytokines and mediators of allergic and inflammatory reactions. This IgE-driven activation of innate immunity could ideally be exploited and redirected against tumors. To this aim, a tumor could be disguised as a parasite or an allergen by loading IgE on the surface of tumor cells. Hence, IgE should affect tumor immunogenicity by initiating an immune response at tumor site. IgE targeting onto tumor cells can be performed through a three-step strategy based on biotin–avidin interactions, a system that has already showed its therapeutic efficacy in targeting cytotoxic molecules [3, 4], cytotoxic cells [5, 6] or radioisotopes [7] on tumor cells. Therefore, the three-step strategy allows the targeting of different effector molecules on the surface of tumor cells. During the first step, a systemic administration of a biotinylated monoclonal antibody (mAb) directed against a tumor-associated antigen (TAA) is performed. Then, the second step involves avidin or streptavidin administration. Avidin binds the tumor-bound biotinylated mAb with high affinity ($K_D = 10^{-15}$) and removes the unbound antibody by liver clearance. A biotinylated effector molecule, administered as third and last step, docks selectively at tumor sites via avidin bridging. In addition, the 1:4 stoichiometry ratio between avidin and biotin enables signal amplification [8].

The three-step strategy can be used to target IgE on the surface of TAA-expressing tumor cells, employing biotinylated soluble murine IgE (or control biotinylated murine IgG) as the effector of the third step. Immunocompetent C57BL/6 mice implanted (3 days before the three-step) with MC38-CEA-2, a syngenic adenoma cancer cell line stably transfected to express the human carcinoembryonic antigen (CEA) as TAA [9], showed a decreased tumor progression compared to control mice (treated with biotinylated IgG instead of IgE). The survival curves were also significantly different, and there were even cases of tumor rejection [10]. As for the *in vivo* tumor cell loading with biotinylated IgE, also the *in vitro* IgE loading (through avidin-biotin bridges) before cell inoculation, significantly affected tumor growth rate and mice survival. Moreover, re-challenge of tumor-rejecting mice failed to cause tumor development, indicating that a protective immunity was induced [10]. The use of the parental (CEA negative) MC38 cell line for re-challenge demonstrates that the protection mechanism was not due to an anti-CEA response, or at least not exclusively. Mice inoculated with tumor cells loaded with either IgG or heat-inactivated IgE developed tumors of similar volumes, suggesting that the Fc portion of IgE is likely to be essential for immune cell recruitment and activation. Another proof for the protective immunity elicited by targeting IgE on tumor cells came from the evidence that mice inoculated with IgE-loaded tumor cells at suboptimal quantities (not sufficient to induce tumor development) induced tumor protection. Indeed, these mice did not develop tumors after a challenge with parental tumor cells. On the contrary, using suboptimal doses of IgG-loaded tumor cells all challenged mice developed tumors. The IgE-treated mice did not develop

tumors even when challenged a second time with the CEA-negative parental cell line MC38.

The IgE-dependent tumor growth delay, decrease in rate of tumor progression and induction of protective immunity against subsequent challenges was observed not only with the weakly immunogenic MC38 colon adenocarcinoma cell line but also with the immunogenic Rauscher-induced rapidly growing RMA lymphoma cell line. In order to provide a target to the first biotinylated agent of the three-step strategy, RMA cells were stably transfected with the Thy 1.1 antigen [10]. This second tumor cell system provided an additional demonstration that the anti-tumor effect observed in these experimental setups is not tumor- or TAA-dependent, rather it is attributable to the presence of IgE on the surface of tumor cells.

9.2.1 Cell Types Involved in IgE-Induced Tumor Protection

Anti-tumor immunity acquired via IgE loading of tumor cells depends on mechanisms involving different cells of the immune system that need to be defined in detail. Specific cell-type depletion studies highlighted the contribution of eosinophils, CD4⁺ and CD8⁺ T lymphocytes in the inhibition of tumor growth. Cell depletion was attained by treating mice intraperitoneally (i.p.) with antibodies against IL-5 (eosinophil depletion), CD4⁺ or CD8⁺ (T-lymphocyte depletion), in separate experiments. Indeed, the significant inhibition of tumor growth observed in wild-type mice in presence of IgE was completely abrogated upon eosinophil and T-lymphocyte depletion [10]. Eosinophils are likely to play a role in the initial phase of the IgE-driven inhibition of tumor growth, whereas tumor-specific T-cell priming should determine the cellular adaptive immunity. The anti-tumor protection against subsequent tumor challenges is therefore attributable to the establishment of a memory T-cell response.

In order to define the mechanisms involved in the protective immunity, tumor-specific CTL responses in cultured spleen cells have been studied. Spleen cells derived from IgE-primed tumor-rejecting mice were stimulated in vitro with irradiated tumor cells. A remarkably higher cytotoxic activity was detected as compared to control spleen cells derived from tumor-bearing mice. This is a strong indication that the induction of anti-tumor CTL responses is a consequence of the presence of IgE. In addition, proliferation of tumor-specific CD4⁺ T-cells has been observed in cultures of spleen cells derived from mice immunized with IgE-loaded tumor cells [10].

Future studies need to be performed to better understand the cellular mechanisms involved in the IgE-driven anti-tumor effect. Preliminary data confirm the implication of dendritic cells as antigen-presenting cells (APC) needed to transfer the tumor antigen information in order to establish an anti-tumor immunity. Cytofluorimetric analysis of draining lymph nodes from mice vaccinated with

irradiated IgE-loaded or not loaded tumor cells, labeled with a fluorescent membrane probe, shows the presence of probe-positive dendritic cells. The significantly higher fraction of probe-positive dendritic cells derived from IgE-vaccinated mice remarks the APC role of these cells in the IgE-driven inflammatory reaction accounting for the establishment of an anti-tumor immune response (unpublished data).

9.2.2 IgE is an Adjuvant in Tumor Vaccination

The induction of an anti-tumor protection against subsequent tumor challenges upon immunization with suboptimal doses of IgE-loaded tumor cells, together with the cell depletion studies, strongly suggests the establishment of a memory T-cell response. According to these evidences, a possible exploitation of the IgE-driven anti-tumor immunity for preventive, or even curative, immunization has been envisaged. To this aim, cellular vaccines have been prepared with IgE- or IgG-loaded irradiated tumor cells. As in the tumor growth studies, the IgE targeting or IgG targeting on MC38-CEA-2 and RMA-Thy 1.1 tumor cell lines has been performed through the three-step method. Mice vaccinated twice (15 days apart) with IgE-loaded irradiated tumor cells showed anti-tumor protection with significant delay in tumor growth after a challenge with parental tumor cells [10]. The anti-tumor protection can be appreciated by vaccinating mice with different doses of the IgE cellular vaccine. The IgE-loaded irradiated cells are highly effective at really low doses (around 10^3 cells subcutaneously (s.c.)/mouse). Conversely, protective immunity can be achieved by vaccination with irradiated IgG-loaded cells at doses 100-fold higher than that with the IgE cellular vaccine. Anti-TAAs serum antibodies can be detected only in mice vaccinated with the highest dose of IgE or IgG cellular vaccines, demonstrating that IgE does not induce an increase in the tumor-specific antibody response [10].

IgE loading on tumor cells by the three-step strategy is effective and does not require the construction of a TAA-specific monoclonal IgE antibody, allowing the use of available biotinylated TAA-specific monoclonal IgG antibodies. Despite these advantageous features, the presence of a TAA-expressing tumor cell line is required for recognition by the biotinylated mAb at the first step. To overcome this need, two faster and easier targeting strategies, as effective as the three-step approach, have been developed. The first strategy is a simplified version of the three-step, involving the biotinylation of the tumor cell surface as first step. As in the original method, the second and third steps consist in the creation of the avidin bridge with IgE. In the second strategy, the tumor cell surface is chemically functionalized with haptens (haptimization) that can be recognized by hapten-specific monoclonal IgE antibodies. Both strategies prevent the need for a TAA-expressing tumor cell line, making the loading strategy suitable for many different tumors, including the poorly immunogenic ones or those not characterized for their TAAs.

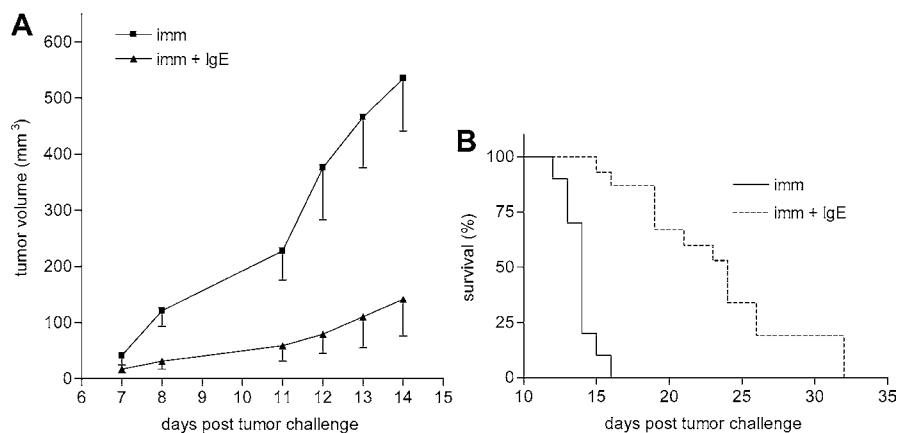


Fig. 9.1 Anti-tumor IgE adjuvanticity in C57BL/6 mice. **(A)** Tumor growth of mice immunized s.c. twice (15 days apart) with 1×10^5 irradiated MC38 cells previously haptenized with DNP and loaded with anti-DNP IgE. Challenge with 3×10^5 parental tumor cells was performed 15 days after the last immunization. Mice immunized with IgE-loaded MC38 cells (15 mice, triangles, imm + IgE) and IgE-free MC38 cells (10 mice, squares, imm). The statistical difference between the two groups was highly significant ($P < 0.001$), evaluated by the Student's *t*-test. **(B)** Mice survival plot: dotted line, mice treated with IgE-loaded cells; continuous line, mice treated in absence of IgE. Statistical analysis on survival data was performed by the Kaplan-Meier method. The difference in survival was highly significant ($P < 0.001$)

Cell haptenization and IgE targeting have been adapted to attain IgE cellular vaccines. As with the three-step approach, mice vaccinated with a double injection of irradiated IgE-loaded tumor cells showed a delay in tumor growth. As depicted in Fig. 9.1, differences in tumor growth can be estimated after tumor challenge in mice vaccinated with IgE-loaded or IgE-free MC38 cells. The rate of tumor growth in the IgE group versus the control group is significantly lower and reflects the difference in mice survival. All mice of the control group died in the first 2 weeks after challenge while the last mice of the IgE group died 32 days post-tumor injection.

Clearly, the evidence for an adjuvant effect exerted by IgE in anti-tumor vaccination represents an important immunological insight and, at the same time, an innovative source for new approaches in cancer therapy. Unknown aspects of IgE cell biology and biochemistry have been discovered and applied to a possible new way to combat cancer. Aside the *in vivo* studies, a full set of *in vitro* tests have been developed to monitor and assure the quality of the cellular vaccine preparations. These tests consist mainly in (i) the evaluation of IgE tumor cell loading via cytofluorimetry and (ii) the verification of the IgE cellular vaccine activity toward FcεRI via FcεRI⁺ cell mediator release assays modified from classic assays to allow detection of mediators in a cell-to-cell contact mode [11]. Moreover, this and the following paragraphs present an overview on how our anti-tumor IgE system has been refined to increase safety and allow the use of human IgE, pointing toward human health.

9.2.3 Vaccinia Virus and MVA as Cell Killing Agents Coupled to IgE Loading

Vaccinia virus (VV) is a large, enveloped virus belonging to the Orthopoxvirus genus, widely used for smallpox vaccination until 1980 when the Assembly of the World Health Organization declared smallpox eradicated and recommended the discontinuation of vaccination. In the same year, the application of the recombinant DNA technology to VV marked the birth of genetically engineered poxviruses [12], developed as expression vectors. The strong immunogenicity of VV, together with the homologous recombination that spontaneously takes place during its replication inside infected cells, allows its wide use as expression vector and candidate vaccine against unrelated infectious diseases [13]. Recombinant poxviruses have been used for protectively immunizing animals against diseases of veterinary importance. Examples include the recombinant VV expressing the rabies virus glycoprotein for immunization of wild animals that greatly reduced incidence of rabies as demonstrated in large field tests in Belgium [14]. The ability of poxviruses to induce strong CTL-mediated immune responses has led to consideration of their use as cancer vaccines. Recombinant VVs that express viral antigens or cellular TAAs have provided prophylactic and therapeutic effects against experimental tumors [13].

In the field of IgE anti-tumor adjuvanticity, the replacement of irradiation with VV infection of tumor cells represents a first step toward the construction of a viral vector-based anti-tumor vaccine. VV guarantees cell death by killing tumor cells in few days. IgE adjuvanticity acts in a concerted manner with VV strong immunogenicity, and as a result, a single immunization is sufficient to empower the anti-tumor effect. Conversely, the irradiated IgE-loaded cellular vaccine is able to exert tumor protection only after two immunizations. In the VV vaccination protocol, soluble IgE are targeted through haptenization on the surface of VV-infected tumor cells. As represented in Fig. 9.2, at 14-days post-tumor challenge, all mice vaccinated with VV-infected IgE-loaded tumor cells are tumor-free, whereas all controls (vaccinated with infected tumor cells without IgE) present a huge tumor growing in the site of injection on the animal's flank. The survival curve shows that only one mouse vaccinated with IgE-loaded tumor cells developed the tumor in the following month.

