IgE Immunotherapy Against Cancer

Lai Sum Leoh, Tracy R. Daniels-Wells and Manuel L. Penichet

Abstract The success of antibody therapy in cancer is consistent with the ability of these molecules to activate immune responses against tumors. Experience in clinical applications, antibody design, and advancement in technology have enabled antibodies to be engineered with enhanced efficacy against cancer cells. This allows re-evaluation of current antibody approaches dominated by antibodies of the IgG class with a new light. Antibodies of the IgE class play a central role in allergic reactions and have many properties that may be advantageous for cancer therapy. IgE-based active and passive immunotherapeutic approaches have been shown to be effective in both in vitro and in vivo models of cancer, suggesting the potential use of these approaches in humans. Further studies on the anticancer efficacy and safety profile of these IgE-based approaches are warranted in preparation for translation toward clinical application.

L.S. Leoh · T.R. Daniels-Wells (✉) · M.L. Penichet (✉)

Division of Surgical Oncology, Department of Surgery, David Geffen School of Medicine, University of California, Los Angeles, 10833 Le Conte Avenue, CHS 54-140, Box 951782, Los Angeles, CA 90095-1782, USA
e-mail: tdaniels@mednet.ucla.edu

M.L. Penichet
e-mail: penichet@mednet.ucla.edu

M.L. Penichet
Department of Microbiology, Immunology, and Molecular Genetics, University of California, 609 Charles E. Young Dr. East, 1602 Molecular Science Building, Los Angeles, CA 90095, USA

M.L. Penichet
The Jonsson Comprehensive Cancer Center, University of California, 10833 Le Conte Ave, 8-684 Factor Building, Box 951781, Los Angeles, CA 90095, USA

M.L. Penichet
The Molecular Biology Institute, University of California, 611 Charles E. Young Dr., Los Angeles, CA 90095, USA

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1 Immunoglobulins and Their Relevance in Cancer

1.1 Immunoglobulins

Immunoglobulins, also known as antibodies, were the first characterized molecules involved in specific immune recognition. Antitoxins against tetanus and diphtheria toxins were discovered in the 1890s by Shibasaburo Kitasato and Emil von Behring (Behring and Kitasato 1890). Collaboration between Behring and Paul Ehrlich enabled the production of a standardized, efficient serum therapy for the treatment of diphtheria (Winau and Winau 2002). Subsequently, Paul Ehrlich formulated the concepts of active and passive immunization (Ehrlich 1891) and developed the side chain theory (Ehrlich 1901b), describing receptors that bind distinct toxins on the cell surface with lock-and-key specificity (Winau et al. 2004). These receptors, representing antitoxins or antibodies, are released into the blood (Ehrlich 1901a). Since then, antibodies, with their unique specificity to recognize distinct target molecules (known as antigens), have been utilized to attack tumor cells expressing certain antigens (Sliwkowski and Mellman 2013; Weiner et al. 2010). Introduction of the hybridoma technology (Kohler and Milstein 1975) enabled mass production of mouse monoclonal antibodies with a single specificity. This technology, along with advances in bioengineering, has facilitated the development of chimeric, humanized, and fully human monoclonal antibodies with decreased immunogenicity and enhanced anticancer efficacy that can be used as effective anticancer therapeutics in humans.

1.2 The Structure of Immunoglobulins

Antibodies are composed of 2 identical heavy (H) and 2 identical light (L) chains, exhibiting a H_2L_2 , heterotetramer configuration (Janeway et al. 2005a). Each chain has both constant and variable regions. Heavy chains can pair with either kappa (κ) or lambda (λ) light chains. There are 5 different classes of antibodies in humans distinguished by their heavy chain structure denoted by the Greek letters: α (IgA), δ (IgD), ϵ (IgE), γ (IgG), and μ (IgM). IgD, IgG, and IgE are monomeric antibodies. There are 4 subclasses of IgG (IgG1, IgG2, IgG3, and IgG4), while IgA has 2 subclasses (IgA1 and IgA2). IgG is the main antibody class found in blood and extracellular fluid and protects the body from infection (Janeway et al. 2005a). IgE is associated with type I hypersensitivity (anaphylactic/allergic) reactions. IgM is the first responder to an antigenic challenge, such as an infection, and exists as a pentamer or hexamer. IgA is secreted through body fluids, while IgD (or an IgM monomer) forms the B-cell receptor on the surface of the B cell. The approximate molecular weights of the different classes are as follows: 184 kDa for IgD; 188 kDa for IgE; 146 kDa for IgG1, IgG2, and IgG4; 165 kDa for IgG3 due to an extended hinge region; 160 kDa for both subclasses of monomeric IgA in serum; 390 kDa for secretory dimeric IgA; 970 kDa for pentameric IgM; and 1,140 kDa for hexameric IgM (Janeway et al. 2005a; Murphy 2012).

1.3 Antibodies for Cancer Immunotherapy

As of 2013, 15 antibodies have been approved by the United States Food and Drug Administration (FDA) for the treatment of cancer, with many more undergoing evaluation in clinical trials (Lewin and Thomas 2013; Sliwkowski and Mellman 2013; Cameron and McCormack 2014). Five antibodies target the B-cell marker CD20, including rituximab (Rituxan[®], mouse/human chimeric IgG1), the first monoclonal antibody approved for the treatment of cancer (indolent lymphoma) in 1997 (Leget and Czuczman 1998), and a new antibody, obinutuzumab (Gazyva[™], humanized IgG1) glycoengineered for higher binding affinity to the Fc γ RIIIa (Cameron and McCormack 2014). Trastuzumab (Herceptin[®]), a humanized IgG1 specific for HER2/*neu*, a member of the epidermal growth factor receptor (EGFR) family, was approved for the treatment of HER2/*neu*-positive metastatic breast cancer as a single agent in 1998 (Vogel et al. 2001). Since then, trastuzumab in combination with conventional chemotherapy has also been approved for the treatment of HER2/*neu*-positive breast cancer (Slamon et al. 2001). Cetuximab (Erbix[®]), a chimeric mouse/human IgG1 targeting EGFR, combined with chemotherapy was approved for the treatment of wild-type KRAS (Kirsten rat sarcoma viral oncogene homolog) and EGFR-expressing metastatic colorectal cancer (Sliwkowski and Mellman 2013). Multiple mechanisms of action have been described for antibodies targeting tumors including the direct inhibition of target

function, complement-mediated cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), and antibody-dependent cell-mediated phagocytosis (ADCP). However, Fc-mediated functions, in particular ADCC, are believed to be a major component of their antitumor activity (Campoli et al. 2010; Kellner et al. 2014; Sliwkowski and Mellman 2013; Weiner et al. 2012).

The above-mentioned FDA-approved antibodies targeting cancer cells and other antibodies in development are of the IgG class. IgA molecules have also been evaluated as anticancer agents. An anti-EGFR IgA2 containing the variable regions of cetuximab significantly decreased the number of metastasis in an established immunocompetent lung metastasis model using mouse melanoma cells (B16F10) expressing human EGFR in C57BL/6 human Fc α RI transgenic mice (Boross et al. 2013). The effect of this IgA2 lasted a week longer (up to 30 days) than its IgG counterpart cetuximab. IgE is another antibody class that is currently being explored as a potential cancer therapeutic. Studies using IgE in the context of cancer belong to the rapidly growing field of AllergoOncology, which aims to reveal the function of IgE-mediated immune responses against cancer cells in order to enhance the understanding of its biology and to develop novel IgE-based treatment options against malignant diseases (Penichet and Jensen-Jarolim 2010).

1.4 The IgE Antibody and Its Receptors

IgE antibodies have a different structure compared to IgG1 antibodies (Fig. 1). The heavy chain of IgG has 3 constant domains and 1 variable region, while the IgE heavy chain contains an extra constant domain, having a total of 4 constant domains and 1 variable domain. They have similar light chains with 1 variable and 1 constant domain each. The variable regions bind specific antigens, while the constant regions are responsible for effector functions. IgE has 6 *N*-linked glycosylation sites compared to 1 for IgG. Crystal structure and solution scattering data have revealed a bent structure of the IgE Fc region, which undergoes substantial conformational changes upon binding to Fc epsilon receptor I (Fc ϵ RI) (Beavil et al. 1995; Wan et al. 2002). There are 2 receptors for IgE: the high-affinity receptor, Fc ϵ RI, and the low-affinity receptor, Fc ϵ R2, also known as CD23.

Fc ϵ RI expression is abundant on human mast cells (MC) and basophils and is expressed at lower levels on dendritic cells (DC), Langerhans cells (LC), monocytes/macrophages, eosinophils, and platelets (Kinet 1999). It is expressed as an $\alpha\beta\gamma_2$ heterotetramer in MC and basophils and as an $\alpha\gamma_2$ heterotrimer in monocytes and eosinophils (Ravetch and Kinet 1991). The extracellular α subunit is sufficient for IgE binding (Hakimi et al. 1990), while the β and γ subunits are involved in cell signaling (Gould et al. 2003). In the mouse, Fc ϵ RI is only expressed in MC and basophils (Kinet 1999) and not in eosinophils (de Andres et al. 1997) and macrophages. However, Fc ϵ RI is present on rat eosinophils, monocytes/macrophages, and platelets (Capron and Dessaint 1985; Dombrowicz et al. 2000; Gould and Sutton 2008).

