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Review

Role and Redirection of IgE against Cancer

Elisa A. Nigro [1], Antonio G. Siccardi [1] and Luca Vangelista [2,*]

[1] Molecular Immunology Group, Division of Genetics and Cell Biology, San Raffaele Scientific Institute, via Olgettina 58, 20132 Milan, Italy; E-Mails: nigro.elisa@hsr.it (E.A.N.); siccardi.antonio@hsr.it (A.G.S.)

[2] Protein Engineering and Therapeutics Group, Division of Immunology, Transplantation and Infectious Diseases, San Raffaele Scientific Institute, via Olgettina 58, 20132 Milan, Italy

* Author to whom correspondence should be addressed; E-Mail: vangelista.luca@hsr.it; Tel.: +39-02-2643-4798; Fax: +39-02-2643-4905.

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Abstract: IgE is a highly elusive antibody class, yet a tremendously powerful elicitor of immune reactions. Despite huge efforts spent on the characterization and understanding of the IgE system many questions remain either unanswered or only marginally addressed. One above all relates to the role of IgE. A common doubt is based on whether IgE mode of action should only be relegated to anti-parasite immunity and allergic manifestations. In search for a hidden role of IgE, reports from several laboratories are described herein in which a natural IgE link to cancer or the experimental redirection of IgE against cancer have been investigated. Epidemiological and investigational studies are trying to elucidate a possible direct intervention of endogenous IgE against cancer, raising thus far no definitive evidence. Conversely, experimental approaches implementing several strategies and engineered IgE formats built up a series of convincing results indicating that cancer might be tackled by the effector functions of this immunoglobulin class. Because of its peculiar immune features, IgE may present a superior anti-tumor performance as compared to IgG. However, extreme care should be taken on how IgE-based anti-tumor approaches should be devised. Overall, IgE appears as a promising resource, likely destined to enrich the anti-cancer arsenal.

Keywords: IgE; FcεRI; CD23; ADCC; cancer; immunotherapy

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1. Introduction

In the evolving fight against cancer, new protocols, concepts and ideas emerge continuously, resulting in the enrichment of an already impressive arsenal. This review is an endeavor to recapitulate and present the relatively scarce, yet manifold, approaches connecting IgE to cancer remission, from the initial attempts to the current state of the art. IgE and the whole system underlined by this antibody class are still retaining elusive sides [1,2]. The deleterious effects triggered by the IgE system in allergic conditions make it one of the most studied scenarios in molecular and cellular immunology [3]. IgE regulation and homeostasis remain very difficult to unveil, paralleled only by the never-ending question on why our defense system evolved IgE. A crucial determinant for these difficulties is the very low serum level of IgE, the least abundant immunoglobulin class. Only recently, research emerged that described the how and where IgE^+ B cell differentiation and memory occur [4–6], one of the most important pieces in the understanding of an antibody immune response. However, the exceedingly low number of IgE-producing B cells represents a difficult barrier to their visualization and characterization and debate on these themes is still ongoing. Aside these inherent difficulties, the potency of the immune responses raised by the antigen-IgE-FcεRI axis is unquestionably among the most immediate and powerful biological reactions to the external environment. In absence of medical care, the extreme circumstances of an anaphylactic shock may culminate with death. Clearly, the tremendous immune orchestration driven by IgE makes sense in the organism's attempt to expel intestinal parasites. And there comes the query as to whether different forms of cancer might be seen or treated in a similar manner, *i.e.*, by redirecting the IgE response towards tumors in what could be seen as a peculiar parallel between a tumor mass and an intestinal worm. Interestingly, the parallel may go beyond the imagination of an experimentalist and be embedded in the evolutionary significance of this immunoglobulin class. In other words, a major question to be addressed deals with the possibility that IgE could have evolved because it functions also as a tumor surveillant. To date, there is no definitive answer to this intriguing question; however, many epidemiological surveys and few investigational studies have been directed towards this objective. From an apparently different angle, anti-tumor approaches have been devised in which various IgE-based formats or protocols have been educated to combat cancer. In these cases it is clearly the external intervention of biomedical science that wields the anti-tumor outcome, rather than an effect sorted by the endogenous IgE system, however, the promising and efficient anti-tumor potency that results from these studies may also reflect a hidden role of IgE. The two arms of this unusual research field, *i.e.*, the endogenous (driven by nature) and the exogenous (driven by man experimentation) IgE anti-tumor effects, have been reviewed a few years ago, with the aim to aggregate them under a common name, AllergoOncology [7,8]. This review intends to provide an update to the field and a vision from the IgE molecular perspective.

2. Players of the IgE System *vs.* Cancer

2.1. IgE and Its Receptors

Antibodies of the IgE class are the least abundant circulating antibodies in the serum. In normal individuals the IgE concentration is 50–150 ng/mL, as compared to the 5–10 mg/mL of IgG [9]. This low concentration is a consequence of IgE half-life (1–3 days compared to 3 weeks of IgG) and the

small number of IgE-secreting plasma cells [10]. Furthermore, circulating IgE is captured by B cells *via* the CD23 receptor [11], and becomes tissue-resident IgE with a half-life of 1–2 weeks [9]. IgE can bind to two structurally and functionally distinct receptors: FcεRI, the high affinity receptor and CD23 (FcεRII), the low affinity receptor, with K_a of 10^9–10^{11}, 10^6–10^7 (CD23 monomer) and 10^8–10^9 M^{-1} (CD23 trimer), respectively [9].

