

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-13 as an autocrine factor in Hodgkin's lymphoma.	40
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
		High eosinophil counts in tumors associated with poor prognosis.	75–77
aaMacs	Inhibit	aaMac-derived arginase-1-mediated arginine depletion inhibits tumor growth.	89–95
	Enhance	Arginase-1 can inhibit CTL function and anti-tumor activity.	96
B-cells	Inhibit	B cell deficient mice have a decreased tumor growth.	99–101
		Antigen-IgE-Fc ϵ R1 axis can induce ADCC and tumor clearance.	114–116
	Enhance	B cells inhibit CTL-mediated tumor clearance through IgG-mediated formation of IC.	102,105,106
Mast cells	Inhibit	Mast-cell deficiency linked to increased tumor growth and inhibition of tumor apoptosis.	122,123
	Enhance	Mast cell infiltration associated with poor prognosis.	119,120
Tc2 cells	Inhibit	IL-4-secreting Tc2 cells can induce tumor clearance.	127,128,130
	Enhance	Tc2 cells secreting high levels of IL-10 can inhibit tumor clearance.	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

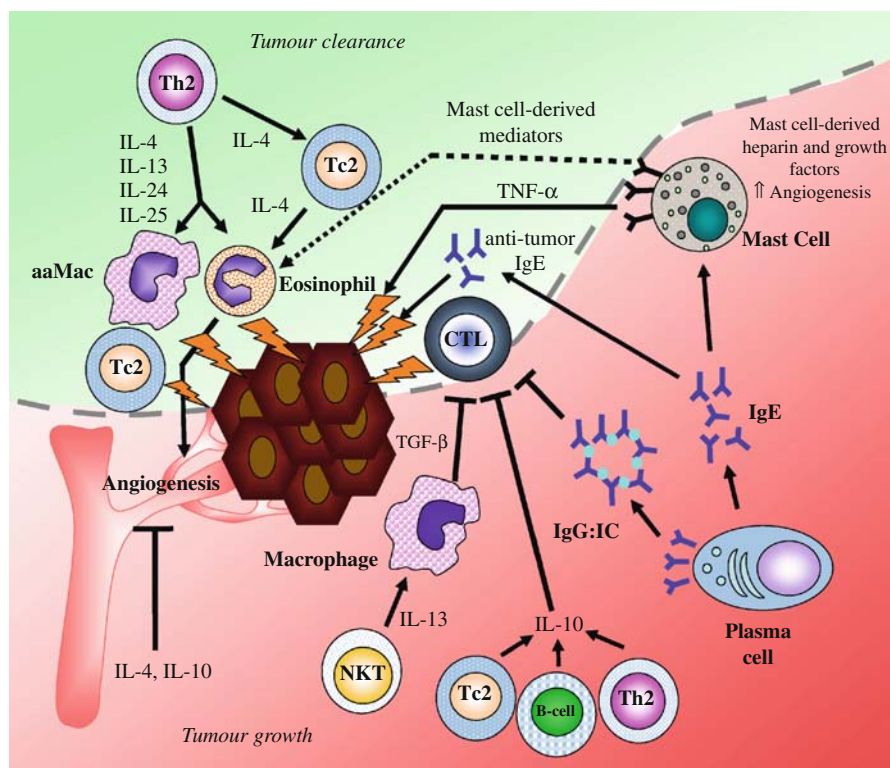


Fig. 11.1 The “yin and yang” of Th2-mediated inflammation within tumors. The model shows how different components of Th2-mediated immunity can either enhance tumor clearance (*light/green zone*) or promote tumor growth (*darker/red zone*). Secretion of IL-4, IL-13, IL-24 or IL-25 by CD4⁺ Th2 cells can 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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