Despite its effectiveness, the use of VV in standard laboratory conditions is hampered by restrictions due to its infectivity. In search for an alternative and more flexible viral system for the anti-tumor vaccination protocol, VV was substituted with the modified vaccinia virus Ankara (MVA) (an attenuated VV derivative, unable to replicate in mammalian cells). The IgE adjuvant effect and its synergy with virus immunogenicity were maintained although the anti-tumor protection was less striking. As obtained with VV infection, the IgE effect is established after a single vaccination, but the almost complete protection observed with the VV protocol cannot be observed using MVA. Using MVA-infected IgE-loaded tumor cells the majority of vaccinated mice present tumor development. However, the tumor growth rate was significantly lower compared to controls. A double immunization with MVA-infected tumor cells decreases the tumor growth rate even in absence

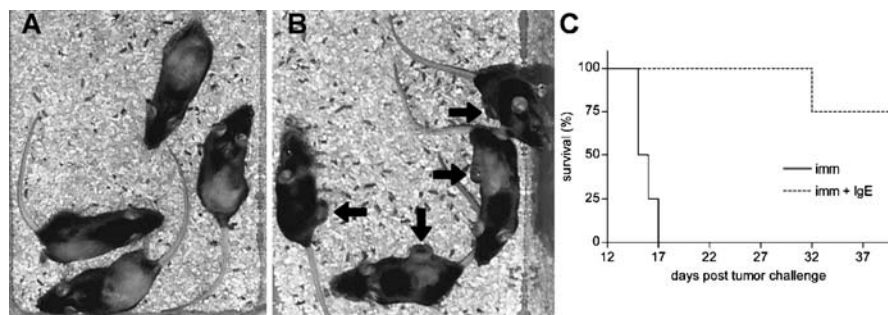


Fig. 9.2 Anti-tumor protection of VV-infected IgE-loaded RMA cells. C57BL/6 mice (four mice per group) were vaccinated s.c. with 10^5 IgE-loaded (A) or not loaded (B) VV-infected RMA cells at day -15 . At day 0 mice received a s.c. tumor challenge of 7×10^4 RMA living cells. The images are representative of one out of three experiments yielding similar results and were taken 14 days after challenge. Mice vaccinated with IgE-loaded cells did not develop tumors (A) while all the controls did (B, see *arrows*). (C) Mice survival plot: dotted line, mice treated with IgE-loaded cells; continuous line, mice treated in the absence of IgE. Statistical analysis on survival data was performed by the Kaplan–Meier method. The difference in survival was highly significant ($P < 0.001$)

of IgE. As already stated, a single immunization in the presence of IgE confers a powerful anti-tumor immune response, demonstrating the MVA-IgE synergy [15]. In perspective, the MVA-IgE anti-tumor vaccination system is particularly important, as it gives plenty of possibilities for its evolution into a fully engineered viral system.

9.2.4 MVA as a Resource for Human Therapy and Recombinant Protein Expression

MVA is a highly attenuated and severely host-restricted viral vector derived from over 570 serial passages of the parental VV strain in chicken embryo fibroblasts [16]. As a result, MVA, characterized by six major genome deletions [17], is unable to multiply in humans and in most mammalian cell lines. Yet, MVA retains a high expression level of viral and heterologous genes in non-permissive cells [18]. Most importantly, MVA is safer than VV because it is non-pathogenic for humans, even in immuno-compromised patients, such as those with hematologic malignancies or those undergoing chemotherapy treatment [19]. The elicitation of humoral and cellular adaptive immune responses, together with the properties mentioned above, make recombinant MVA an ideal vector for both prophylactic and therapeutic vaccination, as demonstrated by its wide use in ongoing clinical trials [20, 21]. Recombinant MVA viruses expressing both infection disease antigens [22–24] and TAAs [20] have been produced. Different strategies have been developed in order to obtain rMVAs expressing heterologous antigens. An initial method for rMVA production required visual selection and manual picking of infected cell foci [18],

followed by selection strategies based on antibiotic resistances and by a 'Transient Host Range Selection' method [25, 26]. The recently developed 'Red-to-Green gene swapping' method represents a fast and easy strategy for rMVA production in which a fluorescence-enhanced visual selection simplifies greatly the isolation of recombinant MVA clones [27].

9.2.5 Use of Transgenic Mice as Tools to Explore IgE Receptor Involvement and Implement Human IgE

The IgE adjuvant effect could depend upon IgE interaction with its high-affinity receptor, FcεRI (present in mouse on the surface of mast cells and basophils), or with its low-affinity receptor, CD23. Eventually, FcεRI activation would lead to cellular degranulation with mediator release and effector cell recruitment. This cascade should cause a potent local inflammation at the tumor site. The role of the receptors involved in the IgE-driven anti-tumor effect could therefore be investigated using FcεRIα^{-/-} [28] and CD23^{-/-} mice [29]. Knock-out mice, in which one or more genes are turned off through gene knock out, are important animal models for studying the role of the targeted genes. By knocking out a specific gene and observing phenotypic, functional or morphological differences from wild-type conditions, a function for the targeted gene can be often inferred. Recruitment of IgE receptor mice knock outs in our experimental anti-tumor vaccines should be likely to provide information about which receptor is involved in IgE adjuvanticity. In addition, the use of a transgenic FcεRIα^{-/-} hFcεRIα⁺ mouse, in which the α-chain of mouse FcεRI is substituted with the human counterpart, should help in understanding whether human IgE could exert an anti-tumor effect similar to mouse IgE. Thus, the establishment of a human IgE model system in mouse would represent a fundamental resource for future development in the IgE anti-tumor vaccination strategy, moving the field closer to the clinics. All transgenic mice described above and employed in our studies were generated in a BALB/c background; therefore the BALB/c mammary adenocarcinoma cell line TS/A-LACK (*Leishmania* receptor for activated C kinase) [30] has been used to reproduce in BALB/c mice the IgE-driven anti-tumor protection observed in the C57BL/6 model [15]. As expected, BALB/c mice, vaccinated once or twice (15 days apart) with MVA-infected IgE-loaded TS/A-LACK cells, exhibited a tumor protection after challenge very similar to that reported for the C57BL/6 model. Indeed, the IgE adjuvant effect is obtained after a single immunization in mice vaccinated by MVA-infected IgE-loaded TS/A-LACK cells. Conversely, after two immunizations, also the MVA-infected cells (in absence of IgE) induced anti-tumor protection [15]. The successful induction of an IgE-driven anti-tumor effect using a BALB/c tumor cell line in a BALB/c mouse demonstrates that the IgE adjuvanticity is not confined to a specific tumor model, rather it is potentially applicable to different tumors. Particularly interesting is the

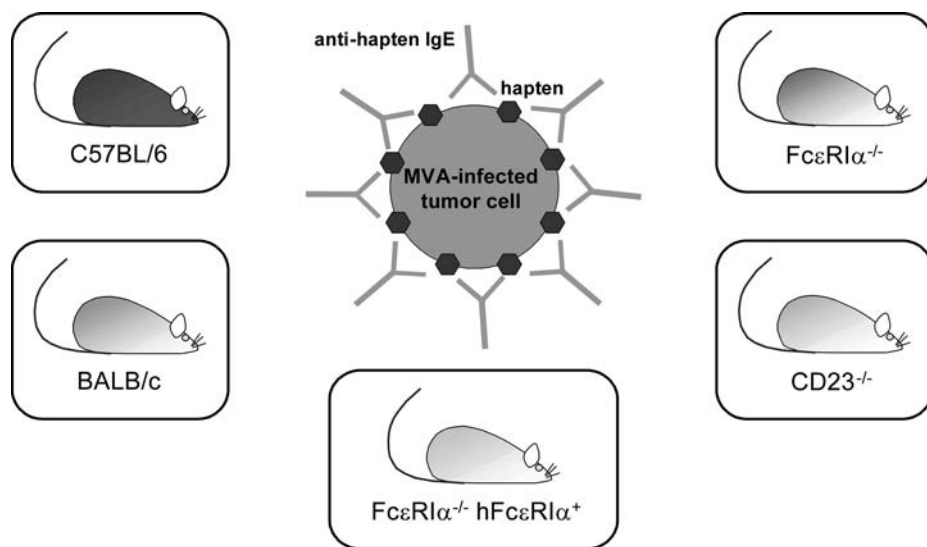


Fig. 9.3 Schematics of mice and MVA-based cell vaccination systems. Cellular anti-tumor vaccines are based on MVA infection and IgE loading via haptenization. The mice model system shifted from C57BL/6 to BALB/c given the availability of several transgenic models in the BALB/c background

fact that TS/A cells have been reported as a poorly immunogenic tumor [31], confirming the power of IgE adjuvanticity. A schematic view of the mice and cell systems described above is represented in Fig. 9.3.

9.2.6 *FcεRI is Crucial for IgE Adjuvanticity in Tumor Vaccination*

It is conceivable that the IgE-adjuvant effect observed in the anti-tumor vaccination would consist in an immune response due to the instauration of a powerful inflammation at the tumor site, similar to that induced during an allergic manifestation. The availability of $Fc\epsilon RI\alpha^{-/-}$ mouse [28] permits to understand the role played by the IgE high-affinity receptor in the IgE-adjuvant effect. $Fc\epsilon RI\alpha^{-/-}$ mice vaccinated in presence of IgE did not show any anti-tumor protection, demonstrating the crucial role played *in vivo* by $Fc\epsilon RI$ in the IgE adjuvant effect [15]. In parallel, similar experiments conducted with $CD23^{-/-}$ mice [29] demonstrated that the low-affinity receptor CD23 is not involved in the IgE adjuvant effect, as $CD23^{-/-}$ mice vaccinated in the presence of IgE presented tumor protection similar to wild-type mice. Indeed, $CD23^{-/-}$ mice do express $Fc\epsilon RI$; thus, as suggested by the lack of IgE adjuvanticity in $Fc\epsilon RI\alpha^{-/-}$ mice, these data reinforce the hypothesis that $Fc\epsilon RI$ is the primary (or unique) mediator of IgE activity in anti-tumor vaccination [15]. The demonstration of a dominant role played by $Fc\epsilon RI$ in the *in vivo* IgE-dependent

anti-tumor effect based on IgE adjuvanticity on a vaccination is extremely important as it implies an orchestration of events, starting from a simple protein–protein recognition (IgE-Fc ϵ RI) and ending to a full adaptive anti-tumor T-cell memory. Different from other approaches described within this book, not involving IgE as an adjuvant but as a direct tumor cell killer, CD23 did not prove to intervene in the cellular crosstalk promoted by the presence of IgE in tumor vaccination.

9.2.7 Implementation of Mouse Membrane-Bound IgE by Recombinant MVA

Despite the encouraging and promising anti-tumor effect of the IgE-based vaccine, the use of soluble IgE in the vaccination protocol could carry the risk of unwanted anaphylactic reactions. The demonstration of a functional interaction between human membrane-bound IgE (mIgE) and Fc ϵ RI [11] provides a mean to the development of a safer protocol for IgE-mediated tumor vaccination. In fact, embedding IgE molecules into the surface of tumor cells eliminates the presence of these potentially dangerous proteins from the bloodstream. In other words, unwanted anaphylactic reactions could in theory derive from unexpected antigen recognition by soluble IgE molecules (or by soluble IgE bound to Fc ϵ RI). These immune complexes could then aggregate Fc ϵ RI on the surface of mast cells and basophils, triggering potent cell degranulation, ultimately leading to systemic anaphylaxis. Although remote, these events should be prevented when using IgE as a drug or as a vaccine component.

Different from human mIgE [11, 32], mouse mIgE needs to be associated with the Ig α –Ig β heterodimer to reach the cell surface [33]. The construction of a chimeric mouse mIgE variant is thus needed to obtain a suitable recombinant protein for the anti-tumor vaccination project. As for human mIgE, mouse mIgG2a can be transported to the cell surface also in absence of Ig α –Ig β heterodimers [33]. Considering that a truncated version of human mIgE has been engineered and provided full capacity to activate Fc ϵ RI in a cell-to-cell contact [11], a truncated mouse mIgE chimeric construct (deprived of IgE Fabs and containing only the Fc C ϵ 3–C ϵ 4 domains) in which the portion C-terminal to IgE C ϵ 4 was replaced by the corresponding mIgG2a moiety (i.e., the extracellular membrane-proximal domain, the transmembrane domain and the cytoplasmic domain) has been designed and cloned. The construct has been transfected in cell lines and characterized for the ability to activate rodent Fc ϵ RI. The truncated chimeric mouse mIgE/IgG2a construct was then subcloned and a recombinant MVA produced and used to infect cells. Despite expression, proper assembly (disulphide bonded dimers) and cell surface export (tested by cytofluorimetry, western blot and surface biotinylation), the truncated chimeric mouse mIgE/IgG2a did not induce mediator release when infected cells contacted a rat basophilic cell line. This may imply a significant Fc ϵ RI binding site difference between human and mouse IgE. Therefore, a second chimeric protein in which the mouse IgE Fc portion comprises also the C ϵ 2 domain has been

constructed and is currently being tested for its function. Once an active mouse mIgE construct is obtained, a new vector-based vaccination protocol should become available. rMVA-mIgE-infected tumor cells should then express the truncated membrane form of IgE on their surface creating a new IgE-mediated vaccine. Tumor growth will then be studied in wild-type, $Fc\epsilon RI\alpha^{-/-}$ and $CD23^{-/-}$ mice, likely reproducing the results obtained in the MVA-infected IgE-loaded tumor cell vaccination protocol, getting closer to the construction of a safe and simplified IgE-based vaccine.

9.2.8 FcεRI Humanized Mice Allow the Use of Human IgE in Tumor Vaccination

FcεRI cellular distribution in humans (mast cells, basophils, dendritic cells, eosinophils, neutrophils, monocytes, macrophages and platelets) is more ubiquitous than in mice (mast cells and basophils); therefore, the output of the human IgE–FcεRI interaction on the anti-tumor vaccination efficiency cannot be predicted. In order to verify whether human secretory IgE targeting on tumor cells exerts anti-tumor protection similar to that observed with mouse secretory IgE, the IgE-based vaccination protocol has been performed in $Fc\epsilon RI\alpha^{-/-}$ hFcεRI α^+ mice [34]. $Fc\epsilon RI\alpha^{-/-}$ hFcεRI α^+ mice present a cellular FcεRI expression similar to that found in humans [34–36], a very useful model to predict the efficiency of the system in the perspective to translate it to the clinic.

As in the wild-type model with mouse IgE, also in the humanized model, two immunizations guaranteed an anti-tumor protection, regardless of human IgE presence. On the contrary, with a single immunization, human IgE showed an adjuvant effect, mediating an anti-tumor protection comparable to that obtained with two immunizations. Mice immunized only once with MVA-infected cells in absence of IgE were not protected from tumor growth, showing a tumor development comparable to that of non-immunized control mice [15]. Wild-type mice (thus expressing mouse FcεRI), immunized with human IgE-loaded MVA-infected TS/A-LACK cells, are not protected from tumor development, showing a tumor growth comparable to that observed in non-immunized mice [15]. This result was expected and is attributable to the lack of human IgE binding to mouse FcεRI [37]. The reconstitution of the IgE effect in $Fc\epsilon RI\alpha^{-/-}$ hFcεRI α^+ mice demonstrates the anti-tumor adjuvanticity of human IgE.