FcεRI assembles its polypeptide chains as a tetramer ($\alpha\beta\gamma_2$) or a trimer ($\alpha\gamma_2$), with different cellular expression pattern. Mast cells and basophils express only the tetrameric isoform at high concentration (~200,000 molecules/cell) whereas the trimeric isoform is expressed on Langerhans cells, dendritic cells (DCs), monocytes and eosinophils at a 10–100 fold lower concentration [12]. Remarkably, in mouse the expression of FcεRI is restricted to mast cells and basophils in its tetrameric structure [13]. The two extracellular immunoglobulin domains of the α chain contain the IgE binding region (the membrane-proximal domain) and the molecular information for an efficient folding (the N-terminal domain) [14], whereas the signaling motifs are located in the intracellular sequences of the β and γ chains [3].

CD23 is a type II integral membrane protein belonging to the calcium-dependent lectin superfamily. Two variants of CD23 have been identified: CD23a and CD23b. CD23a is expressed on antigen-activated B cells and is involved in IgE antibody-dependent antigen endocytosis, processing and presentation, and in the regulation of IgE synthesis and clearance. CD23b expression is induced by IL-4 on a wide range of immune cells such as B cells, monocytes and macrophages [9].

As all antibody classes, IgE is produced as secretory (sIgE) or membrane-bound (mIgE) isoforms. mIgE, together with the accessory proteins Igα and Igβ, constitutes the ε B cell receptor class (BCR), involved in antigen recognition and B cell differentiation [15]. As reported, a functional interaction between mIgE on the surface of B cells and cell-bound FcεRI triggers FcεRI activation also in absence of IgE-specific antigens [16]. A truncated mIgE version containing Cε3 and Cε4 (but deprived of Cε2 and the Fabs) could also interact and activate FcεRI, underlining the importance of these domains in the IgE binding to the receptor [16].

2.2. IgE-Mediated Immune Response

IgE is the antibody class originally committed to the immune response against parasite infections [17–19], although this function has been debated [20–22]. Beside its physiologic role, IgE is well known as the key player of the allergic reactions. During an allergic manifestation, a powerful IgE-mediated inflammatory response is induced in response to common antigens such as dust or pollen allergens. In the first phase, called allergen sensitization, antigen presenting cells (APCs) such as DC and monocytes process and present the antigens to naïve T cells that differentiate into $CD4^+$ Th2 cells. Interaction between Th2 cells and B cells *via* major histocompatibility complex (MHC) class II and co-stimulatory molecules, such as CD40, induces B cells to undergo class-switch recombination from IgM or IgD to IgE [3]. Differentiation into IgE-secreting plasma cells can also be induced by IL-4 and IL-13 produced by mast cells and basophils [23]. As a result of allergen sensitization, allergen-specific IgE binds, *via* its Fc region, to the high affinity receptor expressed on the surface of mast cells and basophils, leaving its Fab regions available for future interaction with the allergen.

The re-exposure to the allergen induces the aggregation of the FcεRI-bound antigen-specific IgE and the consequent receptor activation leads to the early phase of the allergic reaction, which occurs within minutes of exposure to the allergen. This phase is characterized by a copious mast cells and basophils degranulation with release of mediators preformed in the cellular granules (such as histamine, heparin, serotonin and proteases) and the induction of the *de novo* synthesis of lipid mediators, such as prostaglandins, leukotrienes and cytokines. As the acute symptoms of the early phase decrease, IgE-activated mast cells produce chemokines and cytokines leading to the late phase of the allergic reaction with recruitment of inflammatory cells including neutrophils, followed by eosinophils, monocytes and lymphocytes [3].

2.3. Epidemiological Correlation on Allergy and Cancer

Allergy is a continuously rising phenomenon in the western world so that its possible involvement in different pathologies such as diabetes, cardiovascular diseases and cancer has been proposed [24]. In the past five decades, numerous epidemiological studies have investigated the potential association between predisposition to allergic reactions and cancer risk [25]. Considering allergy as a hyper-reactive Th2-oriented immune response, the enhanced immune surveillance could prevent aberrant cells proliferation explaining a reduced risk of childhood leukemia, brain tumors and pancreatic cancer [26,27]. Conversely, an allergic condition, responsible for a sub-chronic inflammation state with repetitive tissue damage and repair, could increment the cancer risk in specific tissues. This may be the explanation of the increased risk of lung cancer in asthmatic patients and skin cancer in patients with atopic dermatitis [28,29]. Results from retrospective studies both with biological indicators of allergy history, including levels of IgE and self-reported history of allergy have consistently reported strong, inverse associations between a self-reported history of allergy and cancer risk, particularly for pancreatic cancer, glioma, and childhood leukemia. On the other hand, results from studies with medical record-defined allergy, or from prospectively designed studies, are less clear [30,31]. Studying these associations from a different point of view, there are epidemiological studies that focused on IgE serum levels and incidence of cancer rather than allergy and cancer [30,32].

Starting from these still controversial epidemiological evidences, the concept of AllergoOncology [7] has been proposed. This rapidly evolving field of research aims to reveal the function of IgE-mediated immune responses against cancer, suggesting a role for IgE antibodies in natural tumor surveillance as well as in active and passive immunotherapy [8].