Fig. 1 Schematic diagram comparing the structures of human IgE and IgG1. Antibodies are composed of 2 pairs of identical heavy (H) and light (L) chain proteins linked by disulfide bonds forming H_2L_2 heterotetramers. The Fab region consists of a constant and a 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 regions include the Fc portion of the antibody and are responsible for the effector functions. *Black circles* denote N-linked glycosylation sites. The hinge region, which provides flexibility, joins the C γ 1 and C γ 2 domains in IgG1. C ϵ 2 replaces the hinge region in IgE. Reprinted from Fig. 7.2 of Daniels et al. (2010) with kind permission from Springer Science and Business Media

IgE also binds to Fc ϵ RII (CD23), a type II integral membrane protein with a calcium-dependent lectin domain ‘head.’ CD23 exists in the membrane as an equilibrium mixture of a monomer and a trimer that is held together by a coiled-coil stalk (Gould et al. 2003). There are 2 isoforms of human CD23. The CD23a isoform is expressed on antigen-activated B cells prior to differentiation into antibody-secreting plasma cells (Gould et al. 2003). CD23a is involved in IgE antibody-dependent antigen endocytosis, processing, and presentation. CD23b is expressed on monocytes and eosinophils following interleukin-4 (IL-4) stimulation (Yokota et al. 1992). Murine and human CD23 show similar structures; however, their pattern of expression is different. Human CD23 is expressed on monocytes/macrophages, lymphocytes, eosinophils, follicular DC, LC, and platelets (Delespesse et al. 1991), while murine CD23 is present on B cells and a subset of CD8 T cells (Mathur et al. 1988).

IgE antibodies play a central role in triggering the allergic immune response (type I hypersensitivity) when exposed to allergens, such as pollen, which leads to MC and/or basophil degranulation and acute inflammation at the site of allergen challenge (Fig. 2a) (Gould and Sutton 2008; Gould et al. 2003). Allergen sensitization results in the production of allergen-specific IgE, which then coat the surface of Fc ϵ RI-bearing cells. Re-exposure to the same allergen triggers MC and basophil

Fig. 2 Scheme of possible IgE-mediated interactions between effector cells and targeted tumor cells. **a** IgE can trigger degranulation upon cross-linking of FcεRI on the surface of effector cells, such as MC, in the presence of tumor cells expressing high density of the targeted antigen. This degranulation is the type I hypersensitivity (allergic) reaction also triggered in the presence of allergens, which in the case of targeted cancer antigens may result in cytotoxicity and cancer cell death. **b** The Fc region of the IgE specific for a tumor antigen interacts with the FcεRI or FcεRII/CD23 expressed on the surface of effector cells such as monocytes/macrophages and may trigger cell killing through ADCC or ADCP. Antibodies bound to apoptotic cells or apoptotic bodies are also expected to trigger phagocytosis. Adapted from Fig. 7.3 of Daniels et al. (2010) with kind permission from Springer Science and Business Media

degranulation due to receptor cross-linking, resulting in recruitment of inflammatory cells. The early phase reaction of the type I hypersensitivity response occurs within minutes of exposure to an allergen, releasing preformed mediators including histamine, heparin, lipid mediators, proteases, chemokines, and cytokines from cytoplasmic granules (Galli and Tsai 2010; Karagiannis et al. 2012). Since engagement of multiple FcεRIs is necessary for cross-linking (Kanner and Metzger 1983; Scholl et al. 2005a; Segal et al. 1977), degranulation can only be triggered by multi-epitopic antigens. The late phase reaction occurs after early phase symptoms have diminished and can last up to several weeks. Recruited inflammatory cells include neutrophils, eosinophils, basophils, monocytes/macrophages, and T cells (Ying et al. 1999). Together, these factors lead to tissue damage and an acute inflammatory response. Additionally, IgE-dependent antigen presentation by antigen-presenting cells (APC) such as DC, LC, and macrophages may lead to a secondary immune response. Upregulation of FcεRI by IgE (Karagiannis et al. 2007; MacGlashan et al. 1999) and CD23 by Interleukin-4 (IL-4) (Karagiannis et al. 2007; Spiegelberg 1990) on effector cells may also enhance these pathways. IgE has also been thought to play a role in parasitic infections (Capron et al. 1999; Capron and Capron 1994; Dunne et al. 1992; Gould and Sutton 2008; Obata-Ninomiya et al. 2013); however, this role has been questioned (Cooper et al. 2008; Watanabe et al. 2005).

1.5 IgE and Cancer

1.5.1 Clinical and Epidemiological Observations

There is evidence suggesting that IgE may play a role in antitumor immunity. An immunohistochemistry study on the distribution of the immunoglobulin classes in head and neck cancer revealed the IgE antibody to be the most abundant class in the tumor tissues (Neuchrist et al. 1994). Significantly elevated levels of tumor-specific serum IgE have been observed in patients with pancreatic cancer compared to healthy controls (Fu et al. 2008). Importantly, these IgE antibodies induced ADCC against pancreatic cancer cells (Fu et al. 2008). In multiple myeloma patients, higher levels of polyclonal IgE in non-allergic individuals have been associated with lower disease incidence and longer survival (Matta et al. 2007). Additionally, the presence of IgE effector eosinophils in the blood or peritumoral infiltrates of both hematological and solid malignancies has been linked to favorable prognosis, particularly in solid tumors (Gatault et al. 2012; Munitz and Levi-Schaffer 2004). The frequent state of eosinophil degranulation in close proximity to the tumor (Caruso et al. 2011) suggests their cytotoxic potential in eliminating cancer cells. Furthermore, epidemiological studies have found inverse correlations between the incidence of allergies and the risk of certain malignancies such as glioma, non-Hodgkin lymphoma (NHL), and childhood leukemia, especially acute lymphoblastic leukemia (Josephs et al. 2013; Martínez-Maza et al. 2010; Turner et al. 2006). Taken together, the above studies suggest a potential natural role of IgE in cancer immune surveillance, at least in certain malignancies.

1.5.2 Advantages of IgE Compared to IgG

Affinity for FcεRs

IgE antibodies have several properties that make them attractive as cancer therapeutics. One of these properties is the high affinity of IgE for its FcεRs. The binding affinity of human IgE for FcεRI ($K_a = 10^{10} \text{ M}^{-1}$) is two orders of magnitude higher than that of IgG for its high affinity receptor FcγRI ($K_a = 10^8 \text{ M}^{-1}$) (Daniels et al. 2010; Gould et al. 2003; Janeway et al. 2005d). This high affinity reflects the slow dissociation rate with a half-life of about 20 h for IgE on the receptor (McDonnell et al. 2001), with residence life on MC in tissues extended up to months (Achatz et al. 2010) as a result of restricted diffusion and rebinding to cell receptors (Gould et al. 2003). The affinity of human IgE for the CD23 monomer is around 10^6 – 10^7 M^{-1} , while the formation of the CD23 trimer leads to a 10-fold higher affinity for human IgE with a K_a of about 10^8 – 10^9 M^{-1} , which is equivalent to the affinity of IgG1 for FcγRI (Conrad 1990; Gould and Sutton 2008; Hibbert et al. 2005; Kilmon et al. 2001; McCloskey et al. 2007; Ravetch and Kinet 1991).

Human Effector Cells

The effector cells involved in the allergic reaction are unique and thus, are a potential advantage of IgE-based cancer therapies. Several mechanisms may be involved in the potential anticancer activity of tumor-targeted IgE (Fig. 2). IgE on the surface of these effector cells would result in cells better suited to target cancer cells. The use of tumor-specific IgE could arm effector cells to recognize tumor antigens, enhancing local tumor cell killing and inducing a secondary anti-tumor response, possibly inducing long-term antitumor immunity.

MC are tissue-based immune cells of hematopoietic origin located primarily in association with blood vessels and at epithelial surfaces (Stone et al. 2010). MC constitutively express FcεRI on their surface and are central effector cells in IgE-mediated allergic reactions. IgE-mediated antigen activation of MC triggers the type I hypersensitivity reaction releasing inflammatory mediators such as histamine, heparin, proteases, chemokines, cytokines, and lipid mediators (Galli and Tsai 2010). MC can potentially mediate pro-inflammatory or immunosuppressive functions (Galli et al. 2008). It has been observed that the microanatomical location of MC within the tumor microenvironment is important and can lead to different outcomes. A high density of MC is associated with unfavorable prognosis in malignancies such as pancreatic and colorectal cancer, Hodgkin lymphoma (HL), and aggressive skin cancer such as malignant melanoma (Dalton and Noelle 2012). However, in other human cancers such as breast and non-small-cell lung cancer, high MC density in tumors is associated with favorable prognosis (Dalton and Noelle 2012). Peritumoral MC in prostate cancer contributes to angiogenesis and tumor invasion into surrounding tissues and is significantly associated with unfavorable prognosis and poor survival (Johansson et al. 2010). However, intratumoral MC are associated with favorable prognosis in prostate cancer (Dalton and Noelle 2012). In fact, the cytotoxic effect of MC in breast cancer was demonstrated by histological images of degranulating MC surrounding dying tumor cells (della Rovere et al. 2007). The release of cytotoxic compounds from MC including tumor necrosis factor- α (TNF- α) leads to inflammation, cytotoxic T lymphocyte (CTL) responses, and tumor destruction (Wasiuk et al. 2010). Importantly, MC degranulation may also cause inhibition of the function of regulatory T cells (Tregs), which may enhance the immune response (de Vries et al. 2009). Therefore, administration of tumor-specific IgE is expected to enhance the protective effect of MC in tumor types that show protection, as well as to 're-educate' MC to target tumor antigens in the other tumor types where MC are not protective, triggering an 'explosive' degranulation leading to tumor destruction.

Basophils develop from CD34⁺ progenitors in the bone marrow, where they differentiate and mature before relocating to the circulation (Stone et al. 2010). They constitute less than 1 % of granulocytes in healthy humans and are involved in allergic/anaphylactic (type I hypersensitivity) reactions. Similar to MC, they express FcεRI, secrete T_H2 (T helper 2) cytokines, and release histamine, lipid mediators, chemokines, and cytokines after activation (Stone et al. 2010). Taken together, the above properties suggest that the use of tumor-specific IgE could

potentially be manipulated to direct basophil degranulation at localized tumor sites resulting in anti-tumor activity.

