

Chapter 7

The IgE Antibody and Its Use in Cancer Immunotherapy

Tracy R. Daniels, José A. Rodríguez, Elizabeth Ortiz-Sánchez,
Gustavo Helguera, and Manuel L. Penichet

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

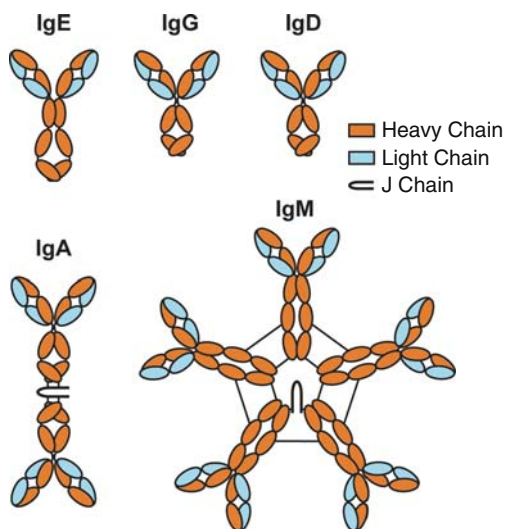
Immunoglobulins (Ig, also known as antibodies) are glycoproteins and are central effector molecules of the humoral immune response [1–3]. They are highly selective for a broad spectrum of antigens including pathogens as well as tumor-associated

M.L. Penichet (✉)

Division of Surgical Oncology, Department of Surgery and Department of Microbiology, Immunology, and Molecular Genetics, Molecular Biology Institute, Jonsson Comprehensive Cancer Center, David Geffen School of Medicine, University of California, Los Angeles (UCLA), Los Angeles, CA, USA
e-mail: penichet@mednet.ucla.edu

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;

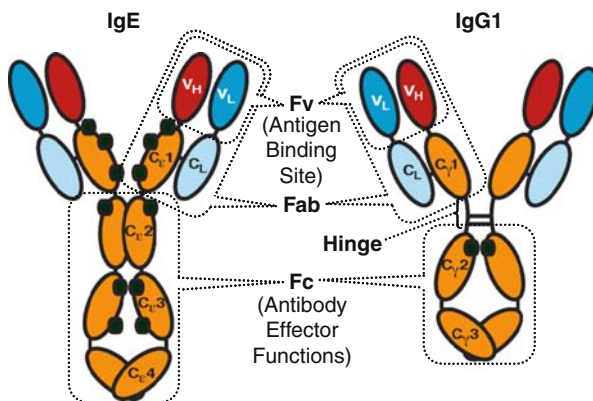


Fig. 7.2 Schematic diagram comparing the domain structures of human IgE and IgG1. Antibodies are composed of two pairs of identical heavy (H) and light (L) chain proteins linked by disulfide bonds forming H₂L₂ heterotetramers. The Fab region consists of one constant and one variable domain from each 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 FcεRI K_{off} rate is four orders of magnitude lower than that of IgG for FcγRIII (CD16) [15–17]. The region of the IgE associated with FcεRI binding resides within the Cε2 and Cε3 domains and involves two distinct sites [18]. The human IgE Cε3 domain is also responsible for binding to the low-affinity IgE receptor, FcεRII (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” FcεR 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 FcεRI [19, 21, 22]. Each Cε3 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 Cε3 domain facilitates the interaction to a large extent [23]. In addition to binding FcεRI 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 FcεRI 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