2.4. IgE-Related Effector Cells in Cancer

The relationship between tumor cells and immune effector cells is complex and results in inflammation and tumor regression but has been also associated with tumor growth, angiogenesis, invasion and metastasis. It is well established that solid tumors are infiltrated by mast cells, B and T lymphocytes, neutrophils, NK cells, DC, macrophages and eosinophils recruited by a variety of cytokines and chemokines expressed by local inflammatory cells [33]. Most of the inflammatory cell infiltrates are IgE effector cells with the potential to play an important role in antitumor activity.

2.4.1. Mast Cells and Basophils

Mast cells are the first immune cells to infiltrate the tumor microenvironment but their contribution to tumor growth and spread is controversial [34] and dependent on the type of tumor. In some human cancers, such as breast and colorectal cancers the presence of mast cells has been associated with favorable clinical prognosis [35,36], whereas Hodgkin's lymphoma and melanoma patients with higher numbers of mast cells in tumor lesions have a worse prognosis [37,38]. Remarkably, it has been proposed that even the different location of mast cells within the tumor microenvironment is prognostic, with a high intra-tumoral density being associated with a favorable prognosis and a high peri-tumoral density associated with a poor prognosis [39,40]. It has been proposed that mast cells can influence tumor angiogenesis, tumor invasion and contribute to the composition of the immune-suppressive tumor microenvironment. Preclinical studies suggested that blocking mast cell degranulation or depleting mast cells in some types of cancer might be an effective therapeutic strategy. However more studies are necessary to better understand whether mast cells can be therapeutically targeted in the tumor microenvironment to improve protective immune responses [41].

The role of basophils in cancer is not completely clarified yet. They have been observed in the inflammatory infiltrate in experimental tumors. Basophils are probably recruited by *in situ* expression of cytokines and chemokines and are considered to be associated with tumor regression [42]. In chronic myeloid leukemia basophils markers are used for diagnostic and prognostic evaluations [43].

2.4.2. Eosinophils

Tumor associated tissue eosinophilia (TATE) is characterized by the presence of eosinophils in the peri-tumoral infiltrate of several types of cancer including hematological malignancies and solid tumors [44]. This phenomenon has been suggested to represent a positive prognostic indicator in different tumors such as colorectal carcinoma, oral and esophageal squamous cell carcinoma, laryngeal carcinoma, pulmonary adenocarcinoma and bladder carcinoma [45–47], but it has been associated with poor prognosis in Hodgkin's lymphoma [48]. Eosinophils contain cytotoxic granule proteins, eosinophil peroxidase, cationic protein and eosinophil-derived neurotoxin [49], which are able to induce tissue damage, enhancing local inflammation and immune response. Some of these eosinophil-derived cytotoxic mediators can induce tumor cell apoptosis and have been considered as potential cancer treatments [50]. Degranulated eosinophils have been detected in tumors following systemic administration of IL-2, suggesting that they are involved in tumoricidal activity [51–53]. Eosinophil infiltration into tumors in wild type mice has been shown to be an early and persistent response [52]. In addition, IL-5 transgenic mice showed a significant reduction in tumor establishment and growth, which was correlated with a high level of eosinophil recruitment to the tumor and surrounding connective tissue [53]. This finding suggests that eosinophils may exert antitumor functions in an IL-5-rich environment such as during an allergic status. Moreover, studies by Capron's group show that eosinophils purified from allergic donors induce significantly increased apoptosis of tumor cells as compared to eosinophils form normal donors, suggesting an efficient *in vivo* priming of eosinophils in allergic patients [54].

2.4.3. Macrophages and Monocytes

Tumor-associated macrophages are found in all types of tumors and can constitute more than 50% of the total tumor mass in both primary and metastatic lesions [55]. Classically associated with poor prognosis, they can promote tumor growth and metastasis by the regulation of angiogenic programming through the production of VEGFA [56], tissue remodeling, production of soluble mediators that support proliferation, survival and invasion of malignant cells, and by their development of immunosuppressive microenvironments [57]. Tumor-associated macrophages' activities are dependent on their polarization state: classical (M1; IFNγ/LPS-dependent) or alternative (M2; IL-4/IL-13/IL-10-dependent) [58]. In this regard, emerging therapeutics are now focusing on the repolarization of tumor-associated macrophages as a method to invoke their anti-tumor potential [59].

Blood monocytes are recruited to the tumor sites by chemokines and cytokines released by tumor cells and neighboring endothelial cells. They can be stimulated to either kill tumor cells and release angiostatic compounds, or, like mast cells, promote tumor growth and metastasis by producing angiogenic factors and matrix metalloproteases [60].

2.4.4. Antigen-Presenting Cells

APC such as B lymphocytes, monocytes, Langerhans cells and DC in tumor infiltrates express CD23 and/or FcεRI. One of the mechanisms of immune escape by tumor cell is the defective differentiation and maturations of APC, with the consequent reduction of adaptive immune responses against tumor antigens. The presence of IgE bound to the surface of DCs *via* FcεRI interaction may increase the efficacy of antigen uptake and presentation by a 100–1,000 fold [61], leading to an efficient activation of T cells that results in a powerful antitumor adaptive immune response.