Eosinophils are granulocytes that originate in bone marrow and are named for their granules colored by the acidic stain eosin (Janeway et al. 2005c). Most eosinophils reside in mucosal tissues and only very small numbers of these cells are normally present in the circulation, implying a likely role in the defense against invading organisms. Blood and tissue eosinophilia are hallmarks of helminth infection, allergy, asthma, eosinophilic gastrointestinal disorders, and other rare disorders (Stone et al. 2010). Upon IgE activation, eosinophils can release cytotoxic mediators such as eosinophil cationic protein (ECP), major basic protein (MBP), eosinophil peroxidase (EPO), eosinophil-derived neurotoxin (EDN), and free radicals. Activation also induces the synthesis of a variety of chemical mediators such as prostaglandins, leukotrienes, cytokines, and chemokines, which amplify the inflammatory response by activating epithelial cells and recruiting and activating more eosinophils and other leukocytes (Janeway et al. 2005c). Eosinophils have been observed to infiltrate malignant tissues, a phenomenon known as tumor-associated tissue eosinophilia (TATE), which has been associated with significantly better prognosis in esophageal squamous cell carcinoma as well as colorectal, oral, and gastric cancer, (Cuschieri et al. 2002; Dorta et al. 2002; Fernandez-Acenero et al. 2000; Ishibashi et al. 2006). However, it has been associated with poor prognosis in HL (von Wasielewski et al. 2000). Recent reports suggest that eosinophils are involved in tumoricidal activity. In fact, TNF- α , together with granzyme A, was shown to direct tumoricidal activity of eosinophils against colon carcinoma cells (Legrand et al. 2010). Clearance of melanoma metastases by CD4 T_H2 cells was associated with a large influx of eosinophils into the tumor, as detected by immunohistochemistry staining of degranulating eosinophils and its cytotoxic protein MBP in lung metastatic sections (Mattes et al. 2003). Eosinophil infiltration of tumors has been reported to be an early and persistent inflammatory host response, as demonstrated in C57/BL6 J mice challenged subcutaneously (s.c.) with B16-F10 mouse melanoma cells (Cormier et al. 2006). In addition, the use of IL-5 transgenic mice, containing elevated levels of circulating eosinophils, demonstrated significant reduction in tumor establishment and growth, which directly correlated with the recruitment of eosinophils and other myeloid cells to the tumor and surrounding connective tissue (Simson et al. 2007). The above observations indicate that eosinophils may have a protective role in the context of tumor eradication. Thus, human eosinophils, which express both Fc ϵ Rs, should be able to interact with tumor-targeted IgE to enhance these anti-tumor effects.

Monocytes develop from dividing monoblasts in the bone marrow and are released into the bloodstream as nondividing cells (Lee et al. 2013). Differentiation of monocytes in tissues gives rise to macrophages, phagocytes that are present essentially in all tissues and function in both innate and adaptive immunity (Cook and Hagemann 2013; Ovchinnikov 2008). Classical M1 macrophages are tumoricidal and highly microbicidal, and are often present after induction by interferon- γ (IFN- γ) in the tissue during infection or acute inflammation (Cook and Hagemann 2013). In contrast, alternatively activated M2 macrophages support T_H2-associated

effector functions such as allergy in response to IL-4 or IL-13 and are involved with tissue remodeling and immunoregulation (Martinez and Gordon 2014). Tumor-associated macrophages (TAM) resemble M2 macrophages and have been implicated in fostering tumor progression, metastasis, angiogenesis, immune suppression of T-cell responses, and resistance to cancer treatment. The presence of TAM has been associated with decreased survival in breast, endometrial, prostate, ovary, gastric, head and neck, and oral carcinomas (Cook and Hagemann 2013; Zhang et al. 2012). Interestingly, improved overall survival was detected with TAM infiltration in colorectal cancer patients (Tan et al. 2005; Zhang et al. 2012). Emerging therapeutic strategies include the repolarization of TAM as a way of unlocking their anti-tumor potential. The effect of immunotherapy targeting CD40, a co-stimulatory molecule found on most APC, was examined to induce recognition and cytotoxicity of TAM by other cells of the immune system as an alternative to direct targeting (Beatty et al. 2011). An agonist CD40 antibody combined with gemcitabine chemotherapy activated macrophages that infiltrated the tumors and became tumoricidal and facilitated the depletion of tumor stroma in a syngeneic pancreatic ductal adenocarcinoma mouse model. Macrophages in the tumors and spleens of these animals showed upregulation of MHC class II molecules, co-stimulatory factors including CD86, and cytokines such as IL-12, IFN- γ , and TNF- α . Together, these factors promote the 'M1'-like macrophage phenotype, improving the anti-tumoral adaptive immune response. In a similar way, since monocytes/macrophages express both Fc ϵ Rs, tumor-specific IgE could be explored to 're-educate' TAM, leading to enhanced antigen presentation and targeting of tumor cells through ADCC and ADCP.

DC are unique APC because they are capable of stimulating naïve CD8 T lymphocytes via presentation of exogenous antigens in complex with MHC class I molecules, a process referred to as antigen cross-presentation (Janeway et al. 2005b; Spel et al. 2013). DC progenitors in the bone marrow give rise to circulating premature DC, which enter tissues such as the skin or mucosa as immature DC with high phagocytic capacity (Banchereau et al. 2000). After antigen capture, DC are activated by inflammatory cytokines. They then migrate to local lymphoid tissue and mature into highly specialized APC that can prime naïve T cells (Novak et al. 2010), expressing co-stimulatory molecules and inducing immune responses. DC, also present antigen in complex with MHC class II peptides to CD4 T-helper cells (Lemos et al. 2003), which secrete cytokines to recruit immune cells including antigen-specific CD8 CTL, as well as macrophages, eosinophils and NK cells (Banchereau et al. 2000), stimulating both primary and secondary T-cell responses (Maurer et al. 1996). The activated T cells help DC in terminal maturation, allowing lymphocyte expansion and differentiation. Importantly, effector cells are educated by DC to home to the site of tissue injury (Banchereau et al. 2000). Fc ϵ RI is expressed on DC (Kinet 1999), LC (Bieber et al. 1992; Osterhoff et al. 1994; Rieger et al. 1992), which are considered to be specialized DC present in the skin, and monocyte-derived DC (Novak et al. 2002). DC also express CD23 (Delespesse et al. 1991). The antigen uptake and presentation efficiency of DC are increased several fold in the presence of specific IgE (Daniels et al. 2010), leading to efficient

activation of allergen-specific T cells. This suggests that tumor-targeted IgE can be employed to induce IgE-mediated tumor antigen presentation by DC, stimulating CD8 T cells, eventually resulting in lysis of tumor cells via CTL, and the establishment of long-term adaptive immune responses against the tumor.

Additional Advantages of IgE

Another property of IgE that could be advantageous for cancer therapy is its low endogenous blood levels, which provide less competition for receptor occupancy. IgE in circulation comprises only 0.02 % of total circulating immunoglobulin, compared to IgG at 85 % (Manz et al. 2005). In fact, high levels of endogenous IgG compete for host FcγR-expressing cells resulting in the loss of the therapeutic IgG from effector cells, thus high doses are required to overcome this limitation (Preithner et al. 2006). Importantly, IgE bound to effector cells has a long half-life from weeks to months (Achatz et al. 2010) compared to 2–3 days for IgG in tissues (Karagiannis et al. 2012), resulting in strong local retention of IgE by FcεRI-expressing resident immune effector cells even in the absence of antigens. The stable interaction of IgE and its receptors on effector cells enables efficient travel to the site of tumor, suggesting that tumor-specific IgE could be used to arm the immune system to target tumor cells. The local retention of IgE in tissues along with lower endogenous IgE levels in the blood may be translated to lower therapeutic doses and/or reduced frequency of administration compared to IgG therapy.

In contrast to IgG1, IgE does not elicit CDC (Janeway et al. 2005d). However, for many IgG-based immunotherapeutics, such as rituximab (Weng and Levy 2001) and trastuzumab (Hudis 2007; Peipp et al. 2008), ADCC is a major mechanism of action responsible for the antitumor activity of the antibody (Campoli et al. 2010; Weiner et al. 2012). CDC can be a hindrance to therapy as it has been shown that ADCC can be reduced by CDC interference (Wang et al. 2008, 2009). Thus, the fact that IgE does not mediate CDC should not necessarily dampen its potential effects as an anticancer agent. IgE can also mediate ADCC via activation of a variety of effector cells such as MC through the release of signaling molecules, enzymes, and cytokines as depicted in Fig. 2b. In addition, IgE mediates ADCP via activation of effector cells such as monocytes/macrophages normally abundant in tumors (Fig. 2b). Furthermore, there is no known inhibitory receptor for IgE; thus, its effector functions can be fully executed. IgG, on the other hand, can be bound by inhibitory FcγRIIb (CD32b), potentially decreasing ADCC/ADCP and antibody-mediated antigen presentation for IgG molecules (Clynes et al. 2000; Nimmerjahn and Ravetch 2007). These properties, together with all of the relevant characteristics of IgE mentioned above, make IgE an attractive strategy to target a wide range of malignancies.

2 IgE-based Immunotherapy

Below are several examples of IgE-based cancer therapies. The different strategies that have been evaluated *in vivo* are summarized in Table 1.