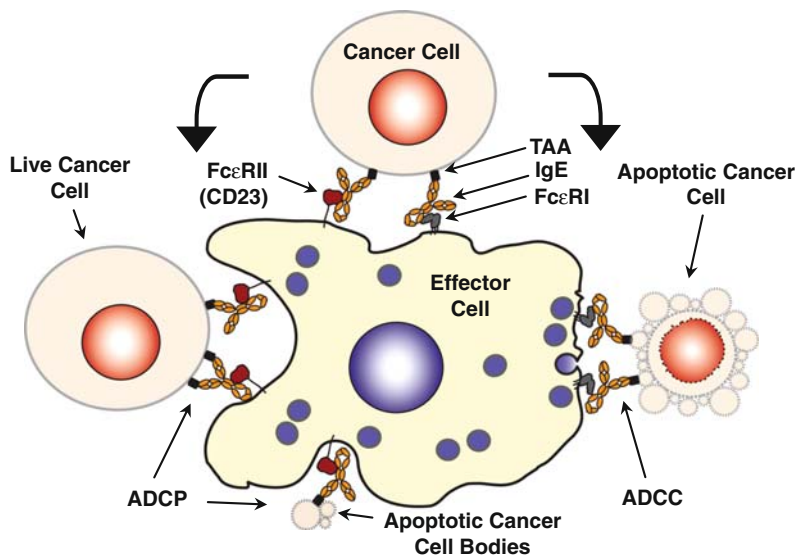


Fig. 7.3 Scheme of the possible interactions between immune effector cells and targeted tumor cells, mediated by an IgE specific for a tumor-associated antigen. The IgE specific for a TAA interacts with the FcεRI or the FcεRII expressed on the surface of the effector cell and may trigger cell killing 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 reagents” 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 reagents [52, 54]. Using a passive cutaneous anaphylaxis (PCA) test, the activity of these reagents (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

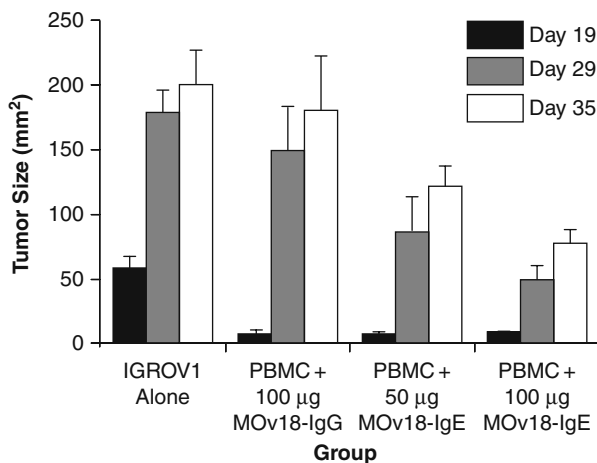


Fig. 7.4 MOv18-IgE inhibits the growth of IGROV1 human ovarian adenocarcinoma cells in vivo in the presence of human PBMC as effectors. A matched variable region set of mouse/human chimeric IgE and IgG1 antibodies specific for FBP was tested for their ability to inhibit the subcutaneous growth of cells 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 μ g MOv18-IgG1, 100 μ g MOv18-IgE or 50 μ g 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 μ g) 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 μ g) of IgE compared to IgG1 (100 μ g) showed 40% inhibition of tumor growth at day 35 (Fig. 7.4). Due to the inability of human IgE to bind murine Fc ϵ 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.

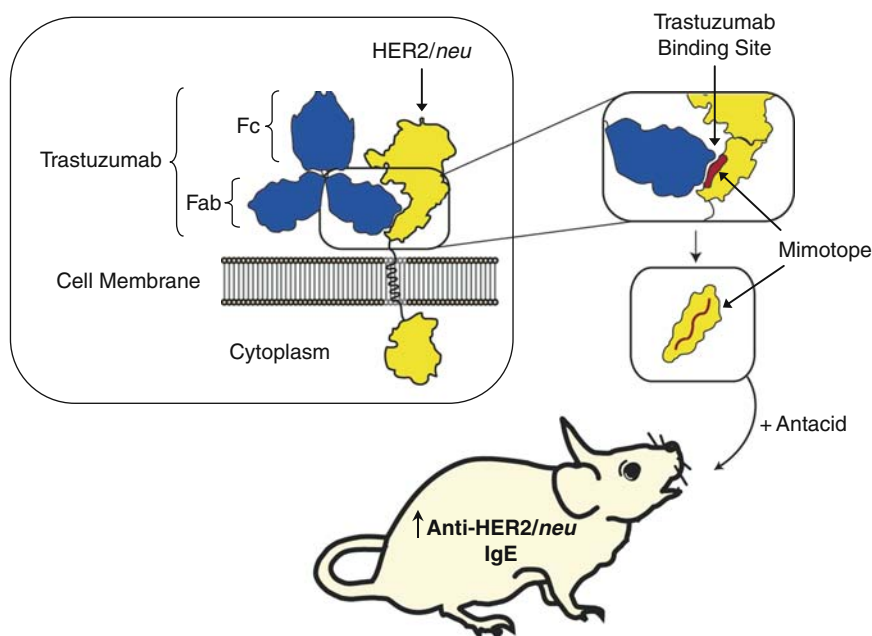


Fig. 7.5 Schematic diagram outlining the strategy for the generation of mimotope peptides and their administration during oral vaccination. HER2/*neu* is a transmembrane receptor consisting of three domains: extracellular, transmembrane and cytoplasmic. The extracellular domain of HER2/*neu* is recognized 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