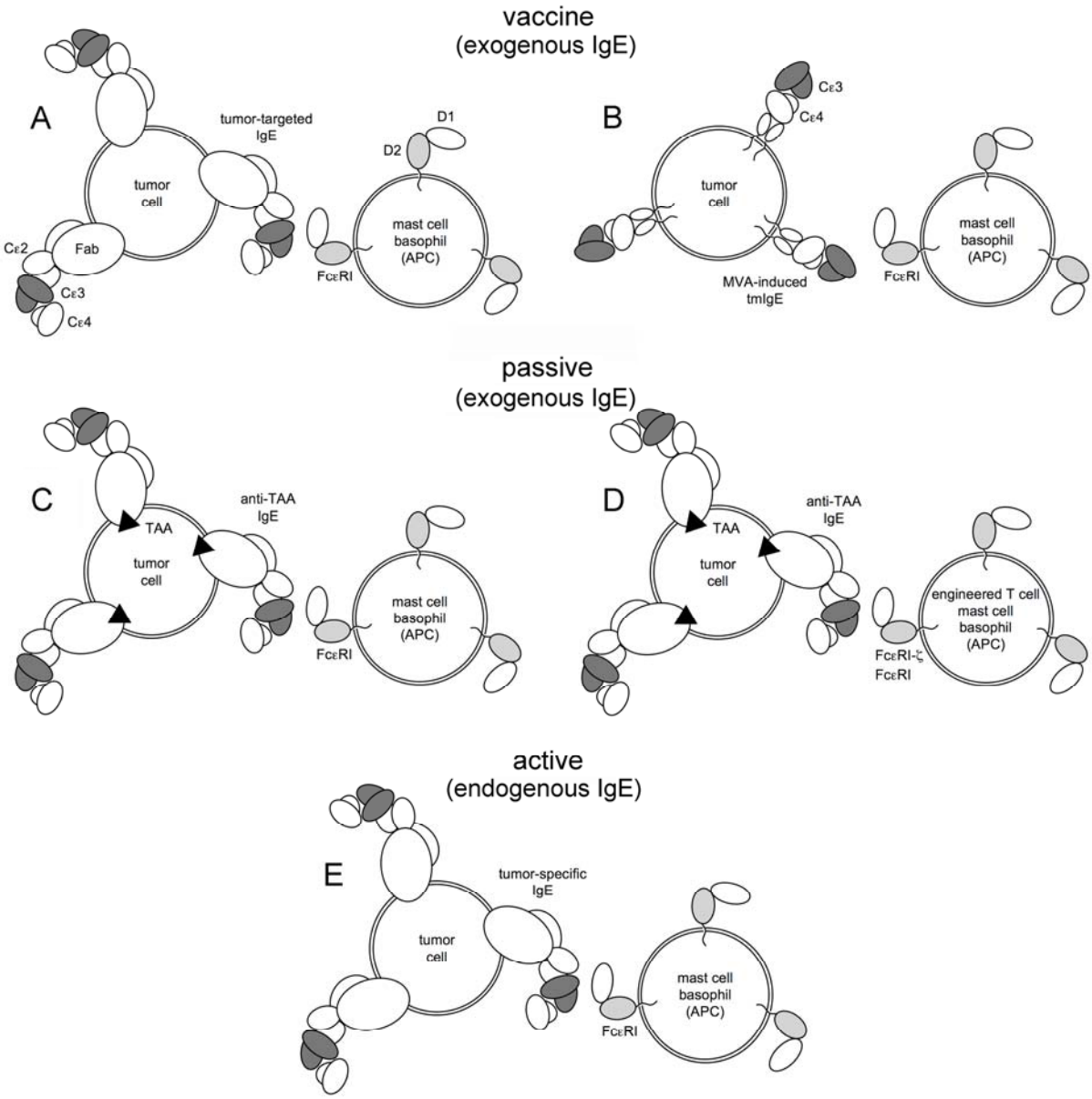
2.4.5. T Cells

Tumor associated antigen (TAA)-specific cytotoxic T lymphocytes (CTL) are the major effectors in the immune response against tumor cells. The role of $CD8^+$ CTL in tumor cytotoxicity is primarily mediated through the Fas/Fas ligand or perforine/granzyme signaling pathways after recognition of TAAs presented by APC (especially DCs) on MHC class I molecules [62]. Interestingly, DCs express both IgE receptors (FcεRI and CD23) that might be able to shuttle antigens for an IgE-mediated cross-presentation, as FcγRs do for the cross-presentation mediated by IgG [63]. An IgE-mediated cross-presentation pathway for the induction of CTL response might be an attractive approach for cell-based cancer therapy [64]. More recently, there has been renewed interested in the anti-tumor immune response mediated by $CD4^+$ T cells [65]. Unlike CTLs, $CD4^+$ T cells can respond to MHC class I-negative tumors, recognizing TAAs presented on MHC class II by APCs, a process less subjected to immunoediting and selection [66].

Overall, the possibility to redirect the IgE-mediated inflammatory response, typical of allergic reactions, at a tumor site characterized by the presence of the described key IgE receptor-expressing immune effector cells, constitutes the basis for the development of recombinant tumor-specific IgE Abs and other IgE-based immunotherapeutic approaches. The following chapter enlists the different

experimental approaches based on IgE (*i.e.*, antitumor vaccine, passive and active therapy) and these have been summarized in Figure 1.