Since human mIgE variants are capable of activating human FcεRI in an antigen-independent manner [11], the transfer of this knowledge to the anti-tumor vaccination strategy (using $Fc\epsilon RI\alpha^{-/-}$ hFcεRI α^+ mice) has been immediate. Hence, a rMVA expressing a truncated human mIgE (tmIgE), previously reported to be able to activate FcεRI [11], has been constructed and characterized. Since cells infected with the rMVA-tmIgE present expression of human tmIgE on the cell surface and activate human FcεRI in a rat basophilic leukemia (RBL) cell-based mediator release assay [11], there is a high likelihood to observe anti-tumor IgE adjuvanticity

as in the case of human secretory IgE-targeted on tumor cells. Moreover, human tmIgE should reveal no anti-tumor protection when tested on wild-type mice, in line with the results obtained using human-soluble IgE.

With the aim to produce vector-based tumor vaccines, recombinant MVA capable to realize a further molecular evolution of the mIgE–FcεRI interaction concept are being generated. In other words, a safe and simple MVA-based system should allow expression of mIgE on the surface of tumor cells, providing all the necessary requirements for the IgE adjuvant effect. Furthermore, the construction of rMVAs with multiple gene insertions should disclose the possibility to exploit IgE adjuvanticity together with specific TAA (e.g., HER2/*neu* or CEA) or other adjuvant molecules. Potential candidates are genes encoding for immunomodulatory cytokines (IL-2 or GM-CSF) or co-stimulatory molecules (B7.1 or B7.2) [38]. The co-insertion of one of these genes in rMVA would augment the immune system boosting driven by IgE, likely providing a more robust and long-lasting anti-tumor protection.

9.3 Conclusion

A schematic representation of the mechanisms behind IgE adjuvanticity in anti-tumor vaccination is represented in Fig. 9.4. The presence of IgE on the surface of tumor cells allows binding with FcεRI on FcεRI⁺ cells in a cell-to-cell contact. In order to express IgE on the surface of tumor cells, different strategies are being developed: starting from the original three-step-mediated IgE-loading strategy [10], followed by cell haptenization and anti-hapten IgE [15], to the engineering of rMVA capable to express truncated mIgE on the surface of infected tumor cells. IgE–FcεRI recognition leads to receptor activation with killing of tumor cells by FcεRI⁺ effector cells (mast cells and basophils) and/or by other specialized killer cells (eosinophils and cytotoxic T cells), recruited as a consequence of the initial mast-cell and basophil release of pro-inflammatory mediators. The inflammatory reaction at tumor site and the consequent cell killing should result in tumor cells debris release. TAAs present in tumor cell debris should then be available to immature highly phagocytic dendritic cells of the peripheral tissues. Activated dendritic cells should therefore migrate via lymphatic vessels to the regional lymph nodes. Mature dendritic cells would stimulate a strong T-cell response by priming and cross-priming of tumor-specific CD4⁺ and CD8⁺ T cells. The consequent establishment of an anti-tumor-immune response is followed by the constitution and consolidation of a tumor-specific immune memory.

Finally and most intriguingly, the IgE adjuvant effect observed and characterized for tumor vaccine engineering might tell us that the power of the IgE–FcεRI system could be extended to other pathologies in which effective or enhanced vaccination strategies are still awaited. This perspective may reveal extremely innovative and promising approaches to improve human health, providing a novel view on an otherwise deleterious molecule, IgE.

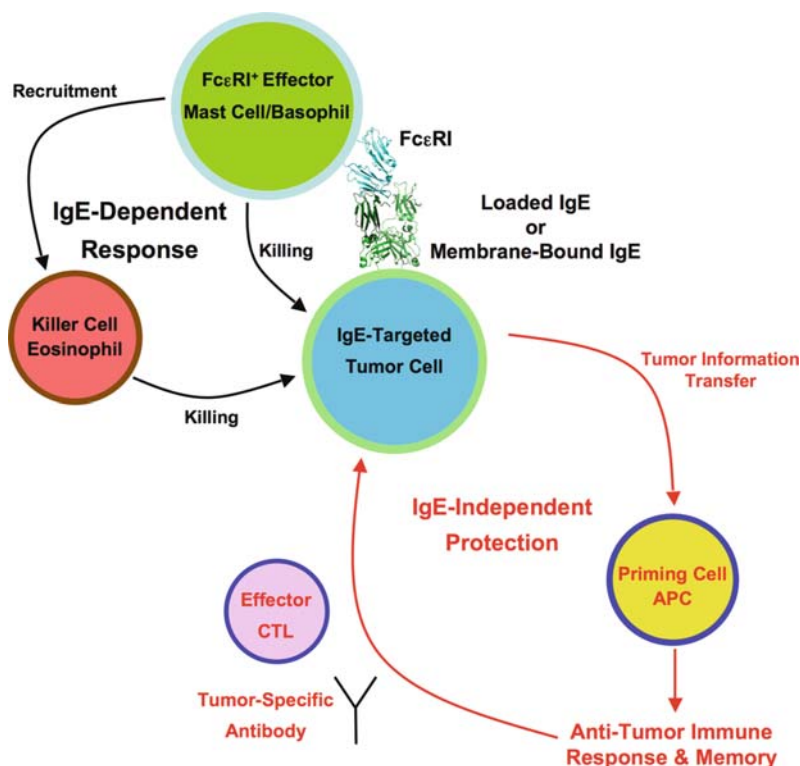


Fig. 9.4 Overview on the mechanisms behind IgE adjuvanticity in anti-tumor vaccination. The crystal structure of the complex between FcεRI α-chain D1D2 and IgE Cε3Cε4 domains [39] is shown to visualize the IgE–FcεRI interaction occurring between tumor cells and FcεRI⁺ cells, regardless of the IgE targeting approach (i.e., cell loading using complete IgE or cell expression of a truncated version of membrane IgE). The 3D representation was prepared using the program PyMol on the pdb coordinates 1f6a. The interaction between IgE (on tumor cells) and FcεRI (on effector cells) is the central event leading to an IgE-dependent response, ultimately resulting in tumor cell killing, either directly by mast cells and/or basophils or upon recruitment of professional killer cells such as eosinophils. Tumor cell disruption causes cell debris to be collected by professional antigen-presenting cells that in turn transfer tumor antigen information to specialized immune districts for the establishment of an adaptive anti-tumor immune response and memory. The resulting tumor-specific T lymphocytes and antibodies build an IgE-independent tumor protection, the basis for IgE adjuvanticity

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Chapter 10

The Targets of IgE: Allergen-Associated and Tumor-Associated Molecular Patterns

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Abstract The most studied targets of IgE immunoglobulins are termed allergens and mostly represent protein antigens from plant or animal origin. However, it has been revealed that also pathogens and self-antigens can be recognized by IgE. Thus, besides a detrimental role in allergy, IgE may exert defense and surveillance functions. Here, we discuss the display of rigid epitope patterns as a common key feature of antigens leading to both formation of high-affinity IgE antibodies by B lymphocytes and IgE-mediated triggering of effector cells. In analogy to the principle of pathogen-associated patterns as danger signals, we introduce here the terms allergen-associated molecular patterns (AAMPs) for the repetitive epitopes exposed by allergens and tumor-associated molecular patterns (TAMPs) in settings when molecular epitope patterns are displayed through overexpressed tumor antigens.

10.1 Background: IgE and the Anaphylactic Reaction

As already discussed in the previous chapters, IgE antibodies have several unique features which enable them to induce highly specific biological responses. Taken together, major features of IgE are (i) high binding strength to their target epitopes through unique affinity maturation program of the IgE-producing B lymphocytes (epsilon B lymphocytes) in an appropriate milieu of cytokine and danger signals [1–3]; (ii) high-affinity interaction of their Fc epsilon (Fcε) domain with specialized FcεRI receptors on a distinct panel of mostly cytotoxic and phagocytic effector cells conferring antibody-dependent cell-mediated cytotoxicity (ADCC) or antibody-dependent cell-mediated phagocytosis (ADCP) functions to them [4–6]; (iii) moderate affinity interactions with specific IgE receptors of the CD23 C-lectin type enabling IgE to participate in antigen uptake, transport and presentation by classical antigen-presenting cells (APCs) including dendritic cells (DCs) and B lymphocytes, as well as non-classical APCs like epithelial cells [7, 8]. Depending on the

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time point, duration and repetition of antigen contact IgE will either induce reactions of the immediate anaphylactic type or nourish chronic Th2-type inflammation [9–11]. In any circumstances, an ongoing IgE-mediated response will be hard to turn off, symbolic for the sensitization state of the individual and characterized by “tissue memory” of IgE [12], prolonging the survival rate of its effector cells. To counterbalance an undesired IgE response toward actually harmless allergens, today’s allergen immunotherapy aims at inducing a tolerant state in the patient [13, 14]. This immune tolerance is supported by the production of immunomodulating cytokines and the induction of non- or anti-inflammatory blocking IgG antibodies which interact with FcγRIIb receptors on epsilon B lymphocytes and on FcεRI-positive effector cells and thus are able to downregulate IgE levels [15]. In this process, immunomodulatory cytokines IL-10 and TGF-beta are driving forces [16]. Both cytokines are, however, also produced by some tumor types and may participate in undesired tolerance phenomena in cancer diseases [17]. On the other hand, it has been reported that epithelial tumors are the source of typical Th2 cytokines as IL-4 and IL-13 [18, 19] which are directly involved in IgE class switch [20]. Anecdotic reports on natural IgE present in tumor tissues indicate that they may have pathophysiological significance in tumor surveillance and attack [21, 22]. Systematic allergooncological approaches to study the prevalence of natural antitumor IgE are at this time point missing [23]. Nevertheless, the advanced knowledge derived from the studies on allergic diseases may help to understand the function of IgE in malignancies. For this, a comparison of the possible target structures of IgE on allergens and on self-antigens like tumor antigens has been undertaken in this chapter.

10.2 Crosslinking of Antigens – A Biological Tool for Immunoregulation

Antibodies, when expressed by B lymphocytes as membrane immunoglobulins (mIg; also termed B-cell receptor, BCR) and in their secreted form, represent homodimeric molecules, thus being principally equipped with two identical antigen binding sites called paratopes [24]. The paratopes are opposed to the epitopes of an antigen which represent exposed molecular surface plains, covering a relative flat area between 600 and 1,000 Å² [25, 26]. Some B-cell epitopes may be cryptic, meaning normally not accessible to the immune system, and might be exposed after physical, chemical or enzymatic processing of an antigen.

The cognition that several short, discontinuous stretches of an antigenic protein chain together form the 3D antibody epitopes [27] has rendered multiple efforts using structural mimics of epitopes, that is mimotopes, as a circumvention strategy for 3D epitope definition [28, 29]. (Such mimotopes can also be applied for epitope-specific vaccination and are suitable for induction of tumoricidal IgE when applied by the oral route; see also [30, 31] and Chapter 7 of this book.)

However, linear sub-epitopes in the range between 8 and 12 amino acids could be as well mapped recently with high accuracy using an antibody-based proteomics

approach [32]. On the membrane of a resting B cell the distance of BCRs has been determined to be approximately 40 nm [33]. The phenomenon that properly spaced multiple copies of an epitope are needed to turn on or turn off a B lymphocyte rendered the cognition that antigens may act as immunoregulators [34]. The collected evidence concerns predominantly the formation of IgM and, consecutively, IgG antibodies by B lymphocytes [35]. Similarly, direct isotype switch from IgM to IgE can occur [36], but depending on the antigen, IgE switch may even occur in an IgM-independent fashion [37]. Chapter 3 of this book gives a comprehensive overview on this topic, also with respect to the effects on affinities of different IgE species.

During the last four decades numerous studies have focused on the evaluation of the epitope requirements for B-lymphocyte activation. Already early immunization studies have indicated that some antigens per se rather induced tolerance, whereas others were prone to act immunogenic. This was systematically analyzed in vitro using conjugates of flagellin as a carrier with different hapten density on it [38]. Within the same series of chapters, Feldmann specified this observation by stating that antigen molecules in a monomeric fashion or with few epitopes never induced tolerance, whereas highly dense display of epitopes did [39]. He concluded that “a tolerance-inducing antigen must bind more avidly to B-cell membranes than an immunogen. . .” [39]. Obviously, the dense display of epitopes on the body’s own cell membranes could, therefore, be involved in the maintenance of tolerance against self.

It has further been documented that these immunoregulative phenomena of antigens could be enhanced by coupling or admixing the same protein antigens to immunogenic or “non-immunogenic” carriers [40]. Among carriers for antigen-specific tolerance induction polyethylene-glycol became particularly popular [41], whereas for antigen-specific immunity coupling to non-related protein carriers like keyhole limpet hemocyanin (KLH), encapsulation in particulate materials [42] or admixture to the protein-absorbing adjuvant $\text{Al}(\text{OH})_3$ has been preferred. As vaccine carriers often enhance the antigenic density, the resulting opposed types of immune responses may thus have to do with the different epitope spacing.

Among B-lymphocyte responses, one has also to dissect between the so-called T-lymphocyte-dependent (TD) and T-independent (TI) responses. According to their definition, for immune responses to TD antigens like low-dose-soluble antigen or monovalent haptenated proteins T-cell help has to be provided to induce sufficient immunity [43]. However, T-cell dependence is a relative matter: some antigens, largely TI but still needing residual T-cell help, are called TI-2 antigens. However, in complete T-cell independence, also immune responses comprising high titers of IgM antibodies may be generated by the so-called TI-1 antigens. In this model, polymeric high-molecular-weight antigens (like lipopolysaccharide, LPS) may represent polyclonal B-cell stimulators or – when exposing highly organized, rigid surfaces – be potent-specific crosslinkers of the BCRs. In a classic publication, Dintzis et al. defined in 1976 the *immunon*, that is, minimally 12–20 antigenic determinants spaced in a distance of 10 nm, as the minimal requirement of TI B-cell activation [44]. Later, Bruton’s tyrosine kinase (Btk) was found to be associated with these clusters, not only being responsible for B-cell activation in terms of proliferation

and antibody synthesis but also controlling the cytoskeleton and thus having a function in antigen processing and presentation [45]. In further pioneer studies, Dintzis et al. specified that for the optimal spacing of at least approximately 20 haptenic epitopes within an immunon, a minimal threshold size of approximately 100 kDa of the monomeric antigen is required [46]. Therefore, antigens which are too small to display a sufficient number of epitopes to form an immunon can bind B lymphocytes but cannot stimulate them. On the other hand, it has been realized that sometimes large polymeric molecules, although exhibiting a dense epitope display, are not inducing immunity but in contrast tolerance. The explanation was that the distance between epitopes of multivalent antigens can also be too large to allow productive B-cell stimulation [46]. In that case, recognition of several epitopes far apart on the antigen, but coming together on a flexible loop, does not substitute for a rigid, optimal crosslinking. Taken together, crosslinking of the BCR occurs optimally with antigens exhibiting a repetitive (at least 12–20 copies) epitope display in rigid spacing above 5–10 nm. Interestingly, some viruses displaying a crystalline-like surface with perfectly spaced epitopes have been recognized as TI-1 antigens [47, 48]. In both TI cases, the antigenic determinants must be arranged in a properly spaced manner for optimal crosslinking, B-cell activation and IgM immunoglobulin synthesis. The degree of antigen organization is even more important for the magnitude of IgG responses and can be supported by costimulatory B-cell signals like via CD21 [49] and Toll-like receptor-4 (TLR4)–MyD88 complex [50], the latter being also susceptible for stimulation with heat shock protein 60 (HSP60) [51]. Further, the spacing of the virus epitopes is affecting the affinity of the (bivalent) IgG response and its capacity to reduce the infectivity of the virion particle [52].

