

Chapter 8

IgE Interacts with Potent Effector Cells Against Tumors: ADCC and ADCP

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Abstract We examine the properties of IgE antibodies which may render them effective against tumors. This may be due to the uniquely high affinity of IgE antibodies for their Fc epsilon (Fcε) receptors and the expression of IgE antibodies on potent effector cells. Owing to these properties, IgE antibodies activate effector cell mechanisms that may be different from and stronger than those mediated by antibodies of other classes, thereby inducing superior anti-tumor responses. In examining the potential of IgE–FcεRI complexes on effector cells to activate effector cells (and describe evidence to-date) we suggest that the interactions of IgE antibodies with tumor-associated antigens on tumor cells and Fc receptors on IgE receptor-bearing cells trigger the association of tumor and effector cells. The outcome of these interactions is effector cell activation and effective tumor cell death by a number of mechanisms including antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP).

8.1 IgE Operates Through Powerful Fc Epsilon Receptors

IgE has two known receptors: the high-affinity receptor FcεRI and the low-affinity receptor CD23 (FcεRII) [1, 2]. FcεRI and CD23 are specific for the Fc epsilon (Fcε) region of the antibody. The IgE-binding molecule galectin-3 binds to oligosaccharides of IgE [3].

8.1.1 The High-Affinity IgE Receptor FcεRI

In humans, FcεRI is found on a variety of immune cells, namely, mast cells, basophils, eosinophils, monocytes, Langerhans cells, dendritic cells (DC) and platelets. There are two forms of FcεRI: the tetrameric form ($\alpha\beta\gamma_2$) is expressed

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on mast cells and basophils, and the trimeric form ($\alpha\gamma 2$) is found on monocytes [4], eosinophils [5–8] and antigen-presenting cells (APC) [9–12]. The IgE α subunit with two immunoglobulin extracellular domains contains the IgE-binding site with a ligand:receptor stoichiometry of 1:1, while the β and γ subunits have no role in binding IgE. The γ -chain, a ubiquitous signal transduction subunit, is shared by a number of immunoreceptors, and notably IgG receptors Fc γ R [13] and the IgA receptor Fc α RI [14] on a range of hematopoietic cells. It is essential for cell surface expression of the receptor complexes and is known to enhance intracellular signaling [15, 16]. The presence of the β -chain serves as an amplifier of IgE-mediated functions both *in vitro* and *in vivo* [17, 18], is known to enhance expression of unoccupied receptor on the cell surface [19] and is therefore, at least in part, responsible for the observed high levels of Fc ϵ RI expression in mast cells and basophils [20]. Ligation of the receptor by IgE and cross-linking by antigen results in the activation of multiple signaling pathways, leading to a variety of effector cell responses (Fig. 8.1). Unlike humans, mice express only the tetrameric form ($\alpha\beta\gamma 2$), and the distribution of Fc ϵ RI is restricted to mast cells and basophils [1, 15].

The affinity of IgE for Fc ϵ RI ($K_a = 10^8 - 10^{11} \text{ M}^{-1}$) is 2–5 orders of magnitude higher than that of IgGs for their receptors [4, 21] (Table 8.1, Fig. 8.1). The high affinity of the receptor–antibody interaction may have profound implications for both the potency of IgE-mediated effector functions and the longevity of IgE-mediated immune responses. Due to the high affinity of the IgE–Fc ϵ RI complex,