Table 1 Examples of IgE antibodies and mouse models used to evaluate their anti-tumor efficacy

IgE species	IgE specificity	Route of administration	Targeted cancer cells (route of cell inoculation)	Mouse model	References
Mouse	gp36 of MMTV	i.p.	H2712 mouse mammary carcinoma (s.c. and i.p.)	C3H/HeJ	Nagy et al. (1991)
Rat/human chimeric	Mouse Ly-2	s.c.	E3 mouse thymoma (s.c.)	C57BL/6	Kershaw et al. (1996)
Mouse	DNP	i.p.	MC38 mouse colon carcinoma cells expressing human CEA (s.c.) ^a	C57BL/6	Reali et al. (2001)
Mouse	DNP	s.c.	TS/A-LACK mouse mammary carcinoma cells coated with DNP (s.c.)	BALB/c	Nigro et al. (2009)
Mouse and mouse/human chimeric	Colorectal cancer antigen	i.v.	Human COLO 205 (s.c.)	SCID	Kershaw et al. (1998)
Rat/human chimeric	Mouse Ly-2	i.p.	E3 mouse thymoma (i.p.)	NOD–SCID	Teng et al. (2006)
Mouse/human chimeric	FBP	i.v.	IGROV-1 human ovarian carcinoma cells (s.c.)	C.B-17 scid/scid	Gould et al. (1999)
		i.p.	HUA patient-derived ovarian carcinoma (i.p.)	nu/nu	Karagiannis et al. (2003)
Mouse/human chimeric	NIP	s.c.	TS/A-LACK mouse mammary carcinoma cells coated with NIP (s.c.)	Human FcεRIα Tg BALB/c	Nigro et al. (2009)
Human	HER2/ <i>neu</i>	i.p.	D2F2/E2 mouse mammary carcinoma cells expressing human HER2/ <i>neu</i> (i.p.)	Human FcεRIα Tg BALB/c	Daniels et al. (2012a)
Human (truncated)	N/A	s.c.	TS/A-LACK mouse mammary carcinoma cells coated with truncated IgE (s.c.)	Human FcεRIα Tg BALB/c	Nigro et al. (2012)
Mouse/human chimeric	MUC1	s.c.	4T1 tumor cells expressing human MUC1 (s.c.)	Human FcεRIα Tg BALB/c	Teo et al. (2012)
Mouse/human chimeric	PSA	s.c.	CT26 tumor cells expressing human PSA (s.c.)	Human FcεRIα Tg BALB/c	Daniels-Wells et al. (2013)

^a Tumor targeting occurred via a biotinylated anti-CEA IgG followed by streptavidin and then a biotinylated IgE.

s.c. subcutaneous, i.p. intraperitoneal, i.v. intravenous, MMTV mouse mammary tumor virus, DNP dinitrophenol, FBP folate binding protein, NIP nitrophenylacetyl, HER2/*neu* human EGFR2/neuroblastoma, MUC1 mucin-1, cell surface associated, N/A not applicable, PSA prostate-specific antigen, Tg transgenic.

2.1 *Passive Immunotherapy*

2.1.1 Mouse IgE Specific for Glycoprotein 36 of the Mouse Mammary Tumor Virus

The use of IgE as an anticancer drug was pioneered by Nagy E. et al. in the 1990s (Nagy et al. 1991). Using the hybridoma technology, this group developed a mouse monoclonal IgE specific for the major envelope glycoprotein (gp36) of the mouse mammary tumor virus (MMTV) (Nagy et al. 1991). This anti-gp36 IgE demonstrated anti-tumor activity in C3H/HeJ mice challenged s.c. with a syngeneic MMTV-secreting mouse mammary adenocarcinoma (H2712). The mice were initially treated with 12.5 μ g of IgE followed by the subsequent treatment of 25 μ g of IgE via intraperitoneal (i.p.) injection every 4 days for 8 weeks. A 50 % protection rate was observed with IgE treatment compared to control animals. Similar protection was observed with mice injected i.p. with H2712 tumor cells treated with 25 μ g of IgE every 4 days for 6 weeks. Long-term survival of IgE-treated mice was observed up to 175 days. This IgE did not protect mice challenged with MMTV-negative tumor cells (MA16/C). These results demonstrated, for the first time, the ability of a tumor-targeted IgE antibody to improve survival of tumor-bearing mice.

2.1.2 Rat/Human Chimeric IgE Specific for Murine Ly-2

The second tumor-specific IgE antibody developed was a rat/human chimeric IgE specific for mouse Ly-2, a subunit of the T-cell CD8 molecule that is also expressed on mouse T-cell tumors (Kershaw et al. 1996). Since human IgE does not interact with mouse Fc ϵ RI (Kinet 1999), a strategy utilizing a chimeric Fc ϵ RI was used to direct mouse CTL to recognize the tumor in a MHC-independent manner. This chimeric receptor was comprised of the human Fc ϵ RI α extracellular domain fused to the membrane proximal and transmembrane domains of human Fc γ RIIIa and the cytoplasmic domain of human CD3 ζ (Kershaw et al. 1996). In vitro, only mouse E3 thymoma cells (expressing Ly-2) pre-coated with the anti-(Ly-2) IgE were sensitive to lysis by mouse CTL (CTLL-R8) effector cells expressing the chimeric receptor (3H2). In an in vivo assay, 3H2 effector cells and E3 target cells at a 10:1 ratio in the presence or absence of the anti-(Ly-2) IgE were incubated together prior to s.c. injection into C57BL/6 mice. Although effector cells (3H2) alone significantly prolonged survival of mice, the antitumor effect was more prominent with addition of the anti-(Ly-2) IgE, where only 1 out of 5 mice developed tumors compared to 4 out of 5 mice with 3H2 cells alone. These results suggest that an anti-tumor IgE can be used to redirect engineered CTL to lyse tumor cells in an adoptive transfer therapy setting.

A similar strategy was used in immunodeficient non-obese diabetic–severe combined immunodeficiency (NOD–SCID) mice utilizing primary human T cells expressing a chimeric Fc ϵ RI (T-CL9) comprised of the extracellular domain of

human Fc ϵ RI α , the human Fc γ RII hinge and transmembrane regions, the cytoplasmic signaling domain of human CD28, and the T-cell receptor ζ chain (Teng et al. 2006). T-CL9 treated with the anti-(Ly-2) IgE induced lysis of mouse E3 thymoma cells expressing Ly-2 in vitro. Production of immune stimulatory cytokines (IFN- γ and GM-CSF) was observed when the anti-(Ly-2) IgE was incubated with T-CL9 and E3 target cells. Importantly, increased survival from 18 to 27 days was observed in mice challenged i.p. with E3 tumors treated with T-CL9 cells (i.p.) loaded with the anti-(Ly-2) IgE at 6, 24, 48, and 96 h following tumor challenge compared with control mice. Similar enhanced survival was observed in mice with established tumors. However, complete tumor rejection and long-term survival were not observed, probably due to the fact that T-CL9 cells did not persist in the animal (Teng et al. 2006).

2.1.3 Mouse and Mouse/Human Chimeric IgE Specific for a Human Colorectal Cancer Antigen

A mouse IgE (mIgE 30.6) specific for a human colorectal cancer antigen was developed in parallel with a mouse/human chimeric IgE (chIgE 30.6) with the same variable regions (Kershaw et al. 1998). The variable regions of both antibodies were derived from the murine IgG2b antibody 30.6. Neither antibody showed direct in vitro cytotoxicity against human colorectal carcinoma COLO 205 cells. The growth of COLO 205 cells (s.c.) in SCID mice was significantly inhibited by 1 μ g mIgE 30.6 injected intravenously (i.v.) 5 days after tumor challenge, although the effect was transient and lasted only for 48 h. This anti-tumor effect was not enhanced with 3 consecutive treatments of 1 μ g mIgE 30.6 every 2 days starting 5 days post-tumor challenge. Administration of chIgE 30.6 did not result in anti-tumor activity, which is expected since human IgE does not bind murine Fc ϵ Rs (Bettler et al. 1989; Kinet 1999). A previous study with a mouse/human chimeric IgG1 30.6 showed anticancer protection with 250 μ g of antibody administered at days 5, 7, and 9 after tumor challenge (Mount et al. 1994). The strong anticancer activity exhibited by the murine IgE may be due to its higher affinity for its Fc ϵ R compared to the affinity of IgG to its Fc γ R. It is notable that chIgG1 30.6 and chIgE 30.6 were not tested simultaneously. It was suggested that the release of cytokines, such as TNF- α or IL-4, from MC may be involved in the anti-tumor effect of mIgE 30.6 (Kershaw et al. 1998).

2.1.4 Mouse/Human Chimeric IgE Specific for Human FBP

Folate binding protein (FBP), also known as folate receptor α , is overexpressed on about 80 % of serous non-mucinous ovarian adenocarcinomas (Veggian et al. 1989). A mouse/human chimeric IgE specific for FBP (MOv18-IgE) was developed and has been extensively studied (Gould et al. 1999). The anti-tumor efficacy of this IgE was compared to a mouse/human chimeric IgG1 containing the same variable

Fig. 3 MOv18-IgE inhibits IGROV1 human ovarian cancer cell growth in vivo in the presence of human PBMC effector cells. A matched variable region set of mouse/human chimeric IgE and IgG1 antibodies specific for FBP was tested for their ability to inhibit tumor growth in a SCID transgenic mouse model of ovarian cancer. SCID mice were pre-treated with rabbit anti-asialo G_{M1} antibody i.p. to eliminate host NK cells. One day later, all mice were challenged s.c. with 2.5×10^6 IGROV1 carcinoma cells, followed by i.v. administration of 3×10^6 human PBMC combined with either 100 µg MOv18-IgG1, 100 µg MOv18-IgE, or 50 µg MOv18-IgE. Mean tumor size $\pm$ standard error of the mean measured on days 19, 29, and 35 after challenge ($n = 4$ for all groups) is shown. Mice given PBMC and 50 µg MOv18-IgE exhibited reduced growth by both day 29 (51 % inhibition; $p < 0.03$) and day 35 (40 % inhibition; $p < 0.04$). Mice injected with 100 µg MOv18-IgE exhibited an even greater degree of inhibition, relative to the positive tumor control, on day 29 (72 % inhibition; $p < 0.001$) and day 35 (62 % inhibition; $p < 0.005$). Adapted from Fig. 4 of Gould et al. (1999) with kind permission from John Wiley and Sons

regions in 2 different human xenograft transgenic models of FBP-expressing human ovarian carcinoma grown in immunodeficient mice. Mice were challenged s.c. with human ovarian carcinoma IGROV1 cells followed by i.v. inoculation of human peripheral blood mononuclear cells (PBMC) as a source of effector cells capable of binding human IgE, in the presence of MOv18-IgE or MOv18-IgG1 (100 µg). MOv18-IgE demonstrated a superior and prolonged anti-tumor effect up to 35 days compared to MOv18-IgG1-treated mice, which developed tumors after 19 days (Fig. 3). Of note, treatment with half the dose of IgE (50 µg) showed 40 % inhibition of tumor growth at day 35. Since the human Fc region of the chimeric IgE does not bind mouse FcεRs (Bettler et al. 1989; Kinet 1999), MOv18-IgE did not induce an anti-tumor response in the absence of human PBMC. This IgE-mediated tumor response is expected to be amplified in humans with a permanent supply of the entire effector cell repertoire, including MC, basophils, monocytes/macrophages, eosinophils, as well as an intact immune system. A similar model employed nude mice challenged i.p. with human HUA ovarian cancer cells expressing FBP inoculated i.p. with human PBMC alone or in combination with the MOv18-IgE or MOv18-IgG1 (Karagiannis et al. 2003). MOv18-IgE significantly increased survival up to

40 days compared to 22 days with MOv18-IgG1. Immunohistochemistry analysis of HUA xenografts revealed infiltration of human monocytes into tumor lesions, reinforcing their role in the anti-tumor efficacy of tumor-specific IgE, which was confirmed using monocyte-depleted PBMC in this tumor model (Karagiannis et al. 2007). Subsequent flow cytometric analysis revealed the distinct pathways by which human monocytes and eosinophils mediated MOv18-IgE-dependent tumor killing in vitro (Karagiannis et al. 2007), where IGROV1 cells were primarily lysed by antibody-dependent cell-mediated cytotoxicity (ADCC) via binding to Fc ϵ RI, and phagocytosed by ADCP via binding to CD23, as depicted in Fig. 2.