The B lymphocytes are, therefore, today recognized as pattern recognizers activated not by single epitopes but by the repetitiveness and spatial organization of the antigen [53]. Importantly, the concentration or aggregation state of an antigen, for instance upon chemical modification, can determine its immunogenicity [54]; environmental stress augments expression of pathogenesis-related proteins in plants [55], and in turn, food processing may render immunogenic aggregates of proteins [56]. With increasing concentration of an antigen also the epitope density increases and may significantly improve the recognition and uptake of the antigen by B-cells as well as other professional APCs. This is important, because professional APCs take care for recruitment of specific and bystander T-helper cells and thus enhance the extent of immune responses. Importantly, it is not sufficient that TD antigens are sensed by the specific BCR but simultaneously a second danger signal is received via by TLRs, representing another class of pattern recognition receptors (PRRs) also expressed by the B lymphocytes [57]. In an experimental system using transgenic mice devoid of TLR on their B lymphocytes (but not on DCs), antigen-specific antibody production indeed was deficient and could not be substituted by adoptive transfer of CD4-positive T-cells [58].

Generally, for TD antigens, the given cytokine milieu will determine the consecutive isotype switch, and the immune response can further be promoted by natural [59] or artificial adjuvants and carriers [60, 61].

10.3 Crosslinking – Indispensable for IgE Synthesis and Pathophysiology

We hypothesize that the above reviewed principles for B-cell activation, namely, rigid and dense (but not too dense) epitope display on antigens, will similarly account for IgE production. The rationale for this is derived from the fact that in both the sensitization and the effector phase of IgE-mediated responses crosslinking is a critical biological phenomenon. Thereby, the epitopes of an antigen (or allergen if clinically undesired hypersensitivity is meant) must interact with the complementary determining regions (CDRs) of at least two IgE antibodies to induce a biological response in the form of signal transduction, protein synthesis or mediator release, respectively [62, 63].

For the mast cell, being the most typical IgE-binding effector cell, it has been shown that also the antigen amount and contact duration are decisive for the overall signal strength and thus determine whether the cell will degranulate or acquire enhanced survival [64, 65]. In contrast, interaction of two antigenic epitopes with only one homodimeric IgE molecule expressed as membrane IgE (mIgE) by a B-lymphocyte or passively bound to high-affinity FcεRI will not activate but, contrarily, lead to anergy or tolerance by the B-cell or non-reactivity of the effector cell. The same principle obviously accounts for DCs, where polyvalent but not monovalent IgE engagement resulted in internalization of antigens [66].

Therefore, the spacing of epitopes will critically affect antigenicity and allergenicity of an agent with respect to its capacity to induce immunoglobulin synthesis. The single, monoclonal B lymphocyte is uttermost restrictive, because each single immunoglobulin expressed on its membrane will recognize the same epitope. Consequently, as discussed above, recent work suggests that B lymphocytes are pattern recognizers [53]. Primarily, upon antigen contact and TI activation, the B lymphocyte will produce a soluble form of IgM antibodies through alternative splicing and secrete them. However, if simultaneously with repeated antigen contact the CD40 ligation and/or IL-4 and IL-13 stimulation occurs, the specific B lymphocyte has several options to switch toward IgE: (i) direct deletional switching [67, 68] and (ii) sequential switching via gamma 4 [68, 36] or even in an IgM-independent fashion, which was at least demonstrated in mice infested with helminths [37]. The (IgE-producing) εB-lymphocytes undergo somatic hypermutation and affinity maturation in spite of their location outside germinal centers [3]. Thereby, recognition of the original antigen epitopes is improved substantially, rendering outstanding IgE affinities, with equilibrium dissociation constants ($K_D = k_d/k_a$) between 10^{-10} and 10^{-11} M [2]. Obviously, also for the reactivation and expansion of the memory subset of the B lymphocytes at later points of time it is crucial that epitopes are presented in a repetitive manner. Indeed, the antibody response in sensitized mice was successfully re-challenged with a dimeric allergen, but not monomeric allergen [69]. For memory εB lymphocytes, contacts with TLR activators may possibly be important for productive activation too, as previously demonstrated for IgG [50]. Moreover, also the achieved affinity to the epitopes will affect surface binding of

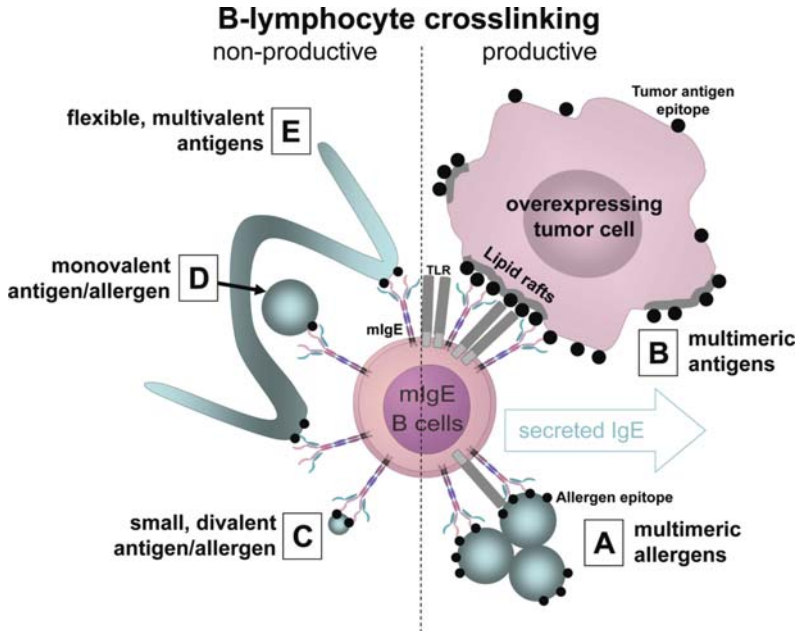


Fig. 10.1 The antigen determines immune reactions through the spacing of its B-cell epitopes. *Right side:* Productive activation (including antibody synthesis) will take place when a multivalent antigen is able to crosslink more than one mIgE by the rigid display of repetitive epitopes. These requirements are fulfilled by multimeric allergens (A), and overexpressed tumor antigens, e.g. accumulating in lipid rafts (B). Other pattern recognition receptors, as TLR family members, may contribute to the extent of activation. *Left side:* Non-productive activation (cytokine production, prolonged survival, but no IgE synthesis) will take place when mIgE remains monomeric. This may happen upon contact with a too small divalent antigen (C), with a single molecular unit of a monovalent antigen (D), and upon contact with loosely connected epitopes (E)

allergens and level of activation [70, 71]. Contrarily, antigens may also induce tolerance in IgE-producing B lymphocytes, possibly in case of multivalent polymers, improper epitope spacing, high flexibility of antigen, or at too small molecular size of an antigen, being monomeric [72] (Fig. 10.1).

Spacing of allergenic epitopes is also critical for the effector phase of the established immediate-type allergy, when IgE is already bound with high affinity to its receptors on mast cells, eosinophils or basophils. According to the textbook, allergens have to be at least bivalent, but should rather be multivalent to increase their potency to activate FcεRI-bearing effector cells, that is, exposure of more than one IgE epitope (Fig. 10.2). Moreover, the FcεRI signal depends on concentration and affinity of both the antigen and the receptors [73].

Interestingly, activation via FcεRI and TLR-4 both induce a shared core response of gene expression toward Th2 in mast cells [74]. Thereby, also monovalent IgE-allergen interactions can induce signaling and prolong the lifetime of mast cells [64, 74, 75] and induce FcεRI surface expression [76, 77]. The higher the epitope density, the more likely effective crosslinking will occur. Whether or not an allergic

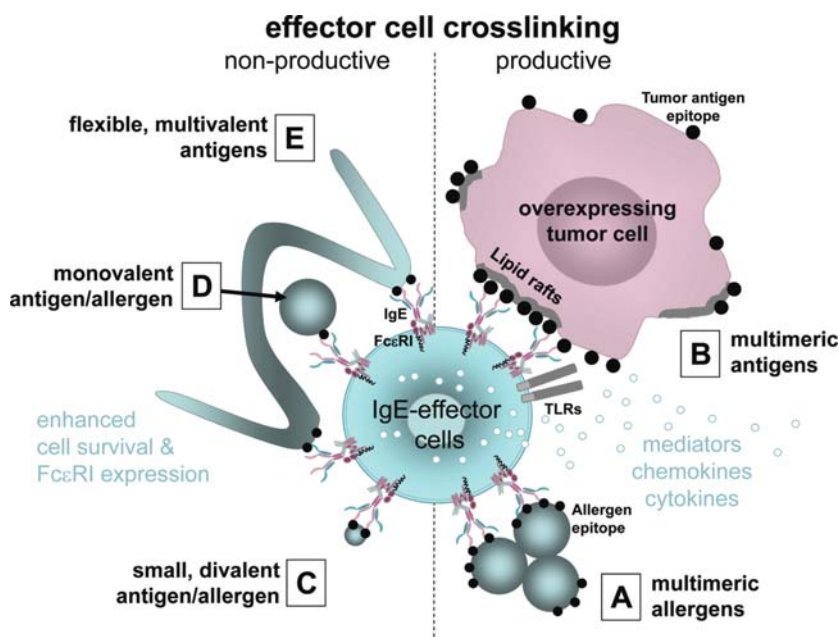


Fig. 10.2 The antigen determines the extent of immediate type inflammation upon crosslinking through the spacing of its B-cell epitopes. A typical effector cell, for instance a mast cell, is illustrated with multiple IgE molecules bound in bent shape to its high affinity receptors FcεRI, constituted of four chains. *Right side:* Productive activation rendering mediator release will take place when a multivalent antigen is able to crosslink more than one FcεRI-bound IgE by the rigid display of repetitive epitopes. These requirements are fulfilled by multimeric allergens (A) and overexpressed tumor antigens, e.g. accumulating in lipid rafts. The later may lead to antibody-dependent cellular cytotoxicity (ADCC) or phagocytosis (ADCP) (B). Other pattern recognition receptors, as TLR family members (dark grey bars), may contribute to the extent of activation. *Left side:* Non-productive activation (cytokine production, prolonged survival) will take place when FcεRI-bound IgE remains monomeric. This may happen upon contact with a too small divalent antigen (C), with a single molecular unit of a monovalent antigen (D), and upon contact with loosely connected epitopes (E)

reaction will be elicited may, however, finally depend on the structural integrity of the allergen and on the distinct spacing of its IgE epitopes (reviewed in [78]).

In early IgE crosslinking studies with rigidly spaced bivalent avidin constructs, molecules with a length of 49.4 Å were poorer triggering agents than those of 92.7 and 135.8 Å; the best cellular degranulation effect was achieved with dimers and polymers from the length of about 80–240 Å [79] (Fig. 10.3). In contrast, more recent studies indicate a shorter distance of allergen epitopes: performing in vitro studies with double-stranded DNA as a versatile rigid spacer, Paar et al. were able to show that for effective crosslinking of specific IgE on rat basophil leukemia cells, an inter-epitope distance of 44–51 Å was optimal to stimulate degranulation [80].

Further, the interaction of an antibody with its specific epitope depends on the flexibility of the Fab arms, which again depends on the respective immunoglobulin isotype. For the Fab arms of IgE in solution there is limited segmental flexibility, and

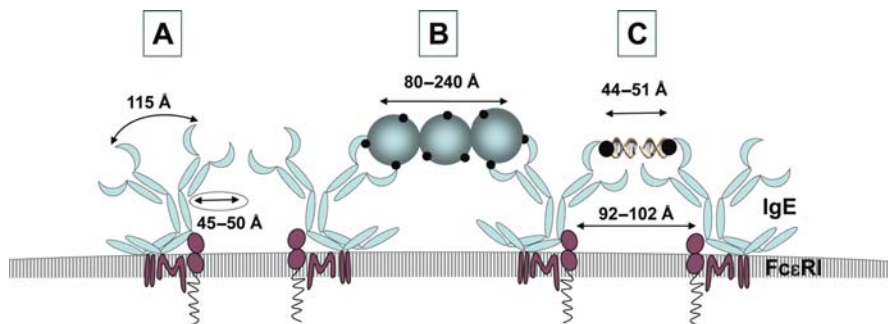


Fig. 10.3 Inter-molecular distances optimal to stimulate degranulation of effector cells via IgE bound to its high affinity receptor, Fc ϵ RI. (A) The estimated intra-molecular distance between two Fab binding sites is at least 115 Å [88] and thus fits to the calculated upper limit for the radius of Fc ϵ RI-bound IgE in the range of 45–50 Å [79]. (B) Cellular degranulation through antigen-mediated IgE crosslinking was optimally triggered by dimers or polymers from the length of 80 to 240 Å [79], but also (C) by smaller proteins using dsDNA as rigid model spacer with a distance of 44–51 Å [80]. The minimal distance between two IgE molecules cross-linked by antigen was estimated in the range of 92–102 Å [79]

Fab arms are not free to undergo extensive “wagging” motions [79] also because of the bent shape of IgE [81], both in solution and on Fc ϵ RI [82–84]. This means that the IgE antibody itself cannot adapt the fit to the allergen.

Allergic individuals have a small number of IgE antibodies on the basophil surface (about 40,000 molecules per cell) evenly distributed in non-activated condition [85]. It was speculated by Siraganian et al. that short bivalent haptens are capable of eliciting histamine release because the second Fc ϵ RI-bound IgE molecule would move closer through lateral diffusion within the cell membrane [86], today more precisely defined as movements into and within the lipid rafts of detergent-resistant membranes [87]. Resonance energy transfer measurements suggested a minimal distance of 92–102 Å between two cross-linked IgE molecules and an upper limit for the receptor radius of 45–50 Å [79].