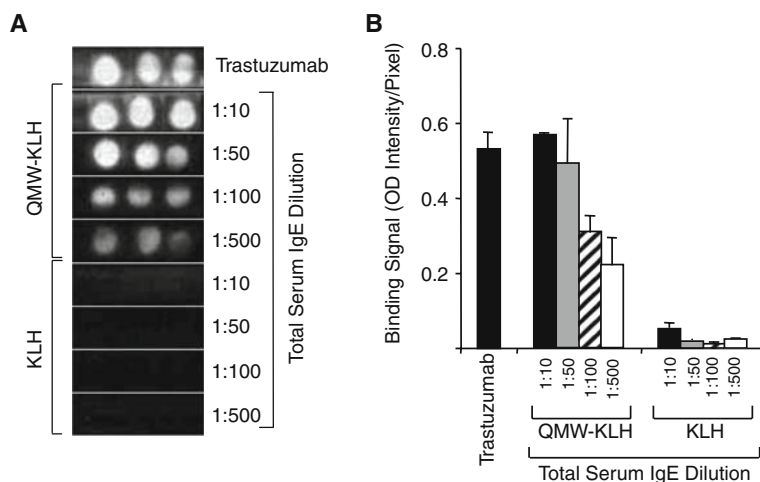


Fig. 7.6 Induction of endogenous antigen-specific IgE is achieved by oral vaccination using a mimotope peptide. BALB/c mice (seven per group) were immunized with the mimotope C-QMWAPQWGPDC (“QMw-KLH”) or the carrier molecule KLH by itself as described in Fig. 7.5. Each animal received 11.6 μ g of the 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.

Acknowledgments This work was supported by NIH/NCI grants R01CA107023, R01 supplements CA107023-02S1 and CA57152-13S1, NIH/NCI T32-CA009120, NIH Fogarty AITRP-AIDS Malignancies Program D43-TW000013-S1, the Susan G. Komen Breast Cancer Foundation Basic, Clinical and Translational Research Grant BCTR0706771, Cancer Research Coordinating Committee (CRCC), the Howard Hughes Medical Institute (HHMI) Gilliam Fellowship, and the Whitcome Fellowship of the Molecular Biology Interdepartmental PhD Program (MBIDP) at UCLA.

