

Chapter 9

IgE as Adjuvant in Tumor Vaccination

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

9.1 Introduction

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

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

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

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

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

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

9.2.1 Cell Types Involved in IgE-Induced Tumor Protection

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

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

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

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

9.2.2 IgE is an Adjuvant in Tumor Vaccination

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

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

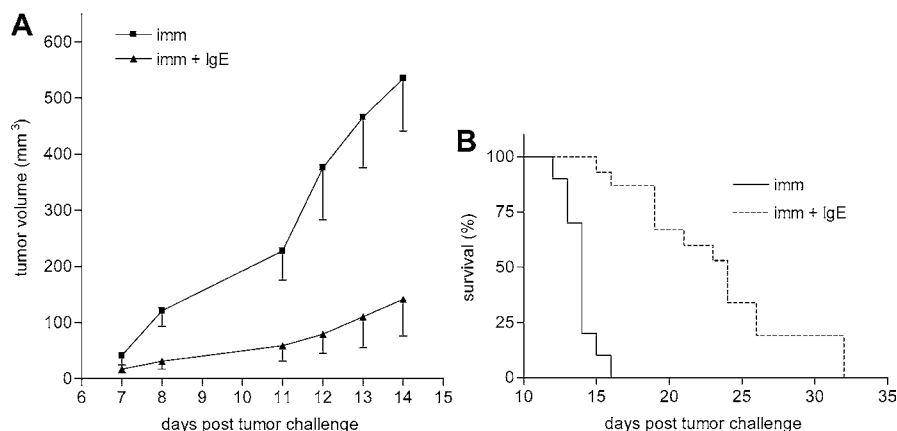


Fig. 9.1 Anti-tumor IgE adjuvanticity in C57BL/6 mice. **(A)** Tumor growth of mice immunized s.c. twice (15 days apart) with 1×10^5 irradiated MC38 cells previously haptenized with DNP and loaded with anti-DNP IgE. Challenge with 3×10^5 parental tumor cells was performed 15 days after the last immunization.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

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

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

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

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

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

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

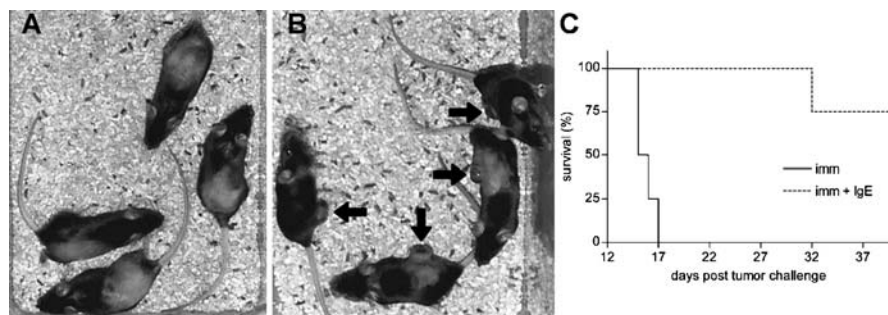


Fig. 9.2 Anti-tumor protection of VV-infected IgE-loaded RMA cells. C57BL/6 mice (four mice per group) were vaccinated s.c. with 10^5 IgE-loaded (A) or not loaded (B) VV-infected RMA cells at day -15 . At day 0 mice received a s.c. tumor challenge of 7×10^4 RMA living cells. The images are representative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