On the other hand, the two Fab-binding sites *within one IgE molecule* were calculated to be at least 115 Å apart [88] (Fig. 10.3). It was furthermore shown in this study that flexible bivalent ligands longer than 130 Å form cyclic monomers with IgE in solution, that is intra-molecular bridging, and thereby rather block than induce histamine release. This would mean that, for instance, a small globular allergen dimer like Bet v 1, the major birch pollen allergen, with an estimated diameter of 38 Å could not form intramolecular bridging of Fab sites of the same IgE and is actually also below the optimal distance for cross-link of two separate IgE antibodies.

10.4 AAMPs: Allergen-Associated Molecular Patterns

Motifs recognized by PRRs may be called pathogen-associated molecular patterns (PAMPs) when associated with membranes of infectious agents [89]. We

suggest here that also allergens display repetitive motifs which we accordingly designate allergen-associated molecular patterns (AAMPs). Our suggestion is based on the observation that in many cases allergenic molecules occur as dimers or even multimers, a list growing longer over time (Table 10.1).

The consequence of di- or multimerization is the repetitive display of individual epitopes within the molecular complex. As suggested previously, the repetitive epitope display is important for both the sensitization and the effector phase of type I allergy [69] and may represent the long-sought common characteristic of allergens. Some allergens, especially small proteins, may possess only few or even a single immunodominant B-cell epitope and therefore as a single molecular unit do not fulfill the requirements for crosslinking. Examples are Api m 4 from bee (*Apis mellifera*) venom with only 2.8 kDa (26 amino acid residues) [90] and Amb a 5 from ragweed (*Ambrosia artemisiifolia*) pollen, which shows one very dominant B-cell epitope for human IgG binding [91]; also for the major birch (*Betula verrucosa*) pollen allergen Bet v 1 a single immunodominant murine IgG epitope was identified [92, 26, 93], which was distinct from the epitope of human polyclonal IgE [94]. For such small proteins, multimerization or oligomerization may provide the necessary means for efficient IgE crosslinking. Indeed, for Amb m 4 the formation of tetramers in aqueous solution was confirmed [95, 96] and Bet v 1 also forms dimers and higher oligomers in physiological solution, which is a necessary pre-requisite for its biological allergenic activity [69]. In accordance, Ghosh et al. have recently demonstrated that protein T1, a non-allergenic Bet v 1-homologue, lacks the capacity to dimerize although sharing a high-sequence and structural homology with other members of the Bet v 1 family [97].

When a series of recombinant monoclonal antibodies equipped with a humanized Fc ϵ domain were tested for their efficacy to activate mast cells, the antibody concentration, affinity and clonality affected the degree of effector cell degranulation [98]. As this experiment was based on murine IgG monoclonals originally raised in mice it may, however, be not representative for the human setting. The impact of epitope presentations for the crosslinking of monoclonal IgE antibodies was further tested with synthetic B-epitope peptides of allergen Phl p 1 from timothy grass (*Phleum pratense*) pollen. The polymeric peptide resulted in the highest release of β -hexosaminidase (70% of total release) compared to monomeric peptide (no release) and dimeric peptide (41% of total release) in in vitro experiments [99]. Moreover, although bivalent DNP-antigen was sufficient to bind and crosslink IgE, the best degranulation results were obtained with multivalent DNP-antigen using a rigid DNA display system [80] (Fig. 10.3).

A limited number of epitopes has also been shown for the major allergen from house dust mite (*Dermatophagoides pteronyssinus*), Der p 1, and Amb a 1 from ragweed, where binding of polyclonal IgE from serum of allergic patients was studied with respect to binding affinities and heterogeneity of recognized epitopes [100]. It was observed that in highly sensitized donors only two to three different affinity levels in antibody-antigen binding studies were relevant. The authors conclude from their work that a large proportion of sensitized patients harbor high-affinity IgE antibodies toward the same epitopes and that therefore the final affinity is determined

Table 10.1 Examples of allergens able to form dimers, multimers and higher aggregates (sorted by year of publication)

Allergen (source) ^a	Year of publication	References
Gad c 1 (Baltic codfish)	1967	Aas and Jebsen [140]
Api m 4 (bee venom)	1982	Terwilliger et al. [96]
ABA 1 (<i>Ascaris</i> , nematodes)	1990	McGibbon et al. [142]
A2U (rat urinary allergen)	1992	Bocskei et al. [143]
Mus m 1 (MUP; mouse urinary protein)	1992	Bocskei et al. [143]
Gly m Bd 30 K (soy bean)	1993	Ogawa et al. [144]
Sol i II (fire ant)	1993	Hoffman [145]
Tropomyosin (e.g. fish)	1995	Gimona et al. [146]
Alt a 1 (<i>Alternaria</i> , fungi)	1996	De Vouge et al. [147]
Bet v 1 (birch pollen)	1996	Wellhausen et al. [148]
Bos d 2 (BDA20) and Bos d 5 (alpha-lactalbumin), bovine dander	1996	Rautiainen et al. [149]
Ara h 1 (peanut)	1998	Shin et al. [150]
Phl p 1 (grass pollen)	1998	Petersen et al. [151]
Aca s 13 (<i>Acarus siro</i> , dust mite)	1999	Eriksson et al. [152]
Equ c 1 (horse dander)	1999	Gregoire et al. [153]
Tropomyosin (seafood)	1999	Reese et al. [102]
ABA-1 (<i>Ascaris</i> , nematodes)	2000	Xia et al. [154]
Ara h 1 (peanut)	2000	Maleki et al. [155]
Equ c 1 (horse dander)	2000	Lascombe et al. [156]
Ves v 5 (wasp venom)	2000	Suck et al. [157]
Ole e 1 (olive pollen)	2001	Huecas et al. [141]
Beta-lactoglobulin (bovine milk)	2001	Sakurai et al. [158]
Ara h 2 (peanut)	2002	Sen et al. [159]
Gad m 1 (Atlantic codfish)	2002	Das Dorez et al. [160]
Phl p 5b (grass pollen)	2002	Rajashankar et al. [161]
Phl p 7 (grass pollen)	2002	Verdino et al. [162]
Profilin (panallergen)	2002	Wopfner et al. [163]
Phl p 1 (grass pollen)	2003	Ball et al., RCSB Protein Data Bank 1N10 [168]
Cav p 1 (guinea pig dander)	2003	Fahlbusch et al. [164]
Fel d 1 (cat dander)	2003	Gronlund et al. [165]
Bet v 1 (birch pollen)	2005	Schöll et al. [69]
Dac g 5 (grass pollen)	2005	van Oort et al. [166]
Ara h 1 (peanut)	2006	van Bostel et al. [105]
Der p 1 (house dust mite)	2006	de Halleux et al. [167]
Can f 1 and 2 (dog dander)	2007	Kamata et al. [169]
Bos d 4 (bovine α -lactalbumin), Bos d 5 (β -lactoglobulin) (bovine milk)	2008	Roth-Walter et al. [56]
Bla g 2 (cockroach)	2008	Li et al. [170]
Der p 21 (house dust mite)	2008	Weghofer et al. [171]
Per a 3 (cockroach)	2008	Bellinghausen et al. [103]
Per a 4 (cockroach)	2009	Tan et al. [172]

^aCodes for allergen molecules are given according to the international allergen nomenclature (<http://www.allergen.org/Allergen.aspx>).

by the structure of the allergenic epitope rather than by variations of the immune response of the individual. In accordance, our own surface plasmon resonance affinity determinations with purified human polyclonal IgE rendered reactivity profiles like monoclonal antibodies, indicating that the repertoire of allergen-specific IgE antibodies within a single patient may be restricted to a handful high-affinity clones [101].

The IgE crosslinking process – already discussed above – is not only important in the effector phase of type I allergy but also for sensitization with allergens. A much better secondary antibody response is produced when allergens display their epitopes repetitively through multimerization [69] (Fig. 10.1A). Accordingly, dimerization was shown to have a positive effect on the immunogenicity of Dau c 1, a major allergen of carrot (*Daucus carota*) [102]. In these immunization studies of BALB/c mice, the highest IgG titers were reached in serum when the dimer of Dau c 1 was applied compared to monomers or mutated proteins. As professional antigen-presenting cells, like DCs, may be the first to handle allergens, they are in focus in several recent studies. For example, monomeric Per a 3 from American cockroach (*Periplaneta americana*) was demonstrated to be less immunogenic and allergenic than its naturally occurring hexamer structure [103]. When monocyte-derived autologous DCs of sensitized and non-sensitized individuals were pulsed with monomeric Per a 3, the release of mainly Th1 cytokines like IFN- γ was observed; in contrast, hexameric Per a 3 induced higher Th2-cytokine levels only in sensitized patients. Also the leukotriene release from leukocytes of allergic patients was higher with the Per a 3 hexamers. Similarly, without touching the question of multimericity, stimulation of allergic DCs from allergic donors with Bet v 1 and its homologues from food rendered Th2 cytokine production [104].

A number of allergens form even larger oligomers or multimers, like Ara h 1 from peanut (*Arachis hypogea*), which has been thought to be a trimer, but it was revealed that it might even form larger oligomers with a molecular weight of approximately 700 kDa, independent of the salt concentration and therefore of the ionic strength [105]. As mentioned above, the aggregation state of proteins is substantially affected by food processing and other manufacturing processes, as well as environmental pollution.

In addition to the formation of multimeric protein aggregates, the occurrence of repetitive epitopes on the native molecule might enhance the allergenic capacity (Fig. 10.2). An example is Hev b 5 from latex (*Hevea brasiliensis*) [106], where an immunodominant B-cell epitope is repeated three times within the protein sequence and, therefore, makes the allergen multivalent. Also, Bla g 1 from German cockroach (*Blattella germanica*) shows tandem repeats, forming two duplexes within the natural allergen [107], and tropomyosin, for example, from shrimps has repetitive motifs [108]. Molecular motifs as such seem to play an important part in the allergenicity of an antigen [109, 110]. A consensus sequence was recently created from different tropomyosin motifs (tropomyosins from house dust mite, cockroaches, shrimp and other). When it was expressed in *E. coli* and tested for IgE binding with sera of shellfish-allergic patients, binding of IgE to the consensus motif correlated well with binding to the full-length allergen, suggesting that the motif represents the allergenic part of the protein [109, 110].

Refining this approach, Kong et al. used an algorithm combining motifs of known allergens. Using motif length of 35 amino acids and motif number of 500, the algorithm can achieve both a high sensitivity and a high specificity for prediction of allergenicity [111]. In the light of novel proteins continuously introduced by the genetic engineering into our plants and diet, *in silico* approaches may provide useful means to predict allergenicity and protect sensitized patients.

In summary, the number and spacing of B-cell epitopes and the display of repetitive epitopes determine the induction and outcome of allergic reactions in the sensitized patient and, therefore, may be called AAMPs.

10.5 TAMPs: Tumor-Associated Molecular Patterns

From above it is clear that correct epitope spacing is a marker for foreignness and thus a key event in B-cell activation. Consequently, highly repetitive epitope display like by particulate antigens, but not monovalent-soluble proteins can break B-cell tolerance [112]. This basic principle represents a safety measure against autoimmunity by several means. Soluble antigens which may be secreted or shedded off by cells do rarely elicit a significant immunity. Several tumor types are shedding off their antigens to escape from eventual immune recognition. Among them, soluble forms for the epidermal growth factor receptor (EGFR), human epidermal growth factor receptor-2 (HER-2) and carcinoembryonic antigen (CEA; CEACAM5) have been described and are already used or suggested for diagnostic purposes [113–115]. In experimental conditions, soluble antigens require the addition of adjuvants to form immunogenic aggregates and even then may induce insufficient antibody titers only. Further, some of the body's own molecules which are displaying repetitive motifs are safely locked within the cells and thus are not encountered by B lymphocytes in healthy conditions. Only when exposed through inflammation elicited through physical damage or infections, self-molecules with repetitive epitope display, including proteins (actin, myosin, collagen) or DNA, will become accessible TI antigens and lead to strong IgM autoantibody production, followed by T-cell activation and IgG synthesis. Moreover, inflammatory oxidative stress may modify self-antigens making them even more antigenic, and thereby supporting the breaking of tolerance [116], or supporting upregulation of stress proteins. One example is HSP70, which is overexpressed in colon cancer and promotes antitumor immunity [117].

Thus, repetitive display of self- and modified epitopes is possible in mammals, and B lymphocytes are indeed infiltrating tumor tissues [118]. Since Taylor and coworkers made the observation that the distribution of membrane immunoglobulins on a B lymphocyte is dynamic [119], one may speculate that low-affinity interactions between autoantigens on tumor cell membranes and the specific BCR may be too weak to allow receptor aggregation and crosslinking. As a result, similarly as with TI-2 antigens, only minimal immunoglobulin production may take place at least in the absence of exogenously added cytokines [120]. However, in settings of antigen overexpression by a tumor cell, the epitope density increases dramatically

and should favor productive B-lymphocyte activation. Indeed, natural antibodies of the IgM, IgG and IgE class toward tumor antigens have been detected, indicating that the epitope display reaches sufficient density to induce B-lymphocyte activation [121].

For instance, the four members of the EGFR family (EGFR/HER-1, HER-2, HER-3, HER-4) lead to signal transduction by various homo- and heterodimerizations and by large-scale clustering within lipid rafts, impacting internalization and signaling processes [122, 123]. There are only a few published attempts to precisely define the intermolecular distances between these tumor antigens, which decrease with the absolute numbers of molecules overexpressed per cell. Moreover, the distances between various EGFR family members depend on the molar ratio to each other and to lipid-raft-specific mono-sialo-ganglioside GM1 [124]. In an early approach, the number of HER-2 molecules was calculated as 10^4 – 10^6 molecules per cell, depending on the type of tumor [125]. Due to low degree of expression, exact determinations sometimes fail [126]. In addition, the more members of the EGFR family are overexpressed, the more heterodimers could be observed by fluorescence resonance energy transfer (FRET) technology [127], making the composition of antigens on the cell membrane more complex. The authors state that the ratios of EGFR : HER-2 produced "...subtle changes in the average (molecular) distance. ..." More precisely, the intermolecular distances were recently calculated for EGFR homodimers, which on A431 cells membranes reside in 8 or 5.5 nm distance, as recently revealed by single-molecule imaging combined with fluorescence lifetime imaging [128]. This distance exactly fulfills the minimal requirements for B-cell activation (see above).