The safety of the MOv18-IgE has been evaluated in ex vivo studies using blood from patients with ovarian cancer. The potential Fc ϵ RI cross-linking capacity of soluble Folate binding protein (FBP), which is found in monomeric form in the circulation (Karagiannis et al. 2012), was recently assessed (Rudman et al. 2011). Even though high levels of Folate binding protein (FBP) can be found in the blood of cancer patients, concentrations up to 10-fold higher than those observed in the blood of patients did not induce degranulation of RBL SX-38 (rat basophil leukemic cells expressing human Fc ϵ RI) in the presence of the MOv18-IgE. The ability of cancer cells to induce degranulation was also explored. IGROV-1 human ovarian cancer cells expressing Folate binding protein (FBP) were incubated with the MOv18-IgE and RBL SX-38 effector cells. Degranulation was induced at high tumor:effector cell ratios, as is expected at tumor sites. Importantly, degranulation was not observed at lower ratios that coincide with numbers of cancer cells that may occur in the peripheral blood of ovarian cancer patients. Incubation with tumor cells not expressing Folate binding protein (FBP) did not induce degranulation, confirming the need of a tumor-targeted IgE. Basophils, either isolated or within the whole blood from patients, were also not activated by MOv18-IgE in the presence of soluble Folate binding protein (FBP), as evaluated via CD63 upregulation. Even though antibody-induced tumor cell death could release fragments of tumor cells from lesions that might activate basophils in the periphery, previous studies suggest that in addition to cytotoxicity, MOv18-IgE triggers substantial infiltration and phagocytosis by monocytes/macrophages (Karagiannis et al. 2007, 2008), which may limit their escape into the circulation. In addition, although IgG autoantibodies against FBP were detected in low concentrations in a small subset of patients, a hypersensitivity reaction was not induced in vitro (Rudman et al. 2011). These studies show that the extent of Folate binding protein (FBP) overexpression on tumor cells and the density of tumor cells seem to determine IgE/Fc ϵ R cross-linking capacity, with the expectation of activating Fc ϵ RI-mediated effector cells within the tumor mass but not in the circulation.

2.1.5 Engineered IgE Specific for Human HER2/*neu*

The anti-tumor efficacy of IgE antibodies specific for HER2/*neu* (human EGFR2/neuroblastoma, ErbB2), which is overexpressed by approximately 20–30 % of all breast and ovarian cancers and is associated with poor prognosis (Berchuck et al. 1990;

Meden et al. 1994; Slamon et al. 1987, 1989), has also been evaluated. HER2/*neu* is a member of the EGFR family and has tyrosine kinase activity that mediates cell survival and proliferation (Martinelli et al. 2009). Trastuzumab (Herceptin®), a humanized IgG1, has shown efficacy as a treatment for metastatic breast cancer. However, the majority of patients treated with trastuzumab alone or combined with chemotherapeutic agents eventually relapse (Ahn and Vogel 2011). In addition, a significant number of breast cancer patients with HER2/*neu* expression do not respond to trastuzumab-based therapies (Ahn and Vogel 2011; Fernandez et al. 2010; Nahta et al. 2009), confirming the need for additional strategies in targeting HER2/*neu* overexpressing tumors. Thus, the variable regions of trastuzumab were fused to the constant region of human IgE to produce a humanized trastuzumab IgE (Karagiannis et al. 2009). Similar direct cytotoxic effects up to 48 h were observed with both antibodies in cell viability assays using SK-BR-3 human breast cancer cells. Trastuzumab IgE mediated antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of U-937 human monocytic cells, while trastuzumab IgG1 exhibited a different mechanism, as detected by flow cytometry and validated by confocal microscopy. Degranulation was induced by trastuzumab IgE in the presence of murine colon carcinoma cells expressing human HER2/*neu* (CT26-HER2/*neu*) incubated with rat basophilic leukemia cells that express human FcεRI (RBL SX-38).

We have developed a fully human anti-HER2/*neu* IgE using the variable regions of the single-chain Fv C6MH3-B1 (Daniels et al. 2012a). C6MH3-B1 was isolated from a naïve human phage display library, affinity matured in vitro, and does not compete with trastuzumab for HER2/*neu* binding (Schier et al. 1995, 1996; Tang et al. 2007). This IgE induced in vitro degranulation of RBL SX-38 cells expressing human FcεRI in the presence of murine mammary carcinoma cells expressing human HER2/*neu* (D2F2/E2) but not in the presence of the parental D2F2 cells that lack HER2/*neu* expression or shed (soluble) extracellular domain of HER2/*neu* (ECD^{HER2}) (Daniels et al. 2012a). These results suggest that the acute inflammatory response (type I hypersensitivity) is expected to occur within the tumor microenvironment, where the HER2/*neu* antigen is overexpressed at high levels on the surface of cancer cells (Pegram and Ngo 2006), facilitating FcεRI cross-linking and triggering effector cell degranulation. However, due to the mono-epitopic nature of the interaction between the C6MH3-B1 IgE and ECD^{HER2}, such a reaction is not expected to occur in the presence of this soluble antigen. This is supported by our data in vivo (Daniels et al. 2012a), which show that ECD^{HER2} did not induce a reaction in the presence of the IgE. Administration of the C6MH3-B1 IgE intradermally (i.d.) into mice, followed by i.v. injection of an anti-human κ antibody to artificially cross-link the IgE-loaded FcεRI, mediated a local, passive cutaneous anaphylaxis reaction, as observed via leakage of Evans blue dye into the skin (Daniels et al. 2012a). This anaphylactic response was not observed with systemic administration of ECD^{HER2}, confirming that soluble ECD^{HER2} in the presence of the C6MH3-B1 IgE cannot induce cross-linking of the FcεRI. In addition, priming of human DC with complexes of ECD^{HER2} and the C6MH3-B1 IgE resulted in CD4 and CD8 T-cell activation in vitro (Daniels et al. 2012a), consistent with the possibility of a tumor-specific IgE enhancing DC antigen presentation via MHC class I

	# of animals	Median survival
HBSS	17	28
Anti-HER2/neu IgE	18	39

Fig. 4 C6MH3-B1 IgE prolongs survival of human FcεRIα transgenic mice bearing syngeneic mammary tumors expressing human HER2/*neu*. Survival plot of female BALB/c human FcεRIα transgenic mice challenged i.p. with a lethal dose of D2F2/E2 (2×10^5 cells) mammary cancer cells on day 0. Animals were treated i.p. with 100 μg C6MH3-B1 IgE or buffer alone on days 2 and 4. Data shown are the combined results from 3 independent experiments. The experimental group ($n = 18$) compared to buffer control ($n = 17$) shows a significant improvement in survival ($p < 0.001$, log-rank test). The remaining 5 long-term survivors were considered cured. Adapted from Fig. 6 of Daniels et al. (2012a) with kind permission from Springer Science and Business Media

and II pathways. Lower levels of T-cell activation were observed with complexes of ECD^{HER2} and trastuzumab in the same assay. This suggests that the C6MH3-B1 IgE can bind soluble ECD^{HER2} and redirect it to APC enhancing antigen presentation. Complexes of the C6MH3-B1 IgE-ECD^{HER2} alone or the use of DC loaded with these complexes could potentially be used as a tumor vaccine.

The C6MH3-B1 IgE was well-tolerated and significantly prolonged survival of BALB/c human FcεRIα transgenic mice bearing a mammary tumor expressing human HER2/*neu* (D2F2/E2), when injected i.p. in a model of peritoneal carcinomatosis (Fig. 4). The human FcεRIα transgenic mouse (also available in C57BL/6 background) has human FcεRIα knocked in and murine FcεRIα knocked out (Dombrowicz et al. 1996, 1998; Fung-Leung et al. 1996) and is an important model given that human IgE does not interact with mouse FcεRI (Kinet 1999). In this mouse model, the human FcεRIα has been shown to assemble with endogenous mouse FcεRβ and FcεRIγ to form functional receptors that mediated anaphylactic reactions when stimulated by human IgE (Dombrowicz et al. 1998). In addition to MC and basophils, in this mouse model, human FcεRIα is also expressed on murine

monocytes/macrophages, DC, LC, and eosinophils (Dombrowicz et al. 1998; Fung-Leung et al. 1996; Kinet 1999), similar to the pattern of expression observed in humans. The C6MH3-B1 IgE was also well-tolerated in an initial study in non-human primates (Daniels et al. 2012a). Human IgE has been shown to mediate anaphylaxis in cynomolgus monkeys (*Macaca fascicularis*) (Weichman et al. 1982), which has been used for target validation and assessment of therapeutic candidates designed to interfere with IgE biology, due to the highly conserved nature of FcεRIα between cynomolgus monkey and humans, with a sequence similarity of 93.8 % (Saul et al. 2014). The monkeys have also been used for safety studies on other HER2/*neu*-targeted antibody therapies, including trastuzumab (Junutula et al. 2010), due to the high sequence homology between human and cynomolgus ECD^{HER2} (Adams et al. 2006). In the initial study on the safety of the C6MH3-B1 IgE, animals received i.v. infusion of either a 0.0024 or 0.08 mg/kg dose (10 ml over 20 min) (Daniels et al. 2012a). These doses were chosen based on previous studies in humans using different immune stimulating therapeutic antibodies and their derivatives (Braly et al. 2009; King et al. 2004). No changes in eating habits or general health were observed for one week after dosing. This preliminary study demonstrated, for the first time, that systemic infusion of an IgE targeting a tumor antigen is and well-tolerated in non-human primates, suggesting a manageable safety profile allowing the anti-HER2/*neu* IgE to mobilize multiple anticancer immune mechanisms.