References

1. Janeway CA, Travers P, Walport M, and Shlomchik M (2005) The generation of lymphocyte antigen receptors. In: *Immunobiology: The Immune System in Health and Disease*. New York: Garland Science Publishing, pp. 123–154
2. Penichet ML and Morrison SL (2004) Design and engineering of human forms of monoclonal antibodies. *Drug Dev Res* 61:121–136
3. Arnold JN, Wormald MR, Sim RB, Rudd PM, and Dwek RA (2007) The impact of glycosylation on the biological function and structure of human immunoglobulins. *Annu Rev Immunol* 25:21–50
4. Stavnezer J, Guikema JE, and Schrader CE (2008) Mechanism and regulation of class switch recombination. *Annu Rev Immunol* 26:261–292
5. Helguera G, Daniels TR, Rodriguez JA, and Penichet ML (2010) Monoclonal antibodies, human engineered. In: *Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology*, M. Flickinger (ed.). New York: John Wiley & Sons, Inc. <http://mrw.interscience.wiley.com/emrw/978047005481/home/>
6. Adams GP and Weiner LM (2005) Monoclonal antibody therapy of cancer. *Nat Biotechnol* 23:1147–1157
7. Reichert JM, Rosensweig CJ, Faden LB, and Dewitz MC (2005) Monoclonal antibody successes in the clinic. *Nat Biotechnol* 23:1073–1078
8. Wan T, Beavil RL, Fabiane SM, Beavil AJ, Sohi MK, Keown M, Young RJ, Henry AJ, Owens RJ, Gould HJ, and Sutton BJ (2002) The crystal structure of IgE Fc reveals an asymmetrically bent conformation. *Nat Immunol* 3:681–686
9. Beavil AJ, Young RJ, Sutton BJ, and Perkins SJ (1995) Bent domain structure of recombinant human IgE-Fc in solution by X-ray and neutron scattering in conjunction with an automated curve fitting procedure. *Biochemistry* 34:14449–14461
10. Janeway CA, Travers P, Walport M, and Shlomchik M. (2005) *The humoral immune response*. In: *Immunobiology: The Immune System in Health and Disease*. New York: Garland Science Publishing, pp. 367–406
11. Gould HJ, Sutton BJ, Beavil AJ, Beavil RL, McCloskey N, Coker HA, Fear D, and Smurthwaite L (2003) The biology of IGE and the basis of allergic disease. *Annu Rev Immunol* 21:579–628
12. Ravetch JV and Kinet JP (1991) Fc receptors. *Annu Rev Immunol* 9:457–492
13. Davis KG, Glennie M, Harding SE, and Burton DR (1990) A model for the solution conformation of rat IgE. *Biochem Soc Trans* 18:935–936
14. Zheng Y, Shopes B, Holowka D, and Baird B (1991) Conformations of IgE bound to its receptor Fc epsilon RI and in solution. *Biochemistry* 30:9125–9132
15. Maenaka K, van der Merwe PA, Stuart DI, Jones EY, and Sondermann P (2001) The human low affinity Fc gamma receptors IIa, IIb, and III bind IgG with fast kinetics and distinct thermodynamic properties. *J Biol Chem* 276:44898–44904

16. Ishizaka T, Helm B, Hakimi J, Niebyl J, Ishizaka K, and Gould HJ (1986) Biological properties of a recombinant human immunoglobulin epsilon-chain fragment. *Proc Natl Acad Sci USA* 83:8323–8327
17. Keown MB, Ghirlando R, Mackay GA, Sutton BJ, and Gould HJ (1997) Basis of the 1:1 stoichiometry of the high affinity receptor Fc epsilon RI-IgE complex. *Eur Biophys J* 25: 471–476
18. Garman SC, Wurzburger BA, Tarchevskaya SS, Kinet JP, and Jardetzky TS (2000) Structure of the Fc fragment of human IgE bound to its high-affinity receptor Fc epsilonRI alpha. *Nature* 406:259–266