A mechanistic problem, however, may be the relatively loose, low-affinity interaction between homo- or heterodimer partners within the lipid rafts resulting in easy dissociation. Nagy et al. have also shown previously that crosslinking of raft lipid GM1 by Cholera toxin subunit B resulted in decrease of HER-2 cluster density as well as of HER-2/HER-3 heteroassociations [124]. Which conditions could, therefore, stabilize the tumor antigen display within the lipid rafts and render higher immunogenicity? As one interesting example, it has been shown that cholesterol, an important constituent of mammalian cell membranes, supports clustering and heterodimerization of EGFR and HER-2 within lipid rafts [129]. Cholesterol contents in tumor versus normal tissues are enhanced [130], and furthermore, the cholesterol content of tumor cell membranes was associated with increased metastatic potential [131]. In contrast, cholesterol depletion impairs microviscosity and thus receptor activation and downstream signaling [132], except EGF receptor [133]. When carcinoma cells were treated with cholesterol or cholesterol esters, their immunogenicity upon transplantation in animal models increased [134, 131]. Although the immunological mechanism was not examined in these pioneer studies, the improved display of tumor-associated antigens in cholesterol-enriched lipid rafts could contribute to the enhanced specific immunogenicity. Recently, IgE antibodies (but no other antibody classes) against a 50-kDa pancreatic cancer antigen were detected in sera of patients suffering from pancreatic cancer. The isolated IgE indeed elicited tumoricidal effects via ADCC [22] (Fig. 10.2). In accordance, it has been shown

that statins through inhibition of cholesterol synthesis impair the attack of CD20-overexpressing lymphoma cells by monoclonal antibody rituximab [135]. Even though the authors suggest a conformational change of CD20 to occur upon cholesterol depletion, the reduced recruitment of CD20 into decholesterinated lipid rafts could contribute to the observed effect [136, 137].

Also the glycosylation pattern on tumor cell membrane changes during the disease development, affecting adhesivity and metastatic potential of malignant cells. In principal, the resulting high-molecular glycoproteins could be targets of IgE antibodies. For instance, MUC4 exposes a rigid tandem repeat structure possibly mimicking EGFR-like domains, which could support interaction with HER-2 [138, 139]. Whether natural IgE against heavily glycosylated tumor antigens can be formed in humans and be implicated in the surveillance of cancer has to the best of our knowledge not been investigated to this date.

10.6 Conclusion

Today, mostly parasite antigens and allergens are known as targets of IgE antibodies. However, accumulating evidence indicates that also tumor antigens are recognized by IgE antibodies. Especially against cancer cells, IgE could be beneficial for the following reasons: (i) IgE binds efficiently to Fc ϵ RI-bearing tumoricidal effector cells and may exploit them as shuttles into tumor tissues, (ii) IgE recognizes preferentially 3D epitopes and can, therefore, target viable tumor cells and (iii) IgE may exert surveillance functions against tumor stem cells, as due to high-affinity recognition of the antigens it may trace and kill low numbers of malignant cells. An important molecular requirement for IgE synthesis by B lymphocytes is the recognition of epitope patterns. Molecular patterns can be achieved when allergens display the epitopes repetitively such as in aggregated state or when self-antigens are highly overexpressed like on tumor cell membranes. Repetitive epitope display could also largely facilitate the crosslinking of IgE bound by its Fc ϵ Rs on effector cells and thus support the triggering of immediate inflammation. From the collected evidence we suggest that molecular patterns displayed by allergens or tumor antigens, which we here designated as AAMPs or TAMPs, respectively, are decisive for the synthesis and triggering of IgE antibodies.

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Chapter 11

The Role of Th2-Mediated Anti-Tumor Immunity in Tumor Surveillance and Clearance

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Abstract The concept that the immune system has the potential to recognize tumor cells and either eliminate them (tumor immune surveillance) or select for immune-resistant variants (immunoediting) has gained a resurgence of interest by the scientific community in the last decade. To date, much of the research on the immune response to cancer has focused on the response of cytotoxic CD8⁺ T lymphocytes to tumor-specific antigens and the production of Th1 cytokines by CD4⁺ and CD8⁺ T cells. In contrast, Th2-mediated immunity has traditionally been viewed as enhancing tumor growth, both by promoting angiogenesis and by inhibiting cell-mediated immunity and subsequent tumor cell killing. Although components of Th2-mediated immunity have been shown to promote tumor growth, there is also an expanding body of evidence demonstrating the anti-tumor activity of CD4⁺ Th2 cells, particularly in collaboration with tumor-infiltrating granulocytes, such as eosinophils. In this chapter we examine all the key components of type 2 immunity and their effects on tumor growth. Based on this collective data, there exists great potential for the development of Th2-mediated immunotherapies that harness the anti-tumor activity of eosinophils, alternatively activated macrophages and the antigen–IgE–receptor axis.

11.1 Introduction

The fundamental requirement for the co-operation between the innate and the adaptive immune systems for effective tumor identification and tumor eradication is widely accepted. Importantly, the role of the immune system in the surveillance of transformed cells has seen a resurgence of interest in the last 10 years, with a substantial body of data in mice and humans supporting a role for the immune system in host protection from tumor development and in shaping tumor immunogenicity [1]. Tumor immune surveillance and immune editing are processes whereby the

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immune system has the inherent capacity to recognize, modify and induce the elimination of transformed cells within the body. Additionally, host immune status has been identified as critical in controlling the spread and growth of metastatic tumors [1]. By increasing our understanding of the anti-tumor immune responses, cancer immunotherapy aims to harness those specific immune responses most effective at producing tumor elimination.

Until recently, most tumor eradication studies have focused on the role of the adaptive dendritic cell (DC)-driven CD8⁺ cytotoxic T-lymphocyte (CTLs) response to tumor-associated antigens (TAAs) following recognition of tumor antigens presented by MHC class I molecules on transformed cells. Many types of vaccines incorporating peptide antigens, proteins, DNA and whole tumor cells, as well as adoptive transfer of tumor-reactive CTLs, have been evaluated, although generally these approaches have had limited clinical success [2, 3]. The lack of success encountered by these strategies highlight the many problems associated with generating CTL-mediated anti-tumor immunity, including the fact that “successful” tumors have often been “immunoselected” or “immunoedited” to evade recognition by CTLs [4]. The fact remains that the ability of the host to generate an effective innate immune response against the tumor is crucial for the subsequent development of an adaptive immunity and an effective CTL response, with the adaptive immune response also recruiting innate effector cells, including natural killer (NK) cells, neutrophils, mast cells, macrophages and eosinophils, into tumors [5]. These observations have stimulated the search for more effective approaches to cancer immunotherapy.

11.2 CD4⁺ T cells – The Th1 and Th2 Paradigm in Tumor Biology

In the last decade there has been renewed interest in anti-tumor immune responses mediated by CD4⁺ T cells. Studies have highlighted the role of CD4⁺ T cells in both the activation of innate effector cells in the primary response and the subsequent co-operation with innate cells in tumor cell killing [6]. Unlike CTLs, CD4⁺ T cells can respond to MHC class II negative tumors, recognizing TAAs presented on MHC class II molecules by bystander antigen-presenting cells (APCs), a process found to be less susceptible to immunoediting and subsequent immunoselection [6, 7]. Studies using either a CD4⁺ T-cell activating cancer vaccine [8] or in vitro-generated tumor antigen-specific CD4⁺ T cells [9, 10] have shown that CD4⁺ T cells can clear established tumors from mice, with both Th1 and Th2 cytokines being implicated in the tumor eradication process. Importantly, recent studies by Mautner et al. of a patient with renal cell carcinoma highlighted the potential of harnessing CD4⁺ T cells against MHC class II-restricted tumor antigens in an immunotherapy strategy [11].

Studies from our own laboratory have demonstrated a critical role for antigen-specific CD4⁺ T cells exhibiting a cytokine profile characteristic of Th2 cells, in

the clearance of established B16 melanoma metastasis [12]. However, in contrast to previous studies [9], CD4⁺ Th1 cells had a limited effect at inducing tumor clearance [12]. The advantage of CD4⁺ Th2 cells is that they have the potential to directly enhance antibody-mediated tumor immunity as well as innate anti-tumor immune responses by directly recruiting tumoricidal myeloid cells, including mast cells, macrophages and eosinophils, into the tumor site, a process dependent on the production of Th2-associated cytokines, such as interleukin (IL)-4 [13–16]. Since the establishment of the classification of CD4⁺ T cells as Th1 or Th2 based on their cytokine expression [17], there has been a strongly held conviction that maintaining a Th1/Th2 immune balance favoring Th1-mediated cellular immunity is linked to a positive clinical prognosis in cancer patients [18, 19]. However, more recent studies in humans and in animal models clearly illustrate that Th2-mediated cellular and chemical mediators have tumor immune surveillance and tumor clearance potential [20–23]. This chapter aims to focus on the mechanisms of Th2-mediated anti-tumor immunity.

11.3 Key Cellular and Chemical Mediators Involved in Th2-Mediated Tumor Immunity

Clinical and laboratory-based evidence is mounting to highlight the potential of Th2-mediated immune responses in initiating and effecting anti-tumor activity, particularly through Th2-mediated cytokines/chemokines, activated eosinophils, alternatively activated macrophages (aaMacs), Tc2 cells, B cells and mast cells loaded with anti-tumor IgE (Table 11.1, Fig. 11.1). In addition, Th2-mediated immunity has the potential to be harnessed as a cancer immunotherapy strategy. The key cellular and chemical mediators involved in Th2-mediated tumor immunity will be discussed below.

11.3.1 Th2 Cytokines

As a key regulator of humoral and adaptive immunity, the role of IL-4 in tumor biology has been studied most extensively. IL-4 is a pleiotropic cytokine produced by activated CD4⁺ Th2 cells, mast cells, basophils and eosinophils. Murine studies, using IL-4-secreting tumors [13], directly injecting IL-4 into tumors [15] or injecting IL-4 around the tumor draining lymph node [13], have demonstrated a role for this pleiotropic cytokine in tumor clearance. In these studies, tumor clearance was found to directly correlate with the infiltration of eosinophils and macrophages into the tumor site [13–15]. In addition, neutralization of IL-4 with an anti-IL-4 monoclonal antibody resulted in the loss of the eosinophil and macrophage infiltrates and a concurrent loss of anti-tumor immunity, results which were found to be less evident following the neutralization of IL-5 using an anti-IL-5 monoclonal antibody [15]. Although B cells, NK cells and mast cells do not appear to be required for IL-4-mediated tumor clearance [15], CD8⁺ T cells have been reported to be

Table 11.1 The key factors involved in Th2-mediated immunity and their effect on tumor growth

Factor	Tumor growth	Key observations	References
IL-4	Inhibit	IL-4 induces tumor clearance via infiltrating granulocytes and is anti-angiogenic.	13–15, 25, 27, 28,29
	Enhance	Inhibition of tumor apoptosis.	26
IL-13	Inhibit	IL-13 induces granulocyte-mediated tumor clearance.	36,37,43
		IL-13R α 2 cytotoxin constructs induce tumor clearance.	41,42
	Enhance	NKT cell-derived IL-13 inhibits CTL activation via macrophage secretion of TGF- β .	38
IL-24/mda-7	Inhibit	IL-24 promotes tumor growth suppression, apoptosis and radiosensitization.	53–55
		Ad.IL-24 therapy successful in several cancers.	54,56–58
IL-25/IL-17E	Inhibit	IL-25 promotes expansion of Th2-mediated tumor immune responses.	61
		Tumor clearance mediated by Virulizin linked to IL-25-induced eosinophilia and macrophage influx.	62–64
Eosinophils	Inhibit	Role in IL-4-mediated tumor clearance in mice and can directly kill tumor cells in vitro.	8,9,12,13,15,16,24
		Eosinophil-mediated tumor clearance.	67,72,74
		Eosinophils implicated in tumor immune surveillance.	23
		High eosinophil counts in tumors are associated with a good prognosis.	66–70
	Enhance	Eosinophils may promote tumor angiogenesis.	78
aaMacs	Inhibit	aaMac-derived arginase-1-mediated arginine depletion inhibits tumor growth.	75–77
	Enhance	Arginase-1 can inhibit CTL function and anti-tumor activity.	89–95
B-cells	Inhibit	B cell deficient mice have a decreased tumor growth.	96
		Antigen-IgE-Fc ϵ R1 axis can induce ADCC and tumor clearance.	99–101
Mast cells	Enhance	B cells inhibit CTL-mediated tumor clearance through IgG-mediated formation of IC.	114–116
	Inhibit	Mast-cell deficiency linked to increased tumor growth and inhibition of tumor apoptosis.	102,105,106
Tc2 cells	Enhance	Mast cell infiltration associated with poor prognosis.	122,123
	Inhibit	IL-4-secreting Tc2 cells can induce tumor clearance.	119,120
	Enhance	Tc2 cells secreting high levels of IL-10 can inhibit tumor clearance.	127,128,130
			131

IL, interleukin; NK, natural killer; CTL, cytotoxic T lymphocyte; TGF- β , tumor necrosis factor β ; Tc2, type-2 CD8⁺ T cell; ADCC, antibody-dependent cellular cytotoxicity; IC, immune complex