2.1.6 Mouse/Human Chimeric IgE Specific for Human CD20

CD20 is a transmembrane protein expressed on B cells during early pre-(B-cell) development until its downregulation when cells differentiate into antibody-secreting plasma cells (Reff et al. 1994). CD20 is expressed at reasonably high levels, is not internalized after antibody binding (Liu et al. 1987), and is not shed or secreted into the circulation, allowing efficient targeting of malignant B cells (Grillo-Lopez 2000). Even though the mouse/human chimeric anti-CD20 IgG1 rituximab (Rituxan[®]) is currently used in clinic for the treatment of NHL, resistance to this antibody is a common occurrence (Rezvani and Maloney 2011; Smith 2003), indicating the need for additional treatment strategies.

A mouse/human chimeric anti-human CD20 IgE has been developed (Teo et al. 2012). This IgE incubated with cord blood-derived MC and OCI-Ly8 human B lymphoma target cells showed an increase in tumor cytotoxicity 24 h after treatment compared to a non-targeted IgE (Fig. 5). Interestingly, this IgE-mediated cytotoxicity was decreased by the addition of an anti-TNF-α antibody, confirming its involvement in MC-mediated cell death. Similarly, antibody-dependent cell-mediated cytotoxicity (ADCC) was observed with eosinophils differentiated from cord blood mononuclear cells and the IgE (Fig. 5). The addition of heparin blocked tumor cell death, probably by neutralizing cationic proteins released by eosinophils. This study shows that MC and eosinophils differentiated in vitro can be used as effector cells in IgE-mediated antibody-dependent cell-mediated cytotoxicity (ADCC) assays.

Fig. 5 An anti-CD20 IgE induces ADCC in vitro in the presence of MC and eosinophils. OCI-Ly8 human B cell lymphoma cells labeled with CFSE were incubated with unstained CBMCs (cord blood-derived MC) and 2.5 μ g/ml anti-CD20-IgE, or cord blood-derived eosinophils (CBEos) and 5 μ g/ml anti-CD20-IgE at different effector:target ratios. The cell mixture was stained with propidium iodide (PI) before flow cytometry analysis. The percentage of PI⁺ cells in the CFSE^{hi} fraction representing total tumor cytotoxicity is shown. Results shown are mean $\pm$ standard deviation (SD) of one representative experiment. Student's *t*-test *, $p < 0.05$; ***, $p < 0.005$. Adapted from Fig. 3 of Teo et al. (2012) with kind permission from Springer Science and Business Media

2.1.7 Mouse/Human Chimeric IgE Specific for Human MUC1

MUC1 (Mucin 1, cell surface associated) is expressed at the apical surface of healthy epithelial cells and is characterized by a high glycosylation level (Roulois et al. 2013). It is overexpressed on tumors arising from glandular epithelium, such as the breast, ovary, pancreas, and colon cancers (Gourevitch et al. 1995; Kotera et al. 1994), with a loss of polarity and modification of its glycosylation pattern (Roulois et al. 2013). A mouse/human chimeric IgE specific for the human MUC1 antigen has been developed (Teo et al. 2012). The efficacy of the anti-MUC1 IgE was tested in vivo using a murine breast carcinoma cell line expressing the

transmembrane form of human MUC1 (4T1.hMUC1). A 24 % reduction in tumor size was observed in human Fc ϵ RI α transgenic mice challenged s.c. with 4T1.hMUC1 cells (10^5) and treated with 20 μ g anti-MUC1 IgE on days 1 through 5. While 3 of 8 mice in the control group survived up to 34 days, 5 of 8 mice in the anti-MUC1 IgE-treated group were still alive at this time. Mice treated with the anti-MUC1 IgE showed an increased presence of MC in necrotic peritumoral regions exhibiting signs of degranulation, compared to mice treated with a control antibody. The lack of a stronger response was attributed to the fact that 4T1 tumors are highly avascular and grow in a densely packed mass, which may impede drug delivery or effector cell recruitment. An alternative approach was implemented to further evaluate the anti-tumor activity of the anti-MUC1 IgE. 4T1 cells were engineered to express mouse IgE specific for human MUC1 to ensure continual exposure of the tumor to the antibody. In addition, 4T1.hMUC1 cells were engineered to express IL-5, which has been shown to be involved in recruiting eosinophils into the peritumoral stroma (Sanderson 1988) or monocyte chemoattractant protein-1 (MCP-1) to recruit monocytes and MC (Soucek et al. 2007), thus increasing the frequency of key effector cells at the tumor site. The 3 engineered tumor cell lines were inoculated s.c. into human Fc ϵ RI α transgenic mice. Rejection of tumors was observed up to 40 days in 7 of 8 mice, suggesting a durable response. Tumor elimination was not observed in the absence of cells expressing the anti-MUC1 IgE. Interestingly, rejection of tumors up to 30 days was also observed with parental 4T1 cells inoculated s.c. into the opposite flank of the 7 mice that rejected the initial tumor challenge, suggesting these animals developed memory responses and that these responses targeted other tumor antigens, indicating that epitope spreading had occurred. These results also suggest that a combination of antigen-specific IgE and chemokine-transfected tumor cells may possibly be used as a tumor vaccine.

2.1.8 Human IgE Specific for Human EGFR

EGFR is overexpressed in a variety of tumors including breast, ovary, prostate, colon, gastric, liver, pancreas, lung, brain, and bladder cancer (Salomon et al. 1995). Two IgE antibodies with human constant regions containing the variable regions of cetuximab, a mouse/human chimeric IgG1 (Erbix[®]), and matuzumab (EMD 72000), a humanized IgG1, were developed (Spillner et al. 2012), targeting spatially distinct structures on EGFR (Schmiedel et al. 2008). Proliferation and cytotoxicity assays demonstrated inhibition of EGFR signaling and direct tumor cytotoxicity of both IgE antibodies (Spillner et al. 2012). Interestingly, using purified human monocytes as effector cells, the cetuximab IgE increased ADCC up to 70 % compared to 30 % by its IgG counterpart using A431 human epidermoid carcinoma target cells, while ADCP levels were similar. This study also demonstrated that surface clustering of the EGFR, and not soluble EGFR, triggers IgE-mediated degranulation.

2.1.9 Mouse/Human Chimeric IgE Specific for Human PSA

The high rate of relapse and absence of a cure for metastatic prostate cancer, combined with the presence of organ-specific tumor antigens and the initial slow-growing nature of the disease, make prostate cancer an attractive target for immunotherapy. The prostate-specific antigen (PSA) is a secreted molecule that has been routinely used for blood screening of prostate cancer and assists in assessing responses to therapy and determining tumor progression (Obort et al. 2013). The anti-PSA murine monoclonal IgG1 AR47.47 complexed with PSA showed enhanced antigen presentation by human DC and induced both CD4 and CD8 T-cell activation (Berlyn et al. 2001). To examine if an IgE also possesses this activity, our group constructed a novel mouse/human chimeric anti-PSA IgE composed of the variable regions of AR47.47 (Daniels-Wells et al. 2013). An artificial multi-epitopic molecule termed human serum albumin (HSA)-PSA, which consists of multiple PSA peptides containing the AR47.47 binding epitope conjugated to HSA, induced degranulation of rat basophil cells expressing human Fc ϵ RI (RBL SX-38), confirming the functionality of this IgE. In contrast, incubation of the anti-PSA IgE with free (soluble) PSA or the naturally occurring mono-epitopic PSA-ACT (α 1-antichymotrypsin) complex with RBL SX-38 did not induce degranulation, even in amounts exceeding those detected in the blood of cancer patients (Fig. 6a). Degranulation mediated by the anti-PSA IgE was also examined *in vivo* using BALB/c human Fc ϵ RI α transgenic mice (Daniels-Wells et al. 2013). Administration of the anti-PSA IgE (*i.d.*) into mice, followed by *i.v.* injection of an anti-human κ antibody to artificially cross-link IgE-loaded Fc ϵ RI, mediated a local, passive cutaneous anaphylaxis reaction, as observed via leakage of Evans blue dye into the skin (Fig. 6b). However, this anaphylactic response was not observed with systemic administration of PSA at concentrations exceeding those found in the blood of cancer patients in the same model, confirming that free PSA in the presence of the IgE does not induce cross-linking of the Fc ϵ RI. The observation of degranulation when artificially cross-linked suggests the ability of the IgE to induce degranulation in the tumor microenvironment, where cross-linking of the Fc ϵ RI may occur due to a high PSA density in the tumors. In addition, priming of human DC with complexes of PSA and the anti-PSA IgE resulted in CD4 and CD8 T-cell activation *in vitro*. This suggests the possibility of the anti-PSA IgE to complex with PSA in the blood of patients leading to the induction of a secondary immune response involving CTL activity. Additionally, anti-PSA antibodies have been previously used for therapeutic purposes (Katzenwadel et al. 2000) and for the delivery of chemotherapeutic drugs (Sinha et al. 1996, 1999), inhibiting the growth of PSA-producing tumors. Furthermore, a radiolabeled anti-PSA has been successfully used to image PSA-producing tumor cells (Evans-Axelsson et al. 2012). These studies on PSA-targeted antibodies suggest that the anti-PSA IgE may also be able to target the tumor directly due to the high PSA density within the tumor microenvironment.