19. Gould HJ and Sutton BJ (2008) IgE in allergy and asthma today. *Nat Rev Immunol* 8:205–217
20. Conrad DH (1990) Fc epsilon RII/CD23: the low affinity receptor for IgE. *Annu Rev Immunol* 8:623–645
21. Hibbert RG, Teriete P, Grundy GJ, Beavil RL, Reljic R, Holers VM, Hannan JP, Sutton BJ, Gould HJ, and McDonnell JM (2005) The structure of human CD23 and its interactions with IgE and CD21. *J Exp Med* 202:751–760
22. McCloskey N, Hunt J, Beavil RL, Jutton MR, Grundy GJ, Girardi E, Fabiane SM, Fear DJ, Conrad DH, Sutton BJ, and Gould HJ (2007) Soluble CD23 monomers inhibit and oligomers stimulate IGE synthesis in human B cells. *J Biol Chem* 282:24083–24091
23. Sayers I, Housden JE, Spivey AC, and Helm BA (2004) The importance of Lys-352 of human immunoglobulin E in Fc epsilon RII/CD23 recognition. *J Biol Chem* 279:35320–35325
24. Jensen-Jarolim E, Achatz G, Turner MC, Karagiannis S, Legrand F, Capron M, Penichet ML, Rodriguez JA, Siccardi AG, Vangelista L, Riemer AB, and Gould HJ (2008) AllergoOncology: the role of IgE-mediated allergy in cancer. *Allergy* 63: 1255–1266
25. Fu SL, Pierre J, Smith-Norowitz TA, Hagler M, Bowne W, Pincus MR, Mueller CM, Zenilman ME, and Bluth MH (2008) Immunoglobulin E antibodies from pancreatic cancer patients mediate antibody-dependent cell-mediated cytotoxicity against pancreatic cancer cells. *Clin Exp Immunol* 153:401–409
26. Matta GM, Battaglio S, Dibello C, Napoli P, Baldi C, Ciccone G, Coscia M, Boccadoro M, and Massaia M (2007) Polyclonal immunoglobulin E levels are correlated with hemoglobin values and overall survival in patients with multiple myeloma. *Clin Cancer Res* 13: 5348–5354
27. Schulman ES (2001) Development of a monoclonal anti-immunoglobulin E antibody (omalizumab) for the treatment of allergic respiratory disorders. *Am J Respir Crit Care Med* 164:S6–11
28. Presta LG, Lahr SJ, Shields RL, Porter JP, Gorman CM, Fendly BM, and Jardieu PM (1993) Humanization of an antibody directed against IgE. *J Immunol* 151:2623–2632
29. Strunk RC and Bloomberg GR (2006) Omalizumab for asthma. *N Engl J Med* 354:2689–2695
30. Dodig S, Richter D, Cepelak I, and Benko B (2005) Anti-IgE therapy with omalizumab in asthma and allergic rhinitis. *Acta Pharm* 55:123–138
31. Manz RA, Hauser AE, Hiepe F, and Radbruch A (2005) Maintenance of serum antibody levels. *Annu Rev Immunol* 23:367–386
32. Preithner S, Elm S, Lippold S, Locher M, Wolf A, da Silva AJ, Baeuerle PA, and Prang NS (2006) High concentrations of therapeutic IgG1 antibodies are needed to compensate for inhibition of antibody-dependent cellular cytotoxicity by excess endogenous immunoglobulin G. *Mol Immunol* 43:1183–1193
33. Kinet JP (1999) The high-affinity IgE receptor (Fc epsilon RI): from physiology to pathology. *Annu Rev Immunol* 17:931–972
34. Sukumar S, Conrad DH, Szakal AK, and Tew JG (2006) Differential T cell-mediated regulation of CD23 (Fc epsilonRII) in B cells and follicular dendritic cells. *J Immunol* 176:4811–4817
35. Delespesse G, Sarfati M, Wu CY, Fournier S, and Letellier M (1992) The low-affinity receptor for IgE. *Immunol Rev* 125:77–97