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

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

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

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

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

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

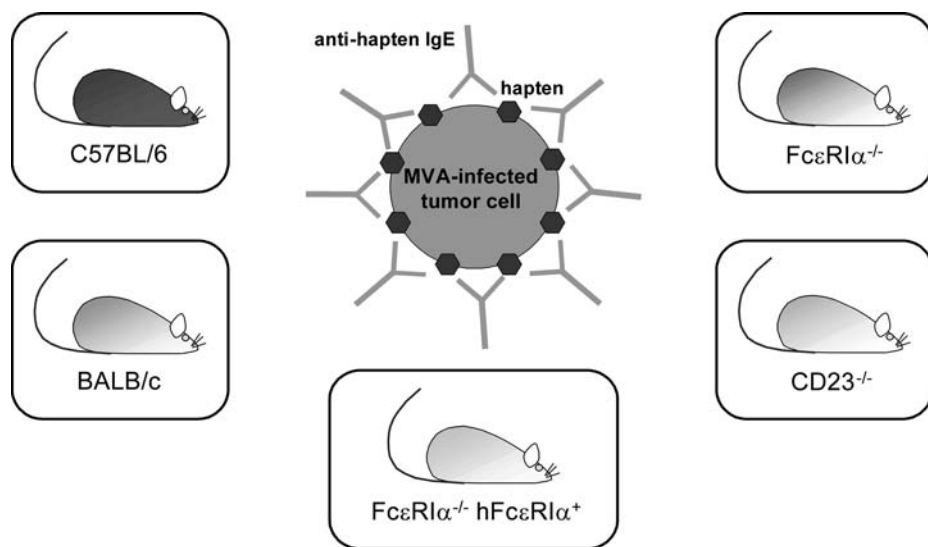


Fig. 9.3 Schematics of mice and MVA-based cell vaccination systems. Cellular anti-tumor vaccines are based on MVA infection and IgE loading via haptenization. The mice model system shifted from C57BL/6 to BALB/c given the availability of several transgenic models in the BALB/c background

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

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

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

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

9.2.7 Implementation of Mouse Membrane-Bound IgE by Recombinant MVA

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

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

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

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

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

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

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

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

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

9.3 Conclusion

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

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

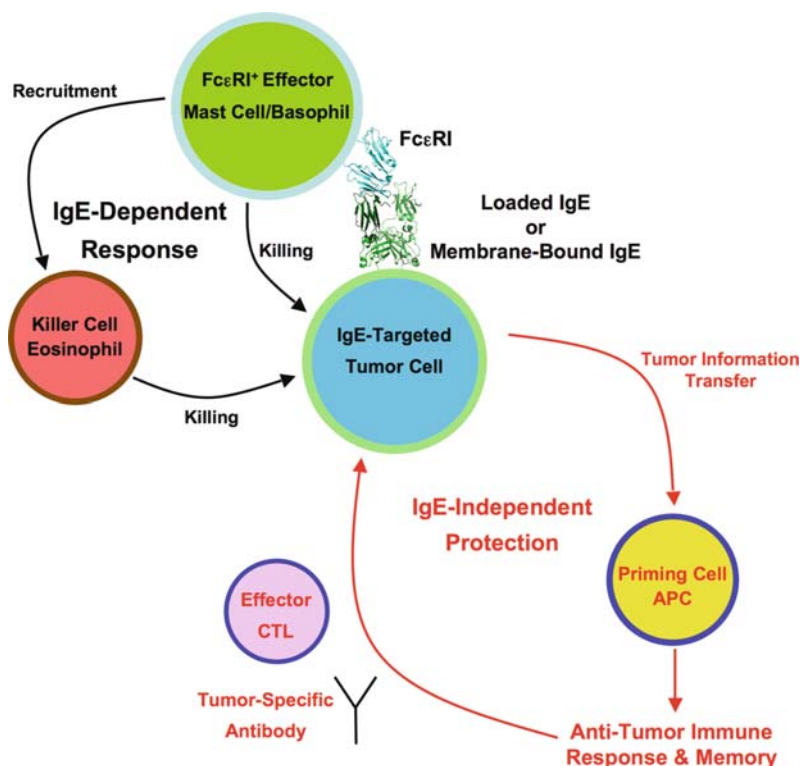


Fig. 9.4 Overview on the mechanisms behind IgE adjuvanticity in anti-tumor vaccination. The crystal structure of the complex between FcεRI α-chain D1D2 and IgE Cε3Cε4 domains [39] is shown to visualize the IgE–FcεRI interaction occurring between tumor cells and FcεRI⁺ cells, regardless of the IgE targeting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

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