An initial prophylactic vaccination study was conducted using PSA complexed with the anti-PSA IgE in human Fc ϵ RI α transgenic male BALB/c mice (Daniels-Wells et al. 2013). The vaccination was well-tolerated and significantly prolonged

Fig. 6 In vitro and in vivo degranulation induced by the anti-PSA IgE. **a** Rat basophil leukemic cells expressing human Fc ϵ RI (RBL SX-38) were sensitized with 1 μ g of IgE or buffer alone for 2 h, followed by a 2-h incubation with PSA, PSA-ACT, or HSA-PSA (an artificial PSA multi-epitope conjugate). HSA-PSA is a positive control of a multi-epitopic antigen. Antigen concentration was adjusted to the equivalent molar amount of PSA-ACT (1 or 0.1 μ g/ml). The release of β -hexosaminidase from cells was monitored through the addition of 2.5 μ M *p*-nitrophenyl-*N*-acetyl- β -D-glucosamine in 50 mM citrate buffer (pH 4.5). Mean $\pm$ SD of triplicate samples are shown. Data are representative of 2 independent experiments ($p < 0.01$, Student's *t*-test compared to either component alone). **b** In vivo local, passive anaphylaxis in human Fc ϵ RIa transgenic mice. Mice were injected i.d. on the back with buffer (PBS) alone, 1 μ g of PSA IgE, or a non-PSA-specific IgE (NS IgE). Four hours later, mice were injected i.v. with either 50 μ g of free PSA or 50 μ g of the HSA-PSA peptide conjugate in 1 % Evans blue in PBS. Animals were euthanized 10 min later. Local hypersensitivity (anaphylactic) responses induced by degranulation of Fc ϵ RI-expressing cells in the skin were visualized by leakage of the dye into the skin due to vasodilation of local blood vessels. A reaction was detected with anti-PSA IgE and HSA-PSA (artificial multi-epitope) but not with anti-PSA IgE and PSA. Representative images of 3 independent experiments are shown. Adapted from Figs. 2 and 3 of Daniels-Wells et al. (2013) with kind permission from BioMed Central

the survival ($p \leq 0.01$ compared to the other groups, log-rank test) of mice challenged with syngeneic human PSA expressing murine colon carcinoma tumors (CT26-PSA) (Fig. 7). No protection was observed with an anti-PSA IgG1 complexed with PSA or with PSA alone. Importantly, this protective IgE-mediated

Fig. 7 Vaccination study with the anti-PSA IgE in human FcεRIα transgenic mice. Survival plot of human FcεRIα transgenic mice (male) vaccinated s.c. with PSA alone (4 μg) or complexed in a 1:1 molar ratio to either the anti-PSA IgE or IgG1 in the left flank (day 0). Mice were given a booster on day 15 in the same left flank. On day 40, mice were challenged with 10⁶ mouse colon carcinoma CT26 cells expressing human PSA (CT26-PSA) s.c. in the right flank. Animals were euthanized when tumors reached 1.5 cm in diameter. Animals administered with buffer alone were used as negative controls. Combined data from 2 independent experiments with a total of 8 mice per group are shown. Reprinted from Fig. 5 of Daniels-Wells et al. (2013) with kind permission from BioMed Central

response was associated with significant increased levels of mouse anti-PSA IgG2a, an isotype connected to the T helper 1 (T_H1) immune response in mice (Finkelman et al. 1990). Even though the in vivo effects observed with the anti-PSA IgE under these initial conditions were modest (although statistically significant) (Daniels-Wells et al. 2013), the observed anticancer effect could have major clinical significance since the BALB/c strain used in these studies is biased toward a T_H2 response (Chu et al. 1997). In addition, these results also suggest the potential use of the anti-PSA IgE complexed to PSA or the use of DC loaded with these complexes as a vaccination approach.

2.2 Active Immunotherapy (Vaccination Approaches)

The above applications describe examples of passive immunotherapy, which in certain cases may also be used as vaccine approaches. Below, we will focus on exclusive vaccination approaches involving IgE.

2.2.1 Oral Mimotope Vaccination

An alternative strategy to the passive administration of an IgE is to induce an endogenous IgE response. A novel approach to establish an active vaccination protocol is to induce tumor antigen-specific IgE through the oral route (Riemer et al. 2007). Synthetically manufactured epitope mimics (mimotopes) were generated for the epitope of human HER2/*neu* that is recognized by trastuzumab. These mimotopes were used to immunize immunocompetent BALB/c mice orally under simultaneous neutralization and suppression of gastric acid, a feeding regimen shown to effectively induce T_H2 immune responses (Scholl et al. 2005b; Untersmayr et al. 2003). The induction of high-titer serum IgE targeting the HER2/*neu* antigen was observed (Riemer et al. 2007). In addition, the endogenous anti-HER2/*neu* IgE recognized HER2/*neu*-expressing human breast cancer cells (SK-BR-3), resulting in both degranulation and cytotoxicity of rat basophil cells expressing rodent FcεRI (RBL-2H3).

2.2.2 IgE-coated Cellular Approaches

The adjuvant effect of IgE and its role in the design of new cell-based tumor vaccines have been explored. Targeting of tumors by IgE was performed using a three-step strategy based on biotin–avidin interactions (Reali et al. 2001), which has shown high efficiency in targeting cytotoxic molecules on tumor cells in vivo (Guttinger et al. 2000). First, tumor cells were coated with a biotinylated IgG antibody targeting the tumor antigen. Next, streptavidin was added to bind biotin-labeled tumor cells, followed by binding of a biotinylated IgE to the streptavidin–tumor complex to initiate effector functions (Reali et al. 2001). Immunocompetent C57BL/6 mice were inoculated s.c. with murine colon adenocarcinoma tumors expressing human carcinoembryonic antigen (MC38-CEA-2) and treated with biotinylated mouse anti-CEA IgG2b (i.p.) 2 days later, followed by avidin (i.p.) on day 3 to eliminate the unbound IgG antibodies in the blood, and streptavidin (i.p.) 4 h later to create the biotin–avidin bridge with the bound anti-CEA IgG2b antibody. On day 4, biotinylated murine anti-dinitrophenyl (DNP) IgE or IgG control antibody was administered (i.p.). IgE treatment significantly reduced tumor growth and prolonged survival compared with IgG treatment. Two out of 10 mice receiving IgE treatment completely rejected the tumor and resisted subsequent tumor challenge with the parental MC38 cell line, consistent with the generation of a memory

response and epitope spreading. Depletion of eosinophils, as well as CD4 and CD8 T cells, abrogated the anti-tumor effects, suggesting the role of these cells in IgE-mediated responses against tumors. These findings were confirmed using the highly aggressive syngeneic murine lymphoma Rauscher virus-induced T-cell lymphoma cells (RMA) expressing tumor antigen Thy 1.1 under similar conditions (Reali et al. 2001).

Cellular vaccines were developed using irradiated tumor cells coated with mouse IgE or IgG by the biotin–avidin bridging technique described above (Reali et al. 2001). Immunocompetent C57BL/6 mice were s.c. vaccinated twice (2 weeks apart) with IgE- or IgG-loaded irradiated (10,000 rad) MC38-CEA-2 cells. Two weeks after the second immunization, animals were challenged with parental MC38 tumor cells. All doses of cells coated with IgE showed significant delay in tumor growth, while only the highest dose of cells (30×10^4) coated with IgG showed protection from tumor challenge. Similar results were observed using a similar vaccination schedule in the RMA tumor model (Reali et al. 2001). These studies show the efficacy of using IgE to stimulate T cell-mediated immune responses, including epitope spreading and induction of a memory response, confirming its potential role as an adjuvant of anti-tumor vaccines.

A slightly different strategy was used to confirm the adjuvant effect of IgE-coated tumor cells. TS/A-LACK murine mammary adenocarcinoma cells were infected with modified vaccinia virus Ankara (MVA) in a BALB/c model, avoiding the need for irradiation of tumor cells prior to vaccination and increasing the tumorigenicity of infected tumor cells (Nigro et al. 2009). TS/A-LACK cells were subsequently haptenized with DNP then coated with a mouse anti-DNP IgE. Immunocompetent mice were vaccinated s.c. and challenged 15 days later with live TS/A-LACK cells. Anti-tumor protection was achieved after a single immunization with IgE-loaded cells and was comparable to mice vaccinated twice in the absence of IgE. FcεRI was demonstrated to be the mediator of IgE activity in this anti-tumor vaccination study. To translate these findings into a human system, the same experiments were performed using the human FcεRIα transgenic mouse with TS/A-LACK cells haptenized with NIP (nitrophenylacetyl) and loaded with a mouse/human chimeric anti-NIP IgE (Nigro et al. 2009). Significant anti-tumor protection was observed in human FcεRIα transgenic mice immunized with IgE-loaded tumor cells (Fig. 8a), but not in wild-type BALB/c mice (Fig. 8b) since human IgE does not interact with mouse FcεRI (Kinet 1999). Taken together, these studies suggest that IgE can act as an adjuvant of immunization in cancer therapy.