36. Clynes RA, Towers TL, Presta LG, and Ravetch JV (2000) Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. *Nat Med* 6:443–446.
37. Nimmerjahn F and Ravetch JV (2007) Antibodies, Fc receptors and cancer. *Curr Opin Immunol* 19:239–245
38. Weng WK and Levy R (2001) Expression of complement inhibitors CD46, CD55, and CD59 on tumor cells does not predict clinical outcome after rituximab treatment in follicular non-Hodgkin lymphoma. *Blood* 98:1352–1357
39. Peipp M, Dechant M, and Valerius T (2008) Effector mechanisms of therapeutic antibodies against ErbB receptors. *Curr Opin Immunol* 20:436–443
40. Hudis CA (2007) Trastuzumab – mechanism of action and use in clinical practice. *N Engl J Med* 357:39–51
41. Wang SY, Racila E, Taylor RP, and Weiner GJ (2008) NK-cell activation and antibody-dependent cellular cytotoxicity induced by rituximab-coated target cells is inhibited by the C3b component of complement. *Blood* 111:1456–1463
42. Janeway CA, Travers P, Walport M, and Shlomchik M (2005) Allergy and hypersensitivity. In: *Immunobiology: The Immune System in Health and Disease*. New York: Garland Science Publishing, pp. 517–555
43. Bieber T (1997) Fc epsilon RI on human epidermal Langerhans cells: an old receptor with new structure and functions. *Int Arch Allergy Immunol* 113:30–34
44. Bieber T (1997) Fc epsilon RI-expressing antigen-presenting cells: new players in the atopic game. *Immunol Today* 18:311–313
45. Maurer D, Ebner C, Reininger B, Fiebiger E, Kraft D, Kinet JP, and Stingl G (1995) The high affinity IgE receptor (Fc epsilon RI) mediates IgE-dependent allergen presentation. *J Immunol* 154:6285–6290
46. Maurer D, Fiebiger S, Ebner C, Reininger B, Fischer GF, Wichlas S, Jouvin MH, Schmitt-Egenolf M, Kraft D, Kinet JP, and Stingl G (1996) Peripheral blood dendritic cells express Fc epsilon RI as a complex composed of Fc epsilon RI alpha- and Fc epsilon RI gamma-chains and can use this receptor for IgE-mediated allergen presentation. *J Immunol* 157: 607–616
47. Karagiannis SN, Bracher MG, Beavil RL, Beavil AJ, Hunt J, McCloskey N, Thompson RG, East N, Burke F, Sutton BJ, Dombrowicz D, Balkwill FR, and Gould HJ (2008) Role of IgE receptors in IgE antibody-dependent cytotoxicity and phagocytosis of ovarian tumor cells by human monocytic cells. *Cancer Immunol Immunother* 57:247–263
48. Karagiannis SN, Bracher MG, Hunt J, McCloskey N, Beavil RL, Beavil AJ, Fear DJ, Thompson RG, East N, Burke F, Moore RJ, Dombrowicz DD, Balkwill FR, and Gould HJ (2007) IgE-antibody-dependent immunotherapy of solid tumors: cytotoxic and phagocytic mechanisms of eradication of ovarian cancer cells. *J Immunol* 179:2832–2843
49. Karagiannis P, Singer J, Hunt J, Gan SK, Rudman SM, Mechtcheriakova D, Knittelfelder R, Daniels TR, Hobson PS, Beavil AJ, Spicer J, Nestle FO, Penichet ML, Gould HJ, Jensen-Jarolim E, and Karagiannis SN (2009) Characterisation of an engineered trastuzumab IgE antibody and effector cell mechanisms targeting HER2/*neu*-positive tumour cells. *Cancer Immunol Immunother* 58:915–930
50. Arase N, Arase H, Hirano S, Yokosuka T, Sakurai D, and Saito T (2003) IgE-mediated activation of NK cells through Fc gamma RIII. *J Immunol* 170:3054–3058
51. Coca AF and Grove EF (1925) Studies in hypersensitiveness: a study of the atopic reagins. *J Immunol* 10:445–464
52. Stanworth DR (1993) The discovery of IgE. *Allergy* 48:67–71
53. Bennich HH, Ishizaka K, Johansson SG, Rowe DS, Stanworth DR, and Terry WD (1968) Immunoglobulin E. A new class of human immunoglobulin. *Immunochemistry* 5:327–328
54. Bartholomaeus WN and Keast D (1972) Reaginic antibody to tumour and alloantigens in mice. *Nat New Biol* 239:206–207
55. Lynch NR and Salomon JC (1977) Passive local anaphylaxis: demonstration of antitumor activity and complementation of intratumor BCG. *J Natl Cancer Inst* 58:1093–1098