Figure 1. Schematic representation of the IgE-based approaches for vaccine, passive and active antitumor therapy. (**A**) Tumor cell targeting with complete IgE as vaccine adjuvant [67,68]. (**B**) MVA-induced tumor cell expression of a mini-membrane IgE (tmIgE) as adjuvant [69]. (**C**) TAA-specific IgE mAbs for passive therapy [70–80]. (**D**) Same approach as in C with a potentiation provided by FcεRI-engineered CTLs [81,82]. (**E**) Active tumor therapy with the induction of endogenous tumor-specific IgE [83]. Mast cell and basophil FcεRI has been reported as the major player in IgE recognition, however, when using the FcεRIα-humanized mouse or in the case of a translation of these approaches from the mouse models to human clinical trials, other FcεRI-expressing cells, particularly professional APC, are likely to be involved. TAAs are in black, the IgE binding site for FcεRI and CD23 (Cε3) is in dark grey and the FcεRI binding site for IgE (D2) in light grey.



3. Experimental Approaches

3.1. IgE Antitumor Adjuvanticity

In a strategy designed to redirect the IgE-mediated inflammatory state towards tumors, the use of IgE as an adjuvant for cancer immunotherapy followed the hypothesis that a tumor could be disguised as an intestinal parasite or an allergen aggregate, targeting IgE on its surface. IgE-covered tumor cells may indeed recruit FcεRI-expressing effector cells within the tumor microenvironment, likely promoting a powerful inflammation with consequent immunological response at the tumor site and restriction of tumor growth.

In our first study, IgE antibodies have been targeted on the surface of tumor cells by a three-step strategy consisting in the creation of an avidin bridge between a biotinylated TAA-specific antibody and a biotinylated monoclonal IgE (of irrelevant specificity) [67]. C57BL/6 mice were injected s.c. with syngeneic MC38-CEA-2 tumor cells, and two days later, mice were injected intraperitoneally (i.p.) with a biotinylated tumour-specific (anti-CEA-2) murine IgG antibody to target the tumour. The next day, mice were given avidin i.p. and on day 4, mice received biotinylated anti-dinitrophenyl (DNP) murine IgE (or a biotinylated IgG antibody as control). IgE treatment (compared to treatment with IgG) caused both a delay in tumor development and a decrease in the rate of tumor growth with prolonged mice survival, conferring protection against subsequent challenges with untreated tumor cells. The Fc region of IgE should play a critical role in its efficacy, since heat inactivation of the biotinylated IgE prior to injection abrogated the anti-tumor effect. Depletion of eosinophils, $CD8^+$, or $CD4^+$ cells also abrogated the anti-tumor effect, demonstrating the requirement for these cell types in the IgE-mediated growth inhibition. These findings were confirmed under similar conditions in a more immunogenic tumor model, the syngeneic murine lymphoma RMA-Thy1.1. Furthermore, mice immunized with a cellular vaccine constituted by IgE-loaded, irradiated tumor cells were protected after tumor challenge with untreated tumor cells [67].

The adjuvant effect of IgE-coated tumor cells was later confirmed using a slightly different strategy [68]. RMA tumor cells were first infected with modified vaccinia virus Ankara (MVA), a severely host-restricted viral vector, unable to multiply in human and in most mammalian cell lines [84]. MVA infection substituted tumor cell irradiation and exploited the high immunogenicity of the vaccinia virus. RMA-infected cells were then conjugated with the hapten DNP and the haptenized cells coated with murine anti-DNP IgE and used to s.c. vaccinate C57BL/6 mice. After s.c. challenge with live RMA tumor cells, a strong anti-tumor effect was observed in these animals, as compared to mice vaccinated with haptenized tumor cells (not bearing IgE); however, when mice were vaccinated twice with haptenized tumor cells, an anti-tumor effect was also observed. This effect was similar to that observed in mice vaccinated only once with IgE-coated tumor cells, demonstrating the IgE adjuvanticity.

The results obtained in C57BL/6 mice with the RMA tumor have been similarly reproduced in BALB/c mice using the mammary adenocarcinoma cell line TS/A-LACK (coding for Leishmania receptor for activated C kinase, as an immunological tag) [85]. The proof for IgE anti-tumor adjuvanticity in a BALB/c model demonstrated that this might not be confined to a specific tumor model and its potential broader applicability. In order to understand how IgE orchestrates the immune system for tumor protection, the investigation on the receptors involved has been mandatory. The key

role of FcεRI in the IgE anti-tumor adjuvant effect has been demonstrated using FcεRIα$^{-/-}$ [86] and CD23$^{-/-}$ mice [87]. Loss of tumor protection in FcεRIα$^{-/-}$ mice (but not in CD23$^{-/-}$ mice) confirmed the prominent role exerted by FcεRI in IgE anti-tumor adjuvanticity. In parallel, the use of a transgenic mouse hFcεRIα, in which the α chain of mouse FcεRI is substituted with the human counterpart [88], allowed us to demonstrate that also human IgE exerts anti-tumor adjuvanticity [68].

The replacement of tumor cell irradiation with MVA infection represented a first step towards the construction of a viral vector-based anti-tumor vaccine. MVA is considered as safe and is widely used as vector for both prophylactic and therapeutic vaccination in clinical. The "Red-to-Green gene swapping" method, a fast and reliable recombinant MVA (rMVA) production system [89,90] has been used for the subsequent evolution of the IgE-based vaccination.