Based on these results, this group developed a novel protocol employing membrane IgE for anti-tumor vaccination to eliminate any possible anaphylactogenicity caused by circulating IgE (Nigro et al. 2012). Truncated human IgE lacking the Fab regions (tmIgE) engineered into a recombinant MVA (rMVA-tmIgE) was used to infect TS/A-LACK tumor cells, resulting in transport of tmIgE to the infected cell surface. Human FcεRIα transgenic mice were vaccinated s.c. and challenged 15 days later with live TS/A-LACK cells. Mice immunized with rMVA-tmIgE-infected TS/A-LACK cells showed significant attenuation of tumor

Fig. 8 IgE adjuvant effect in human Fc ϵ RI α transgenic mice. **a** Mice were s.c. vaccinated with 10^5 mouse/human chimeric anti-NIP IgE-loaded (1 imm + human IgE) or IgE-free (1 imm) MVA-infected TS/A-LACK cells at day -15 ($p < 0.0001$). At day 0, all vaccinated mice were challenged s. c. with 2×10^5 living TS/A-LACK cells. Non-immunized mice challenged with living TS/A-LACK cells were used as controls (no imm). **b** As a control, wild-type BALB/c mice were s.c. vaccinated with 10^5 human IgE-loaded (1 imm + human IgE) MVA-infected TS/A-LACK cells at day -15. At day 0, all vaccinated mice were challenged s.c. with 2×10^5 living TS/A-LACK cells. Non-immunized mice challenged with living TS/A-LACK cells were used as controls (no imm). Results are representative of 3 experiments. Mean $\pm$ standard error of the means is shown. Reprinted from Fig. 5 of Nigro et al. (2009) with kind permission from The American Association of Immunologists

growth compared with mice immunized with control vaccine not expressing tmIgE. This effect is comparable to that obtained using secretory IgE and was lost in wild-type BALB/c mice, confirming the role of Fc ϵ RI in IgE-active anti-tumor immunotherapy (Nigro et al. 2012).

3 Safety Concerns

A major perceived concern in using IgE in the passive immunotherapy of cancer is the potential to induce a systemic type I hypersensitivity reaction (anaphylactic shock). However, there is evidence suggesting that this adverse reaction may not occur. Type I hypersensitivity reactions are only induced by antigens that are able to engage multiple FcεR1s on effector cells (Kanner and Metzger 1983; Scholl et al. 2005a; Segal et al. 1977), resulting in cross-linking of FcεR1 and the release of anaphylactic mediators. Since IgE antibodies are tightly bound to their FcεR1 receptor in a bent form (Gould et al. 2003), more than 2 antigen epitopes have to be displayed rather rigidly in a specific range of spatial conformation in order to cross-link IgE bound to FcεR1 and induce its downstream effects (Holowka et al. 2007; Jensen-Jarolim et al. 2010). Molecules present in high density, such as certain overexpressed tumor antigens that are tightly packed on cell membranes or in lipid rafts, may cross-link FcεR1 by forming tumor-associated molecular patterns, inducing a type I hypersensitivity reaction within the tumor microenvironment and resulting in tumor cytotoxicity (Jensen-Jarolim et al. 2010). Importantly, no systemic reactions have been observed in the various *in vivo* cancer models examined via a range of approaches by multiple independent groups, aimed to address IgE-mediated activity in cancer immunotherapy in the studies described above. Of particular concern are antigens shed or secreted by tumors such as ECD^{HER2}, FBP, and PSA. However, due to the mono-epitopic nature of the interaction with the IgE, these soluble antigens were not able to induce a type I hypersensitivity reaction.

A challenge in the field of IgE-based therapeutics is the lack of appropriate *in vivo* models to examine the efficacy and toxicity of the therapy [reviewed in Daniels et al. (2012b)]. Available models include immunocompetent murine models bearing tumor cell lines from the same genetic background (syngeneic), which can be used to evaluate the efficacy and potential toxicity of mouse IgE-mediated therapy and cancer vaccines, as well as the adaptive immune response induced by these murine antibodies. This model allows numerous administrations of therapeutic IgE without the potential neutralization of the therapy by a murine antibody immune response. However, due to the lack of species reactivity between human IgE and murine FcεRs, the anti-tumor effects of human IgE cannot be evaluated in syngeneic models. In addition, since FcεR1 expression in the mouse is limited to MC and basophils, this model does not adequately reflect the immune response in humans where FcεR1 is also expressed on other cells including APC and eosinophils. Furthermore, human tumors cannot be established due to their immunogenicity in mice. Xenograft models using immunocompromised mice allow establishment of human tumors and the use of human effector cells bearing both FcεR1 and FcεRII/CD23 to evaluate the cytotoxic effects of a human antibody or a mouse IgE specific for a human antigen. However, these animals have impaired cellular and humoral immune responses and cannot be used to evaluate the adaptive immune response elicited by antibodies. In addition, human effector cells must be administered due to the lack of interaction of human IgE with mouse FcεR1.

Another disadvantage of this model is the limited supply of human effector cells and the possible lack of interaction of human cytokines secreted by these effector cells with the murine immune system. Establishment of a human Fc ϵ RI α transgenic mouse model in both BALB/c and C57BL/6 background (Dombrowicz et al. 1996, 1998; Fung-Leung et al. 1996), with murine Fc ϵ RI α knocked out and its human equivalent knocked in, allowed human IgE binding and expression of functional humanized Fc ϵ RI. This model also provides a constant repertoire of effector cells expressing human Fc ϵ RI mimicking that of humans for the study of human IgE and anaphylaxis (Dombrowicz et al. 1998; Fung-Leung et al. 1996; Kinet 1999) and the efficacy of molecules that inhibit anaphylaxis (Allen et al. 2007; Zhang et al. 2004; Zhu et al. 2002). In addition, it is useful for the evaluation of the anticancer effects mediated by Fc ϵ RI and the potential toxicity of IgE containing human constant regions. However, these animals are only transgenic for Fc ϵ RI and not Fc ϵ RII/CD23; therefore, the role of CD23 in the anticancer effects cannot be measured, which may underestimate the overall anticancer activity of the IgE. Additionally, human cancer cells cannot be established in this transgenic model, and the potential generation of an immune response against the foreign human components of the IgE antibodies may prevent multiple administrations of human antibodies.

The cynomolgus monkey (*M. fascicularis*) has been used for target validation and assessment of toxicity of therapeutic candidates designed to interfere with IgE biology, due to the highly conserved nature of Fc ϵ RI α between cynomolgus monkey and humans (Saul et al. 2014). This model has been used to study anaphylaxis induced by human IgE (Ishizaka et al. 1970; Weichman et al. 1982). Recent studies confirmed the cross-reactivity of human IgE with cynomolgus monkey effector cells and comparable binding kinetics to peripheral blood leukocytes from both species (Saul et al. 2014). However, human IgE was reported to dissociate faster from cynomolgus monkey PBMC compared with human, which was reflected in IgE-mediated ADCC assays, where higher concentrations of human IgE were needed to elicit an effector cell response of cynomolgus monkey cells (Saul et al. 2014). Moreover, IgE binding on PBMC yielded significantly different cytokine release profiles in each species, suggesting differences in downstream activation of effector cells. These data need to be considered when evaluating the toxicity of IgE-based therapies in this model.

Another animal model is the dog (*Canis lupus familiaris*), which has been used to examine IgE-mediated food allergy and atopic dermatitis (Ermel et al. 1997; Helm et al. 2003) and expresses Fc ϵ RI on MC (Brazis et al. 2002) and LC (Bonkobara et al. 2005). Spontaneous development of tumors also occurs in dogs, similar to humans (Singer et al. 2012). In addition, canine homologues of the tumor antigens EGFR and HER2/*neu* were susceptible to cetuximab and trastuzumab targeting, leading to growth arrest (Singer et al. 2012). These studies suggest that the canine may be a meaningful model to accurately assess the potential and safety of IgE-based immunotherapies against cancer. In summary, there are multiple animal models with distinct advantages and disadvantages that should be carefully considered when evaluating the efficacy and toxicity of IgE-based therapeutics.

Other evidences that support the safety of IgE as an anticancer therapy in humans include the presence of IgE antibodies against tumor antigens in patients with different types of cancer. Clinical and immunohistochemistry studies have described IgE that bind tumors both in the circulation and in tumor tissues (Fu et al. 2008; Neuchrist et al. 1994). Even so, anaphylaxis has not been observed in these studies on naturally occurring IgE antibodies in head and neck cancers, pancreatic, ovary, breast, or colon cancers (Jensen-Jarolim and Singer 2011). Importantly, clinical administration of exogenous IgE has been previously conducted without adverse events (Dreskin et al. 1987; Iio et al. 1978).

Allergic reactions are well-known clinical consequences of different types of treatments, including therapeutic proteins, chemotherapy agents, and antibodies, (Baldo 2013; Buttel et al. 2011; Romano et al. 2011; Singer and Jensen-Jarolim 2014). Pre-medication with antihistamines (Benadryl[®]) and/or corticosteroids (dexamethasone) are often administered prophylactically before chemotherapy (cisplatin, carboplatin) and monoclonal antibody treatment such as infliximab (Remicade[®]), trastuzumab (Herceptin[®]), and rituximab (Rituxan[®]) (Mezzano et al. 2014). In the unlikely event of signs of systemic anaphylaxis upon IgE treatment, epinephrine injection can be administered to neutralize this adverse effect (Simons et al. 2011). Established rapid drug desensitization procedures also exist to allow safe re-administration of a drug to patients that have developed allergic reactions to it and are performed when no alternative to the drug is deemed equally effective (Mezzano et al. 2014).

Despite the above statements, allergic reactions are relevant side effects that need to be carefully monitored. Neutralization and prevention strategies should be carefully considered. Safety measures and patient monitoring could include the evaluation of: (1) clinical signs of a systemic type I hypersensitivity (anaphylactic) reaction, (2) the presence of MC/basophils degranulation products in blood such as the elevation of histamine and tryptase, (3) the presence of autoantibodies to the targeted antigen and to the therapeutic antibody, and (4) blood concentrations of the targeted antigen. Correlation between blood antigen concentrations and clinical responsiveness, besides evaluation of toxicity in vivo, should also be examined. Treatment of patients with IgE-based therapeutics will have to proceed with extreme caution.

4 Concluding Thoughts

Several studies using IgE antibodies targeting various tumor antigens in different models have shown that these immunoglobulins have significant anticancer activity while being well-tolerated. The different properties of IgE compared to IgG support the development of IgE-based therapeutics to complement or enhance the anti-tumor activity shown by existing IgG-based therapeutics. Therefore, further studies including clinical trials are needed to confirm the efficacy and tolerability of IgE in cancer immunotherapy.

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