56. Kohler G and Milstein C (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 256:495–497
57. Morrison SL, Johnson MJ, Herzenberg LA, and Oi VT (1984) Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains. *Proc Natl Acad Sci USA* 81:6851–6855
58. Neuberger MS, Williams GT, Mitchell EB, Jouhal SS, Flanagan JG, and Rabbitts TH (1985) A hapten-specific chimaeric IgE antibody with human physiological effector function. *Nature* 314:268–270
59. Bruggemann M, Williams GT, Bindon CI, Clark MR, Walker MR, Jefferis R, Waldmann H, and Neuberger MS (1987) Comparison of the effector functions of human immunoglobulins using a matched set of chimeric antibodies. *J Exp Med* 166:1351–1361
60. Nagy E, Berezi I, and Schon AH (1991) Growth inhibition of murine mammary carcinoma by monoclonal IgE antibodies specific for the mammary tumor virus. *Cancer Immunol Immunother* 34:63–69
61. Isaacs JD, Clark MR, Greenwood J, and Waldmann H (1992) Therapy with monoclonal antibodies. An in vivo model for the assessment of therapeutic potential. *J Immunol* 148:3062–3071
62. Panaccio M, Gillespie MT, Walker ID, Kirszbaum L, Sharpe JA, Tobias GH, McKenzie IF, and Deacon NJ (1987) Molecular characterization of the murine cytotoxic T-cell membrane glycoprotein Ly-3 (CD8). *Proc Natl Acad Sci USA* 84:6874–6878
63. Kershaw MH, Darcy PK, Trapani JA, and Smyth MJ (1996) The use of chimeric human Fc(epsilon) receptor I to redirect cytotoxic T lymphocytes to tumors. *J Leukoc Biol* 60: 721–728
64. Fung-Leung WP, De Sousa-Hitzler J, Ishaque A, Zhou L, Pang J, Ngo K, Panakos JA, Chourmouzis E, Liu FT, and Lau CY (1996) Transgenic mice expressing the human high-affinity immunoglobulin (Ig) E receptor alpha chain respond to human IgE in mast cell degranulation and in allergic reactions. *J Exp Med* 183:49–56
65. Dombrowic, D, Brini AT, Flamand V, Hicks E, Snouwaert JN, Kinet JP, and Koller BH (1996) Anaphylaxis mediated through a humanized high affinity IgE receptor. *J Immunol* 157: 1645–1651
66. Teng MW, Kershaw MH, Jackson JT, Smyth MJ, and Darcy PK (2006) Adoptive transfer of chimeric FcepsilonRI gene-modified human T cells for cancer immunotherapy. *Hum Gene Ther* 17:1134–1143
67. Kershaw MH, Darcy PK, Trapani JA, MacGregor D, and Smyth MJ (1998) Tumor-specific IgE-mediated inhibition of human colorectal carcinoma xenograft growth. *Oncol Res* 10: 133–142
68. Mount PF, Sutton VR, Li W, Burgess J, McKenzie IF, Pietersz GA, and Trapani JA (1994) Chimeric (mouse/human) anti-colon cancer antibody c30.6 inhibits the growth of human colorectal cancer xenografts in scid/scid mice. *Cancer Res* 54:6160–6166
69. Gould HJ, Mackay GA, Karagiannis SN, O'Toole CM, Marsh PJ, Daniel BE, Coney LR, Zurawski VR Jr, Joseph M, Capron M, Gilbert M, Murphy GF, and Korngold R (1999) Comparison of IgE and IgG antibody-dependent cytotoxicity in vitro and in a SCID mouse xenograft model of ovarian carcinoma. *Eur J Immunol* 29:3527–3537
70. Karagiannis SN, Wang Q, East N, Burke F, Riffard S, Bracher MG, Thompson RG, Durham SR, Schwartz LB, Balkwill FR, and Gould HJ (2003) Activity of human monocytes in IgE antibody-dependent surveillance and killing of ovarian tumor cells. *Eur J Immunol* 33: 1030–1040
71. Weng WK and Levy R (2003) Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. *J Clin Oncol* 21:3940–3947
72. Slamon DJ, Clark, GM, Wong SG, Levin WJ, Ullrich A, and McGuire WL (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/*neu* oncogene. *Science* 235:177–182