In order to abolish the potential side effects of circulating IgE and eliminate the need for antigen specificity, we exploited the direct and functional interaction between membrane-bound IgE (mIgE) and FcεRI [16]. A truncated mIgE version (tmIgE) containing Cε3 and Cε4 (but deprived of Cε2 and the Fabs) has been engineered into a recombinant MVA (rMVA-tmIgE) and used to infect TS/A-LACK cells, with consequent transport of tmIgE to the surface of infected cells. Transgenic human FcεRIα mice were vaccinated s.c. and then challenged s.c. with live TS/A-LACK cells. Mice immunized with the rMVA-tmIgE cellular vaccine showed a significant attenuation of tumor growth compared to mice immunized with the control vaccine (not expressing tmIgE). This anti-tumor protection was completely lost when the cellular vaccine was administered to FcεRIα$^{-/-}$, thereby confirming the key role of FcεRI in IgE anti-tumour adjuvanticity [69]. Most importantly, rMVA-tmIgE could be tested both in animal models (tumors in mice) and in clinical trials, as rMVAs are grown on chicken embryo fibroblasts (CEF), in serum-free medium, throughout the production protocol, in line with FDA recommendations.

3.2. Induction of Endogenous IgE by Active Vaccination

A different vaccination strategy has been used by the group of Jensen-Jarolim for the induction of a TAA-specific IgE response *in vivo*. Knowing the epitope recognized by the therapeutic antibody trastuzumab on the oncogenic protein HER2/*neu*, epitope mimics, the so-called mimotopes, have been generated [91]. Mice oral vaccination with mimotopes in combination with anti-acid treatment, known to induce food allergy [92], led to the induction of endogenous anti-HER2/*neu* IgE response [83]. The IgE antibodies induced proved to be functional in a mediator release assay where they were found to react specifically with HER2/*neu*-expressing breast cancer cells. Their ADCC-mediated potential of tumor cells killing *in vitro* has also been reported [83].

3.3. IgE mAbs as Cancer Therapeutics

The unique features of IgE antibodies provide the rationale for their use as cancer therapeutics. First of all, the high affinity of IgE for FcεRI is two orders of magnitude higher than that of IgG for its receptors [13], allowing IgE antibodies to remain bound to immune effector cells even in the absence of antigen [9]. Furthermore, unlike IgG, which is subjected to the inhibitory receptor FcγRIIb, IgE antibodies lack inhibitory Fc receptors, suggesting that IgE may escape the suppressive effects of the tumor microenvironment [3]. Another advantage of the use of IgE in the treatment of solid tumor is the

IgE prolonged half-life in tissues (2 weeks compared to 2–3 days for IgG). This results in local retention of IgE by FcεRI-expressing resident effector cells and longer immune surveillance. Finally, the binding of IgE to FcεRI and CD23 promotes several cell killing modes: (i) pro-inflammatory mediators and cytokines released by mast cells and basophils recruit professional killer cells (such as neutrophils and eosinophils) on site; (ii) antibody dependent cellular cytotoxicity (ADCC) causes target cell lysis through enzymes and cytokines release; and (iii) antibody dependent cellular phagocytosis (ADCP) is mediated by macrophages and monocytes resident in the tumor microenvironment.

3.3.1. Murine Anti-gp36 IgE

The first study reporting the production of a TAA-specific recombinant IgE antibody as anti-cancer agent has been conducted by Nagy and collaborators in the early 1990s. A monoclonal IgE specific for the envelope glycoprotein (gp36) of the mouse mammary tumor virus (MMTV) has been produced and used for the treatment of C3H/HeJ mice subcutaneously (s.c) injected with syngeneic MMTV-expressing H2712 murine mammary carcinoma cells. Concomitant with tumor cells injection, mice received ascites containing anti gp36-IgE by i.p. inoculation, followed by 4-day interval injections for eight weeks. Injections with normal mouse serum have been used as control. By day 44 after tumor injection all the control mice died while three out of five mice treated with the anti-gp36 IgE survived till day 175. Comparable results were obtained when H2712 tumor cells were injected i.p., providing a further proof for the principle of IgE as cancer therapeutics. Remarkably, anti-gp36 IgE was not effective in the treatment of MMTV-negative MA16/C mammary carcinoma-bearing mice, demonstrating the tumor specificity of the antibody [70].

3.3.2. Rat/Human Chimeric Anti-Murine Ly-2 IgE

In a similar way, the antitumor effect of a rat/human chimeric IgE specific for the murine Ly-2 antigen has been explored. This antibody, originally constructed for evaluating antibody-mediated elimination of CD8-expressing target cells *in vivo* [93], has been employed together with murine cytotoxic T cells (CTLs) redirected to recognize TAA in a non-major histocompatibility (non-MHC)-dependent manner. According to this original strategy, CTLs have been stably transfected to express a chimeric FcεRI-ζ receptor. This receptor is composed by the extracellular domain of human FcεRI, the trans-membrane domain of human FcγRIIa and the intracellular human CD3-ζ signaling domain. C57BL/6 mice injected s.c. with syngeneic Ly-2-expressing E3 thymoma cells together with anti-Ly-2 IgE and FcεRI-ζ receptor-expressing CTLs showed a significant protection from tumor growth with 80% of survival, as compared to controls without anti-Ly-2 IgE [81].

The same strategy has been used in non-obese diabetic-severe combined immunodeficiency (NOD/SCID) mice using human primary T cells retrovirally transduced to express the chimeric FcεRI-ζ receptor linked to the cytoplasmic domain of the human co-stimulatory molecule CD28. Teng and collaborators showed the induction of IgE-mediated Ly-2^+ E3 thymoma cell lysis *in vitro* with production of immune-stimulatory cytokines such as IFN-γ and GM-CSF. Furthermore, adoptive transfer of engineered primary human T cells redirected toward E3 thymoma cells by anti-Ly-2 IgE resulted in *in vivo* anti-tumor activity with significant prolonged survival of treated Ly-2^+ tumor-bearing mice as compared to controls [82].