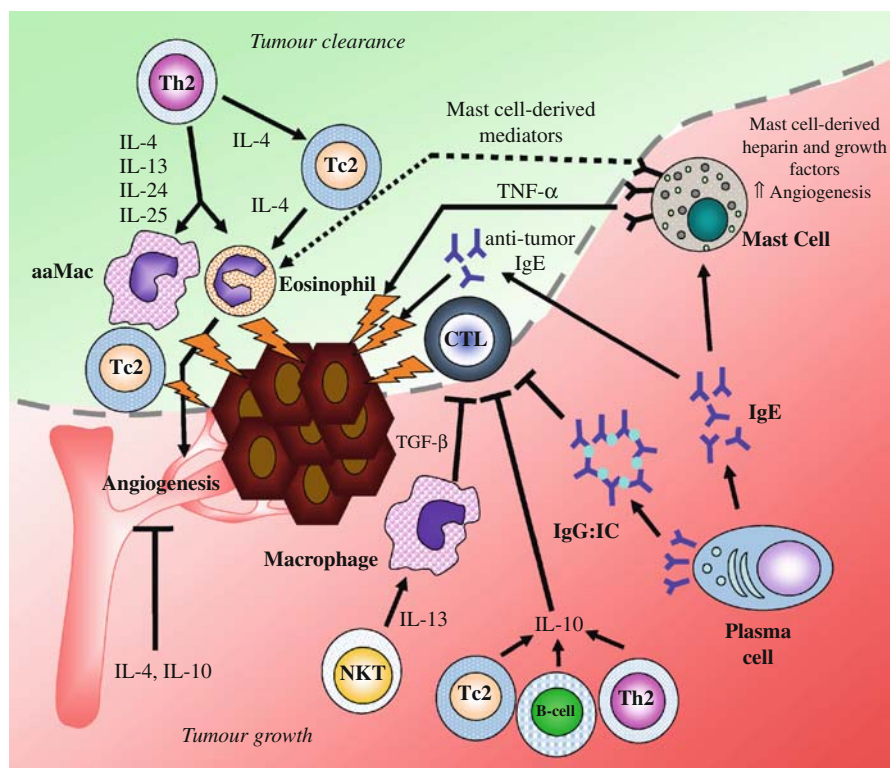


Fig. 11.1 The “yin and yang” of Th2-mediated inflammation within tumors. The model shows how different components of Th2-mediated immunity can either enhance tumor clearance (light/green zone) or promote tumor growth (darker/red zone). Secretion of IL-4, IL-13, IL-24 or IL-25 by CD4⁺ Th2 cells can induce an influx of eosinophils and alternatively activated macrophages (aaMac) into the tumor and promote anti-tumor cytotoxic activity. Similarly, IL-4-secreting CD8⁺ Tc2 cells may induce the direct killing of tumor cells, although less effectively than CD8⁺ CTL cells, in addition to collaborating with eosinophils and macrophages to induce tumor clearance. Mast-cell mediators, such as TNF- α , have also been shown to have a direct anti-tumor effect. Conversely, NKT cell-derived IL-13 can induce TGF- β secretion by CD11b⁺ macrophages, inhibiting CTL-mediated anti-tumor activity by interfering with antigen processing and presentation, similar to immunosuppressive IL-10, produced by Th2, Tc2 and B cells. Plasma cells can promote tumor growth by the secretion of anti-tumor antibodies that form immune complexes (ICs), in particular, IgG-mediated ICs that interfere with CTL-mediated tumor cell lysis, and together with tissue-remodeling leukocytes, such as eosinophils and macrophages, promote tumor angiogenesis. Anti-tumor IgE, however, can be harnessed for both passive and active tumor immunotherapy, resulting in subsequent tumor clearance. Also, IgE-mediated activation of mast cells can result in mast cell degranulation, the activation of eosinophils by mast-cell-derived mediators and resultant enhanced tumor clearance. On the other hand, mast-cell-derived heparin and growth factors, as well as eosinophils, have been linked with enhanced angiogenesis. Based on figure published in *Tissue Antigens* by Ellyard, Simson and Parish [20]

either essential [24] or not required [15]. CD8⁺ T-cell efficacy in IL-4-mediated tumor clearance has more recently been related to direct or indirect interactions with eosinophils, rather than CTL activity, a process which will be more thoroughly discussed later in this chapter.

Additional *in vivo* and *in vitro* studies have provided contrasting results for the efficacy of IL-4 as an anti-tumor treatment. IL-4 has been demonstrated to inhibit angiogenesis through the activation of tumor-associated fibroblasts in the tumor stroma [25]. Conversely, IL-4 has been shown to both inhibit [26] and enhance [27] the apoptosis of tumor cells, depending on the tumor model being investigated. Using an *in vitro* breast cancer model, IL-4 was found to induce apoptosis, a role directly attributed to the IL-4-mediated regulation of the estrogen synthesizing enzymes 17 β -hydroxysteroid dehydrogenase (HSD) and 3 β -HSD [28]. In a study by Taylor et al., IL-4 treatment induced growth inhibition in 52–60% of myeloma and lymphoma specimens tested [29]. However, tumor cell growth was stimulated in 8.6% of the tumors, these tumors displaying surface markers associated with a poor clinical prognosis.

Despite promising preclinical growth inhibitory and immunomodulatory effects, IL-4 clinical trials have produced minimal biologic and clinical activity against renal cell carcinoma [30, 31], melanoma [30, 32] and AIDS-related Kaposi's sarcoma [33]. These disappointing results could be directly attributed to the fact that IL-4 administration was limited to systemic or distal subcutaneous delivery rather than intratumoral or draining lymph node delivery, routes of administration that were shown to have the most efficacy in the mouse models studied [14, 15, 24].

Similar to IL-4, studies investigating the role of another Th2 cytokine, IL-13, in tumor immunity have produced mixed results. Although both IL-13 and IL-4 can signal through the same receptor, namely the type II IL-4 receptor composed of the IL-4R α chain and the IL-13R α 1 chain, IL-13 can also signal through IL-13R α 2 [34–37]; hence the biological activity of IL-13 is similar but unique to IL-4 [35, 38]. Overexpression of IL-13 by tumor cells has been shown to induce tumor clearance, with Gr-1⁺ and MAC-3⁺ expression identifying the tumor-infiltrating leukocytes as neutrophils and macrophages [36, 37]. However, the expression of Gr-1⁺ on eosinophils, coupled with the known role of eosinophils in IL-13-mediated inflammation [39], suggests that eosinophils may also be effector cells in IL-13-mediated tumor clearance, as observed for IL-4. In contrast, transfection of a soluble IL-13R α 2 decoy receptor into the tumor cells or administration of the receptor as a soluble fusion protein had no effect on the growth of B16F1 melanoma and Meth A fibrosarcoma tumors [38]. Furthermore, studies by Terabe et al. have indicated that IL-13 plays a role in CD4⁺ NKT cell-mediated suppression of tumor immune surveillance [38]. IL-13 has also been identified as an autocrine growth factor for Reed–Sternberg cells in Hodgkin's lymphoma [40].

Interestingly, IL-13R α 2 but not IL-13R α 1 is overexpressed in a variety of human cancer cells derived from glioblastoma, squamous cell carcinoma of head and neck, breast cancer, colon cancer, renal cell carcinoma, pancreatic cancer and AIDS-associated Kaposi's sarcoma [41, 42]. However, the stable transfection of the IL-13R α 2 in human breast and pancreatic cancer cell lines identified a significant inhibition in the tumorigenicity of the IL-13R α 2-expressing tumors in immunodeficient mice, with IL-13 produced by infiltrating neutrophils combined with tumor cell-derived IL-8 producing an anti-tumor effect [43]. The use of targeted therapy employing an IL-13R α 2 cytotoxin construct has shown promising preclinical tumor

cytotoxicity in vitro and in vivo [41, 42], but clinical trials using an IL-13R α 2 cytotoxin have shown no significant effect on tumor growth [44]. It remains clear that although IL-13R α 2 expression is elevated in some cancers, the role of IL-13 in tumor growth varies greatly between tumor types.

Previous reports suggesting that Th2-type cytokines inhibit anti-tumor immunity in vivo [45–47] and in vitro [48] had attributed this effect to IL-10-mediated immune suppression. However, it has now become clear that IL-10 has the potential to regulate both Th1-type and Th2-type cytokine responses [49]. On the other hand, the melanoma differentiation association gene (mda)-7, or IL-24, has been identified as a unique tumor suppressor in the IL-10 family of cytokines and is classified as a Th2 cytokine, being produced by monocytes and Th2 cells [50]. The two heterodimeric IL-24 receptors, IL-22R1/IL-20R2 and IL-20R1/IL-20R2 [51], are expressed in human lymphoid organs such as spleen and thymus, as well as by peripheral blood leukocytes and normal melanocytes [52]. In vitro, IL-24 has been shown to selectively induce growth suppression, apoptosis and radiosensitization of a diverse range of human cancer cells, without causing similar effects in normal cells [53]. Intratumoral and peritumoral treatment of prostate cancer tumors in athymic nude mice using a replication-incompetent adenovirus expressing IL-24 (Ad.IL-24) was found to completely eradicate primary tumors as well as tumors created at a distal site to treatment [54]. Similarly, Ad.IL24 transduction of human breast cancer cells resulted in G(2)/M phase cell cycle arrest and apoptotic cell death, which correlated with the secretion of the IL-24 protein. Tumor cell killing by IL-24 was also dependent on binding to the IL-20R1/IL-20R2 heterodimeric receptor complex expressed by tumor cells [55].

Importantly, the efficacy of Ad.IL-24 as a gene therapy for cancer has been validated in terminal prostate cancer patients [54]. These studies also identified the ability of Ad.IL-24 to induce a “potent bystander apoptosis-inducing effect” in adjacent tumor cells not initially receiving this gene product, whereas normal cells are unaffected [56]. Based on its profound cancer tropism, Ad.IL-24 has been evaluated in a phase I clinical trial in patients with melanomas and other solid cancers. These studies document that mda-7/IL-24 is well tolerated and demonstrates evidence of significant clinical activity [57]. Recent in vitro studies have observed that certain cancers, including pancreatic and colorectal cancer cells, are resistant to monotherapy with Ad.IL-24; however, combinatorial treatment with chemo- or radiotherapy, small-molecule inhibitors and anti-tumor monoclonal antibodies have improved the efficacy of treatment and improved success in resistant tumors [54, 58].

Unlike other members of the IL-17 family of cytokines, IL-25/IL-17E is found to be secreted by activated Th2 cells, rather than the recently discovered Th17 lineage of helper T cells [59]. Furthermore, Th2 memory cells express high levels of IL-25R [59, 60], which following IL-25 binding promotes cell expansion and Th2 cytokine production by Th2 central memory cells activated by a variety of stimuli, such as thymic stromal lymphopoietin (TSLP)-activated DCs, homeostatic cytokines or T-cell receptor triggering [61]. IL-25 regulates hematopoietic and immune functions, stimulating the development of eosinophils and B lymphocytes [61]. The fact that IL-25 overexpression results in high levels of circulating eosinophils, IL-4, IL-5,

eotaxin and IgE suggests that IL-25 may be a pro-inflammatory cytokine favoring Th2-type immune responses [60]. Recent clinical studies by Liu et al. have shown that Virulizin[®], a novel biological response modifier extracted from bovine bile, has produced significant anti-tumor efficacy in a variety of human tumor xenograft models including melanoma, pancreatic cancer, breast cancer, ovarian cancer and prostate cancer [62]. Interestingly, tumor clearance is directly linked to IL-25-induced eosinophilia and macrophage infiltration into the tumor environment [63, 64].

11.3.2 Eosinophils and Eosinophil-Derived Mediators

The eosinophil remains as one of the most enigmatic cells in the immune system, a pleiotropic multifunctional leukocyte involved directly and indirectly in the modulation of innate and adaptive immunity. However, the true immunological role(s) of the eosinophil is yet to be fully elucidated. In the clinic, the development of blood eosinophilia and the localization of high eosinophils numbers within the tumor stroma and necrotic core of solid tumors, or tumor-associated tissue eosinophilia (TATE), have been observed since the 1950s [65]. The previously held belief that eosinophils act as ineffectual bystanders in the tumor environment has come under great scrutiny, with several clinical studies showing a direct correlation between eosinophil infiltration and a favorable patient prognosis, suggesting a true effector cell role for the eosinophil in tumor clearance. Thus, high infiltrating eosinophil levels have been associated with a positive prognosis in pulmonary adenocarcinoma, nasopharyngeal and esophageal squamous cell carcinoma, head, neck, gastric and prostate cancer [66–70]. As an extension of earlier studies, Takanami et al. found a significant difference in the rate of patient survival between patients whose tumors had high levels of eosinophils, as identified by the eosinophil-specific antibody EG2, and those patients with low numbers of eosinophils, suggesting the importance of a large eosinophil infiltrate into the tumor for the mediation of a positive clinical outcome [70].

Our own previous studies in mouse models have demonstrated that tumor-specific CD4⁺ T cells, exhibiting a cytokine secretion profile characteristic of Th2 cells, are capable of clearing established lung and visceral metastases of the B16-F1 CTL-resistant melanoma [12]. Clearance of B16 metastases by the Th2 cells was found to be completely dependent on the eosinophil chemokine, eotaxin, with degranulating eosinophils within the tumors being associated with tumor regression [12]. Tumor clearance was also partially dependent on the transcription activator signal transducer and activator of transcription 6 (STAT6) that is important in IL-4 and IL-13 signaling through the type II IL-4R [12], suggesting that these Th2 cytokines play a supportive role in eosinophil-mediated tumor eradication. Additional *in vivo* studies have since confirmed that eosinophils can exhibit potent anti-tumor activity when recruited into established tumors [8, 9, 12–15, 24].

For the first time, we recently established an important role for eosinophils in tumor immune surveillance [23]. The key findings were that genetically modified

mice that have enhanced levels of circulating eosinophils were extremely resistant to tumor induction by the carcinogen, methylcholanthrene (MCA), with most tumors that arose having remarkably slow growth rates leading to a dormant tumor state [23]. In contrast, eosinophil-deficient IL-5/CCL11^{-/-} and Δ dblGATA strains were highly susceptible to tumor induction at high and low concentrations of MCA in the total absence of eosinophils. Further studies are currently aimed at clarifying whether eosinophil-mediated tumor immune surveillance primarily involves either adaptive or innate immune responses or whether both are needed to establish effective tumor control.

Once recruited into the tumor environment, eosinophils have the ability to harness both direct and indirect mechanisms to induce tumor clearance. Direct tumor killing can be mediated via the release of granule-associated cytotoxic proteins, including major basic protein (MBP), eosinophil peroxidase (EPO), eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN), which are capable of inducing tissue damage and dysfunction [71] and have been linked to tumor cell apoptosis [72]. In addition, tumor-specific antibodies may earmark tumor cells for killing by eosinophils via mechanisms similar to those demonstrated in studies of immunity to helminthic parasites [73]. In vivo experiments using either an eotaxin-expressing melanoma (Simson and Parish – unpublished observation) or an eotaxin-expressing hepatocellular carcinoma [74] have revealed that although eosinophils are successfully recruited into the tumors, there is little effect on tumor growth unless the tumors are grown in IL-5 transgenic recipients, confirming the potential importance of IL-5 in tumor eradication.

However, modeling eosinophil-mediated tumor cytotoxicity in-vitro has highlighted the complex nature of this process. Studies by Furbert-Harris et al. were able to demonstrate that eosinophils harvested from allergic individuals, and the conditioned media of cell-lines established from these cells, were able to inhibit prostate tumor growth in vitro [67]. We have also reported that in vitro mouse eosinophils can directly induce the killing of MCA-induced fibrosarcoma cells [23], a process significantly enhanced when eosinophils were used from Th2-induced inflammatory exudates [23].