73. Baselga J, Tripathy D, Mendelsohn J, Baughman S, Benz CC, Dantis L, Sklarin NT, Seidman AD, Hudis CA, Moore J, Rosen PP, Twaddell T, Henderson IC, and Norton L (1996) Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/*neu*-overexpressing metastatic breast cancer. *J Clin Oncol* 14:737–744
74. Baselga J, Tripathy D, Mendelsohn J, Baughman S, Benz CC, Dantis L, Sklarin NT, Seidman AD, Hudis CA, Moore J, Rosen PP, Twaddell T, Henderson IC, and Norton L (1999) Phase II study of weekly intravenous trastuzumab (Herceptin) in patients with HER2/*neu*-overexpressing metastatic breast cancer. *Semin Oncol* 26:78–83
75. Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, Slamon DJ, Murphy M, Novotny WF, Burchmore M, Shak S, Stewart SJ, and Press M (2002) Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. *J Clin Oncol* 20:719–726
76. Pegram MD, Lipton A, Hayes DF, Weber BL, Baselga JM, Tripathy D, Baly D, Baughman SA, Twaddell T, Glaspy JA, and Slamon DJ (1998) Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/*neu* monoclonal antibody plus cisplatin in patients with HER2/*neu*-overexpressing metastatic breast cancer refractory to chemotherapy treatment. *J Clin Oncol* 16:2659–2671
77. Pegram MD, Pienkowski T, Northfelt DW, Eiermann W, Patel R, Fumoleau P, Quan E, Crown J, Toppmeyer D, Smylie M, Riva A, Blitz S, Press MF, Reese D, Lindsay MA, and Slamon DJ (2004) Results of two open-label, multicenter phase II studies of docetaxel, platinum salts, and trastuzumab in HER2-positive advanced breast cancer. *J Natl Cancer Inst* 96:759–769
78. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, and Norton L (2001) Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 344:783–792
79. Baselga J, Carbonell X, Castaneda-Soto NJ, Clemens M, Green M, Harvey V, Morales S, Barton C, and Ghahramani P (2005) Phase II study of efficacy, safety, and pharmacokinetics of trastuzumab monotherapy administered on a 3-weekly schedule. *J Clin Oncol* 23:2162–2171
80. Schier R, Marks JD, Wolf EJ, Apell G, Wong C, McCartney JE, Bookman MA, Huston JS, Houston LL, Weiner LM, and Adams GP (1995) In vitro and in vivo characterization of a human anti-c-erbB-2 single-chain Fv isolated from a filamentous phage antibody library. *Immunotechnology* 1:73–81
81. Schier R, McCall A, Adams GP, Marshall KW, Merritt H, Yim M, Crawford RS, Weiner LM, Marks C, and Marks JD (1996) Isolation of picomolar affinity anti-c-erbB-2 single-chain Fv by molecular evolution of the complementarity determining regions in the center of the antibody binding site. *J Mol Biol* 263:551–567
82. Penichet ML. Novel antibodies of IgE class targeting HER2/*neu* and CD20 for the treatment of cancer. Proceedings of the 2nd International AllergoOncology Symposium (Vienna, Austria, April 11–12, 2008) Abstract No. S-10
83. Tang Y, Lou J, Alpaugh RK, Robinson MK, Marks JD, and Weiner LM (2007) Regulation of antibody-dependent cellular cytotoxicity by IgG intrinsic and apparent affinity for target antigen. *J Immunol* 179:2815–2823
84. Coiffier B (2007) Rituximab therapy in malignant lymphoma. *Oncogene* 26:3603–3613
85. Smith MR (2003) Rituximab (monoclonal anti-CD20 antibody): mechanisms of action and resistance. *Oncogene* 22:7359–7368
86. Helguera G, Rodriguez JA, Daniels TR, Ortiz-Sanchez E, Quintero R, Timmerman JM, Wu A, Martinez-Maza O, and Penichet ML. Anti-CD20 IgE: a novel antibody for the treatment of non-Hodgkin's lymphoma. Proceedings of the American Association for Cancer Research Special Conference “Tumor Immunology: An integrated perspective” (Miami, Florida, USA, December 2–5, 2008). Abstract No. B18/PR04
87. Riemer AB, Untersmayr E, Knittelfelder R, Duschl A, Pehamberger H, Zielinski CC, Scheiner O, and Jensen-Jarolim E (2007) Active induction of tumor-specific IgE antibodies by oral mimotope vaccination. *Cancer Res* 67:3406–3411

88. Riemer AB, Klinger M, Wagner S, Bernhaus A, Mazzucchelli L, Pehamberger H, Scheiner O, Zielinski CC, and Jensen-Jarolim E (2004) Generation of peptide mimics of the epitope recognized by trastuzumab on the oncogenic protein Her-2/*neu*. *J Immunol* 173:394–401
89. Scholl I, Untersmayr E, Bakos N, Roth-Walter F, Gleiss A, Boltz-Nitulescu G, Scheiner O, and Jensen-Jarolim E (2005) Antiulcer drugs promote oral sensitization and hypersensitivity to hazelnut allergens in BALB/c mice and humans. *Am J Clin Nutr* 81:154–160
90. Untersmayr E, Scholl I, Swoboda I, Beil WJ, Forster-Waldl E, Walter F, Riemer A, Kraml G, Kinaciyan T, Spitzauer S, Boltz-Nitulescu G, Scheiner O, and Jensen-Jarolim E (2003) Antacid medication inhibits digestion of dietary proteins and causes food allergy: a fish allergy model in BALB/c mice. *J Allergy Clin Immunol* 112:616–623