3.3.3. Murine and Murine/Human Chimeric Anti-CCA IgE

The same group developed both a murine (m30.6) and a murine/human chimeric (ch30.6) IgE antibody containing the variable region of the murine IgG2b antibody 30.6, specific for a colorectal cancer antigen (CCA) expressed on the surface of human carcinoma cell line including COLO 205 [71]. Although neither of these antibodies demonstrated any *in vitro* cytotoxicity against COLO 205 cells, m30.6 IgE showed a rapid but transient (48 hours) anti-tumor effect *in vivo* after intravenous (i.v.) treatment of COLO 205 tumor-bearing SCID mice. The anti-tumor effect was antigen-specific and required the murine IgE Fc region. A similar effect has been shown using a murine/human chimeric 30.6 IgG1 (ch30.6 IgG1) antibody [94] but at antibody concentrations 250-fold higher than those reported for m30.6 IgE. This superior activity *in vivo* might be due to the higher affinity of IgE for FcεRI as compared to the affinity of IgG for its FcγRs. The lack of efficacy of ch30.6 IgE is not surprising since human IgE does not interact with murine FcεRI [13]; thus there was no activation of an IgE-mediated anti-tumor immune response.

3.3.4. Murine/Human Chimeric Anti-Human FRα IgE

In 1999, the group of Gould created two murine/human chimeric MOv18 monoclonal antibodies of the IgG1 and IgE classes, specific for the ovarian TAA folate receptor α (FRα). The *in vivo* efficacy of MOv18 IgE and IgG1 has been compared in two separate human xenograft models of FRα-expressing ovarian carcinoma grown in immunodeficient mice. In the first model, SCID mice were challenged s.c. with FRα-expressing human ovarian carcinoma (IGROV1) cells. Subsequently, i.v. administration of human peripheral blood mononuclear cells (PBMC), added as effector cells, was conducted in presence of either MOv18 IgE or MOv18 IgG1 [72]. MOv18 IgE had a superior and prolonged anti-tumor effect. Since the human Fcε region of chimeric IgE does not bind mouse Fcε receptors, MOv18 IgE did not induce an anti-tumor effect in the absence of human PBMC, demonstrating the key role of human cells to mediate MOv18 IgE effector functions. The evidence for an anti-tumor effect of MOv18 IgE was consolidated by the use of patient-derived FRα-expressing human ovarian carcinoma cells (HUA), injected i.p. in nude mice in presence of human PBMC [73]. In this model, treatment with PBMC and MOv18 IgE significantly increased survival to 40 days, where the survival of mice treated with the combination of PBMC and MOv18 IgG1 was 22 days. Immunohistochemistry (IHC) analysis of tumor slices from MOv18 IgE-treated mice revealed the infiltration of human monocytes in tumor lesions, reinforcing the key role played by these effector cells [73]. Furthermore, the use of monocyte-depleted PBMC *in vivo* resulted in a loss of the survival advantage conferred by MOv18 IgE [74]. Flow cytometry analysis attested that MOv18 IgE-dependent tumor cell killing by human monocytes is mediated by two distinct pathways: ADCC *via* FcεRI and ADCP *via* CD23, both expressed on the surface of IL-4-activated monocytes [74,75]. The involvement of human eosinophils as potent effector cells in MOv18 IgE Ab-dependent cytotoxicity *in vitro* has also been shown [74].

3.3.5. Humanized and Human Anti-HER2/*neu* IgE

Further evidence of the IgE-mediated tumor cell killing by monocytes has been shown *in vitro* in a study examining the functional properties of an IgE specific for the human epidermal growth factor

receptor 2 (HER2/*neu*) [76], a TAA expressed in approximately 30% of breast cancers in association with poor prognosis [95]. Anti-HER2/*neu* IgE is homologue to trastuzumab (Herceptin), a humanized IgG1 drug in use for human therapy [96]. Both antibodies exhibited a similar direct anti-tumor effect on SK-BR-3 human breast cancer cells *in vitro*. trastuzumab IgE was shown to mediate ADCC of target cells, while trastuzumab IgG1 facilitated ADCP, supporting the idea of developing IgE antibodies to enhance the mechanism of existing therapeutic monoclonal antibodies.

Next, a fully human anti-HER2/*neu* IgE antibody has been constructed and tested *in vivo* for its anti-tumor properties [77]. Daniels and colleagues showed that this IgE antibody significantly prolonged survival of D2F2/E2 tumor-bearing human FcεRIα transgenic mice [88]. Moreover the group of Penichet reported that the anti-HER2/*neu* IgE is well tolerated in a preliminary study conducted in *Macaca fascicularis* (cynomolgus) monkeys [77].

3.3.6. Murine/Human Chimeric Anti-Human MUC-1 IgE and Anti-Human CD20

The human FcεRIα transgenic mouse model has been employed also to study a murine/human chimeric IgE mAb specific for the human epithelial antigen MUC-1 [78]. A modest inhibition of tumor growth was observed after peri-tumoral injection of the anti-hMUC-1 IgE in hMUC-1-expressing 4T1 tumor-bearing mice. However, the same tumor cells engineered to express an anti-hMUC-1 mouse IgE together with either MCP-1 (4T1.hMUC-1/MCP-1) or IL-5 (4T1.hMUC-1/IL-5), two chemoattractant cytokines, failed to grow. This result emphasizes the importance of antibody delivery to the tumor site and the presence of effector cells in the tumor microenvironment. Remarkably, mice that rejected 4T1.hMUC-1/MCP-1 and 4T1.hMUC-1/IL-5 also rejected subsequent (30 days later) injections of wild type 4T1 cells, suggesting the development of a memory immune response. The latter evidence parallels our previous demonstration of an anti-tumor adjuvant effect exerted by IgE [68,69].