Conversely, high levels of infiltrating eosinophils have also been associated with a negative clinical prognosis in some cancer patients [75–77]. It has been proposed that eosinophils have the potential to promote tumor growth by inducing angiogenesis. Several factors that are known to promote angiogenesis, including vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), tumor necrosis factor (TNF)- α , granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-8, are synthesized by eosinophils and stored in their granules [78]. In one study, human peripheral blood eosinophils were able to induce rat aortic endothelial cell proliferation and angiogenesis in the chick embryo chorioallantoic membrane, a process partially mediated through VEGF [78]. However, a study by Tataroglu et al. evaluating non-small-cell lung carcinoma biopsies found no correlation between eosinophil, macrophage or mast-cell content and tumor angiogenesis, as confirmed by vascular density and microvessel quantification [15, 79].

Interestingly, although clinical prognostic score studies in classical Hodgkin lymphoma focus on the evaluation of eosinophil and mast-cell tumor infiltration, a recent retrospective analysis revealed eosinophil predominance to have no prognostic value that could be used in clinical practice although a tendency for a correlation between mast-cell infiltration with overall survival was identified [80]. Thus, to date, no direct role for eosinophils in tumor angiogenesis has been identified.

A synopsis of these studies suggests that the tumor microenvironment in which eosinophils are recruited plays a role in determining the physiological outcomes. Hence, a specific combination of activation signals is required to achieve effective eosinophil-mediated tumor clearance, enabling a degree of regulation when considering the high toxicity of eosinophil-derived mediators. Furthermore, our recent *in vivo* studies suggest that Th2-recruited myeloid cells, other than eosinophils, may be involved in providing both the appropriate activation environment and are also directly involved in tumor eradication (Ellyard, Simson, Parish – in submission). In many studies highlighting the anti-tumor role of eosinophils, a concurrent influx of macrophages, has been observed [8, 12, 13, 16, 24] and will be discussed in the following section. Hence, the relationship between eosinophils and other immune cells known to be involved in tumor clearance and immune surveillance is of considerable interest. In addition, the signals required for the recruitment of eosinophils into the tumor microenvironment and the subsequent mechanisms employed by eosinophils to induce tumor cell death warrant further investigation.

11.3.3 Alternatively Activated Macrophages

As previously highlighted, the Th2-mediated infiltration of eosinophils into the tumor stroma is often associated with a corresponding influx of macrophages into the tumor microenvironment [13, 15, 24], suggesting that macrophages may also play a role in Th2-mediated tumor clearance. However, tumor-associated macrophages (TAMs) have often been associated with increased tumor growth, tumor angiogenesis and a suppression of anti-tumor CD8⁺ T-cell responses, equating with a poor clinical prognosis [80]. Conversely, macrophages have been demonstrated to elicit the direct killing of tumor cells *in vitro* and *in vivo* [8, 81, 82]. Thus the role of macrophages remains controversial and, as with many other immune cells including the eosinophil, may differ depending on the tumour microenvironment.

Macrophages are one of the most heterogeneous cells of the body, with differentiation from monocyte precursors being determined by tissue-related and immune-related stimuli [83]. Macrophage-mediated cytotoxicity is traditionally associated with IFN- γ -activated type-1 macrophages and mediated through nitric oxide [84], TNF- α [85, 86], cytolytic proteases [85] and the generation of reactive oxygen species such as hydrogen peroxide and superoxide following activation of antibody-dependent cellular cytotoxicity (ADCC) [86, 87]. Alternatively activated macrophages (aaMacs) are elicited through a Th2-mediated IL-4/IL-13 environment [83], resulting in the upregulated expression of the mannose receptor and

MHC class II, enhanced endocytosis and antigen presentation, and production of the chemokines CCL22 and CCL17. The IL-4/IL13-induced production of the intracellular enzyme, arginase-1, and the reduced production and release of nitric oxide through arginase-1 suppression of nitric oxide synthase (NOS)-2 [83] are characteristic features of aaMacs.

Recent studies by Anthony et al. revealed that aaMacs effectively mediate the IL-4-induced clearance of the gastrointestinal parasite *Heligmosomoides polygyrus* in mice, a process dependent on the production of arginase-1 [88]. Recent studies in our laboratory have illustrated, for the first time, the ability of Th2-activated aaMacs to induce tumor eradication in vitro (Ellyard, Simson, Parish – unpublished results). In this study it was found that macrophages in a type-2 inflammatory infiltrate, when exposed to either IL-4 or IL-13, became cytotoxic for B16-F1 melanoma cells via a mechanism dependent on arginase-1-mediated depletion of L-arginine in the tumor cell microenvironment (Ellyard, Simson, Parish – unpublished results). The sensitivity of many tumour cells to arginase-1-mediated L-arginine depletion in vitro is well documented [89–94]. In fact, pegylated arginase deaminase, an enzyme known to induce the depletion of L-arginine, is now being developed as a potential anticancer agent and has entered phase II and phase III clinical trials [95].

The debate continues regarding the role of macrophages in tumor progression in vivo, with some studies suggesting that the tumor microenvironment suppresses TAM-mediated cytotoxicity. In fact TAM-derived arginase-1 has been linked to the inhibition of CTL-mediated immune surveillance, L-arginine depletion resulting in the inhibition of CD3 expression and subsequent T-cell activation and proliferation [96]. In addition, many clinical studies have correlated a high number of TAMs within the tumor microenvironment with a poor patient prognosis [80, 97]. It should be noted that in most clinical studies the TAM phenotype, namely identifying infiltrating macrophages as IFN- γ -activated type-1 macrophages or aaMacs, has not been evaluated. Based on our own studies, a considerable number of aaMacs need to be present for effective L-arginine depletion and resultant tumor cytotoxicity. In fact, when L-arginine depletion was incomplete, the aaMacs actually enhanced tumor cell proliferation (Ellyard, Simson, Parish – unpublished results). Furthermore, whether macrophages, through the production of arginase-1, also play a role in Th2-mediated tumour clearance in vivo remains to be fully elucidated.

11.3.4 B Cells, IgG and IgE

An important function of CD4⁺ T cells with a Th2-cytokine phenotype is to provide “help” for antigen-specific B cells and facilitate IgG1 and IgE antibody responses. Clinical and experimental data indicate that chronic inflammation, often antibody-induced, results in the recruitment and activation of innate immune cells within the tumor environment, which can promote cancer development [98]. In fact, increased humoral immunity has been reported to be a factor in several chronic inflammatory diseases, such as ulcerative colitis, asbestosis, HCV-related cirrhosis and chronic obstructive airway disease, and several autoimmune diseases [98]. Mouse studies

support this view, B-cell deficient mice displaying a higher resistance to colon carcinoma, B16 melanoma and EL4 thymoma [99, 100], in addition to an enhanced T-cell response to melanoma vaccines and an increased resistance to subsequent tumor challenge [101]. However, as has already been discussed in this chapter, most innate cells and their released mediators are also able to effectively induce tumor clearance in the appropriate tumor microenvironment.

The mechanism of B-cell-mediated inhibition of anti-tumor immunity is likely to be multifactorial. B-cell-mediated anti-tumor antibodies, in particular those of the IgG class, have been shown to enhance tumor growth by inhibiting CTL-mediated tumor killing and by forming immune complexes (ICs) within tumors [102]. ICs have been associated with increased tumor burden and poor prognosis in several cancers [103, 104], a process unrelated to specific antigens. Interestingly, receptors for the Fc portion of IgG, namely FcγRs, have been identified as inducers of IC-mediated inflammation and subsequent downregulation of anti-tumor immune responses [105]. In addition, IgG has been shown to complex with latent transforming growth factor (TGF)-β, resulting in the inhibition of CTL-mediated anti-tumor responses [106].

The role of IgE-mediated allergy (atopy) in cancer etiology is covered extensively in other chapters of this monograph. Numerous epidemiological studies have evaluated the association between allergy, in particular atopic disorders, and an enhanced capacity for tumor immune surveillance [107–110]. In summary, case–control studies, measuring allergen-specific IgE in patients with cancer, have reported conflicting results [111–113]. However, recent studies have effectively harnessed the antigen–IgE–FcRI axis in tumor targeting strategies based on the strong affinity of IgE for FcRI even in the absence of antigen [114]. Passive immunotherapy with a chimeric IgE antibody against an ovarian tumor-specific antigen resulted in IgE-mediated tumor clearance by infiltrating macrophages, tumor elimination being mediated via FcεRI ADCC and CD23 antibody-dependent cellular phagocytosis (ADCP) [114]. In addition, eosinophils were identified as potent effector cells in this model. Interestingly, a direct comparison between the efficacy of IgG- and IgE-mediated tumor clearance revealed that IgE chimeric antibodies were able to more effectively inhibit tumor growth [114, 115]. Related studies have focused on using IgE-loaded tumor cells as tumor vaccines, with the tumor clearance induced by these vaccines being via both innate effector mechanisms and eosinophil-dependent induction of tumor-specific T-cell immunity [116].

11.3.5 Mast Cells and Mast-Cell Mediators

The role of mast cells in tumor biology is extensively covered in Chapter 6 of this book; consequently, this section will only briefly highlight the potential interaction of these myeloid cells and their mediators with other Th2-mediated effector cells in tumor immunity.

Mast cells are regarded as playing an important role in adaptive and innate host defense against parasites and bacteria [117], in addition to being recognized as the

key effector cell in IgE-mediated allergic responses [118]. The potential of mast cells to actively promote tumor angiogenesis, a process mediated by mast-cell-derived heparin [119], VEGF, FGF-2 IL-8, TNF- α and matrix metalloproteinase (MMP)-9, is well documented [120]. Consistent with these activities, the majority of epidemiological evidence suggests that the presence of higher mast-cell numbers within tumors is associated with a poor prognosis [121]. Conversely, recent studies clarifying the prognostic value of tumor-localized mast cells in classical Hodgkin lymphoma confirmed a correlation between higher mast-cell infiltration and overall improvement in patient survival [122]. However, the role of mast cells in systemic tumor immunity remains poorly understood. To examine the role of mast cells in intestinal tumorigenesis, mast-cell-deficient Sash mice were crossed with the Min mouse, a genetic model of intestinal neoplasia [123]. The resulting mast-cell-deficient Min–Sash mice developed 50% more adenomas than littermate controls, and the tumors were 33% larger in these mice. Mast-cell deficiency did not affect tumor cell proliferation, but tumor cell apoptosis was significantly reduced in mast-cell-deficient mice. Interestingly, although tumor-infiltrating neutrophil, macrophage and T-cell populations remained similar between the Min and the Min–Sash mice, eosinophil infiltration was greatly reduced in the tumors of Min–Sash mice [123].

11.3.6 Tc2 Cells

Similar to CD4⁺ T cells, CD8⁺ T cells can be classified into two distinct types of effector cells based on their cytokine secretion profile following antigen encounter [124, 125]. Type 1 CD8⁺ T cells (Tc1) secrete IFN- γ , IL-2 and TNF- α and type 2 CD8⁺ T cells (Tc2) predominately produce IL-4, IL-5 and IL-10 [124]. The role of Tc1 CD8⁺ CTLs in tumor clearance is largely IFN- γ -mediated, while cytotoxicity is primarily mediated through the Fas/Fas ligand or perforin/granzyme signaling pathways [126]. Although the anti-tumor activity of Tc1 cells is outside the scope of this chapter, of interest are several recent studies aimed at clarifying the role of Tc2 cells in anti-tumor immunity. Transfer of OVA-specific Tc2 effector cells into an OVA-transfected B16-melanoma model was found to induce perforin-dependent tumor regression and enhanced survival of mice [127]. Interestingly, the use of gene-deficient mice confirmed that anti-tumor activity was dependent on effector cell-derived IL-4 and IL-5. In addition, Tc1-mediated IFN- γ release was also upregulated in surviving Tc2-treated hosts [127]. In a related study, although Tc2 cells were found to induce tumor clearance in an EG7 thymoma model, Tc2 cells were reported to be 5- to 25-fold less effective in inducing an equivalent anti-tumor effect to Tc1 cells [128].

Studies by Dobrzanski et al. suggested that Tc2 cells may facilitate tumor rejection through their interaction with other leukocytes such as macrophages, NK cells and eosinophils [127], although only eosinophils were found to infiltrate tumors at significant levels [127, 129]. However, as previously reported in this chapter, a role for CD8⁺ T cells in Th2-mediated or IL-4-mediated tumor clearance is well

established, with eosinophils and macrophages deemed to be effector cells in this process [9, 10, 14, 24]. Furthermore, in a supporting study, adoptive transfer of IL-4 producing CD8⁺ T cells cleared established lung metastasis via a process dependent on CD8⁺ T-cell-derived IL-4 and host NK cells and partially dependent on granulocytes [130].

Conversely, several studies have found that Tc2 cells lack the capacity to induce effective anti-tumor immunity. TCR-transgenic CD8⁺ T cells were activated in vitro to generate cytotoxic Th1 and Tc2 cells, which were adoptively transferred into syngeneic hosts, and their ability to protect against tumor challenge was compared. Tc1 cells were able to significantly delay tumor growth, whereas Tc2 cells from IFN- γ -deficient donors had no effect. In a separate study, tumor-specific Tc2 cells were found to be significantly less effective than Tc1 cells in mediating perforin-dependent lysis of tumor cells in vitro, had a significantly lower capacity for in vivo survival after adoptive T-cell transfer, and were significantly less effective at eradicating established tumors in animals [131]. One consistent factor in studies highlighting the reduced anti-tumor activity of Tc2 cells is the production of high levels of IL-10 in the tumor microenvironment, an observation not reported in models where effective Tc2-mediated tumor clearance occurred. The immunosuppressive effects of IL-10 in anti-tumor immunity are well documented and may counteract the anti-tumor potential of Tc2 cells.

11.4 Conclusion

Historically Th2-mediated immunity has been associated with the inhibition of anti-tumor immunity, both by promoting tumor angiogenesis and by suppressing cell-mediated immunity and effective tumor clearance. However, a more thorough analysis of the scientific literature and our own observations highlight the potential role for Th2-mediated immune effector cells and their mediators in tumor immune surveillance and tumor clearance (summarized in Table 11.1 and Fig. 11.1). Of particular interest are eosinophils and aaMacs which, through their innate responsiveness or recruitment and activation by the adaptive immune system, have the potential to act as potent anti-tumor effector cells. Most importantly, the tumor microenvironment and its milieu of cells and mediators play a primary role in determining the efficacy of global anti-tumor immunity and must be fully evaluated in the design and implementation of Th2-mediated tumor immunotherapies.

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