The same group produced a murine/human chimeric IgE antibody specific for the human B cell antigen CD20 [78]. The anti-hCD20 IgE was capable to drive ADCC by umbilical cord blood-purified mast cells and basophils against the OCI-Ly8 lymphoma B cell line. According to the authors, the anti-hCD20 IgE activity *in vivo* was not studied because the significant levels of circulating antigen in a physiological model of lymphoma would have led to a high risk of anaphylaxis upon IgE-treatment.

3.3.7. Human Anti-EGFR IgE

Two more variants can be added to the list of anti-tumor IgE, both engineered to target the human epidermal growth factor receptor (EGFR) [79]. Increased EGFR expression and activation has been found in a variety of human tumors, in correlation with poor response to treatment with high incidence of metastasis [97]. Hence, EGFR constitutes a key target for cancer therapeutic approaches, including monoclonal antibodies. Spillner and colleagues focus their attention on the numerous anti-EGFR IgG antibodies to study the therapeutic potential of the human IgE class variant in tumor cell targeting. The antibodies chosen for comparative class assessment were cetuximab (clone 225) [98] and matuximab (clone 425) [99]. Proliferation and cytotoxicity assays proved both signal blocking and effector mediating capability by the anti-EGFR IgE mAbs. Interestingly, while phagocytosis remained nearly identical, cytotoxicity, with consequent tumor cell killing, increased up to 95% as compared to the IgG counterparts [79].

3.3.8. Mouse/Human Chimeric Anti-PSA IgE

In the lively field of AllergoOncology, the latest report deals with the application of IgE against prostate cancer (PCa) [80]. PCa is the most frequently diagnosed cancer and its present treatments bare serious pitfalls, especially at the advanced or metastatic stage. Therefore, any novel treatment option would be highly desirable. The prostate-specific antigen (PSA) is a promising target for PCa immunotherapy because of its organ specific production. In this view, a mouse/human chimeric anti-PSA IgE containing the variable regions of the murine IgG1 monoclonal antibody AR47.47, specific for human PSA, has been engineered. AR47.47-PSA complexes showed enhanced antigen presentation by human dendritic cells and induced both CD4 and CD8 T-cell activation [100]. The anti-PSA IgE variant provided evidence for effector cell degranulation upon aggregation, but not in the presence of the natural soluble antigen, suggesting absence of systemic anaphylaxis risk. Noteworthy, anti-PSA IgE-PSA complexes triggered immune activation and prolonged the survival of human FcεRIα transgenic mice challenged with PSA-expressing tumors in a prophylactic vaccination setting.

4. Conclusions

Within the diversified strategies designed by the research mentioned here, there are probably two aspects that captivate most attention and hope. These share very similar IgE-driven mechanisms that should lead to tumor regression. One concerns the ignition of an IgE immune response against tumors *via* therapeutic IgE tumor targeting. The second mechanism largely overlaps with the first, with the fundamental difference that it should be established in the context of a dynamic immune response generated endogenously. Both scenarios witness the well-known cellular degranulation (either by mast cells or basophils, or both) that leads to the recruitment of effector cells and the establishment of a potent inflammatory state that should directly kill tumor cells and aid in the programming of an immune memory against the tumor.

A role for IgE in tumor regression is being delineated. It remains to be understood if this role might result only from an external intervention of biomedicine or whether this intervention, together with epidemiological and experimental studies, may reflect a natural role in tumor surveillance by the IgE system. Of special interest in this line of thought is the reported presence of tumor-specific IgE in patients [101,102]. Furthermore, expression of a functional FcεRI by human intestinal epithelial cells, particularly in patients with colon cancer or gastrointestinal inflammation, may suggest a contribution of FcεRI to theimmunosurveillance of the gut [103]. Past and current research provided compelling evidence for a potent anti-tumor effect elicited when IgE is recruited into the combat, with an activity superior to equivalent IgG-based strategies. Future research should most likely focus onto: (i) enlarging the molecular arsenal deriving from diverse IgE formats and specificities; (ii) devising refined IgE-based anti-tumor therapeutics and protocols; (iii) solving the quest on a role played by IgE in tumor surveillance; and (iv) translating all the above into clinical protocols applicable to human health.

Regardless of the approach taken (active, passive or vaccine therapy, Figure 1), many questions and concerns need to be either answered or relieved in regard to the dual nature of the IgE system: its potency and its danger. Ideally, an engineered (or a naturally-arising) tumor-specific IgE antibody may exploit its effector mechanism at the site of the tumor, where the highest target antigen expression is found, but not systemically [104]. The possible crosslinking capacity of soluble forms of tumor

antigens represents a further concern in perspective clinical studies with therapeutic IgE [105,106]. It may well be that only certain types of cancer could be investigated, however we provided here some examples on how IgE-based systems could be engineered to overcame the dangerous branch in order to provide an exclusive benefit to human health.

Conflict of Interest

The authors declare no conflicts of interest.

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