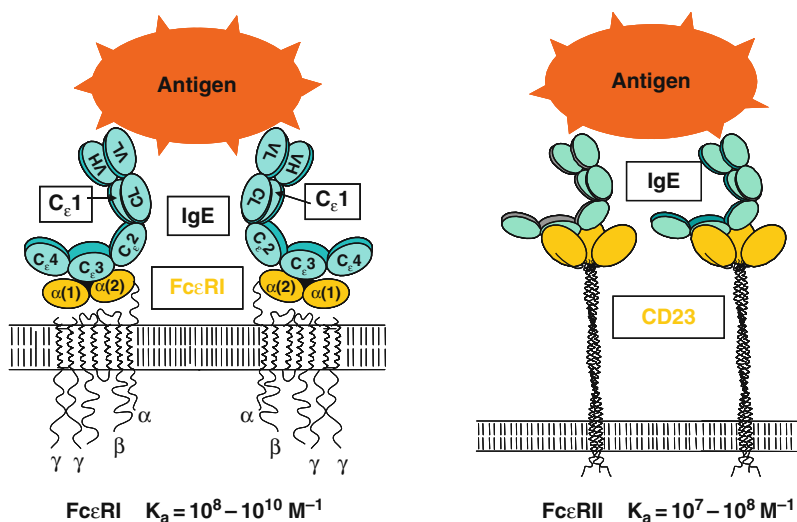


Fig. 8.1 Schematic representation of IgE antibody binding to the extracellular domains of the Fc ϵ RI α -chain (*left*) and to the lectin domains of CD23 (*right*) and reported binding affinities for each interaction. Cross-linking of surface-bound IgE–Fc ϵ receptor complexes by antigens triggers effector cell activation

Table 8.1 Binding affinities of Immunoglobulins for their Fc receptors

Fc receptor	Ig ligand	Affinity (K_a)	References
FcαR	IgA	10^6 M^{-1}	Wines et al. [130]
FcεRI	IgE	$10^8\text{--}10^{11}\text{ M}^{-1}$	Gould et al. [1]
FcεRII (CD23)	IgE	$10^7\text{--}10^8\text{ M}^{-1}$	Gould et al. [1]
FcγRI (CD64)	IgG	$10^8\text{--}10^9\text{ M}^{-1}$	Ravetch and Kinet [4]
FcγRII (CD32)	IgG	$<10^7\text{ M}^{-1}$	Maenaka et al. [21]
FcγRIII (CD16)	IgG	10^5 M^{-1}	Maenaka et al. [21]

IgE is the only antibody class that can be strongly retained by effector cells in the absence of antigen. The high affinity of IgE for FcεRI may account for the long half-life of IgE in tissues (around 2 weeks measured in skin), which is longer than that measured for IgG (2–3 days). The relative retentions of each antibody class in tissues are proportional to the affinity constants [1, 4]. The high affinity of the IgE–FcεRI complex may suggest the potential of IgE to engage FcεRI in superior anti-tumor cell responses in tissues compared to those mediated by IgG and its receptors. This may be because FcεRI on the surface of effector cells recruited into tissues may sequester IgE.

A number of studies on the emerging protective role of IgE in parasitic infections implicate the requirement for FcεRI expressed on the surface of mast cells to mediate the inflammatory cascades that confer protection against parasites [22–24]. The roles FcεRI play in allergic responses, both in the “early phase,” involving mast cell activation and degranulation, and the “late phase,” involving recruitment and activation of inflammatory cells, have been extensively studied [25]. FcεRI-expressing tissue mast cells and blood basophils bind antigen-specific IgE and on cross-linking by multivalent antigen release potent inflammatory mediators, such as histamine, leukotrienes, proteases and a variety of Th2-type cytokines, initiating and enhancing the recruitment and activation of T cells, monocytes, eosinophils, basophils and APC to the site of antigen challenge (Fig. 8.1). The resulting inflammatory response may also serve to activate and perpetrate immune effector functions in situ. In addition, local or recruited IgE-sensitized APC promote IgE production by B cells, thus maintaining mast-cell and APC sensitization and long-term immunologic memory and immune surveillance both in situ and systemically.

IgG receptors on the surface of effector cells are capable of mediating anti-tumor responses. FcγRIII (CD16), which is the nearest IgG receptor homologue to FcεRI, containing the ligand-binding α-chain with the closest homology to the α-chain of FcεRI, and which has a very low affinity for IgG ($K_a = 10^5\text{ M}^{-1}$) [21] (Table 8.1), has been shown to participate in IgG antibody-dependent T-cell-, natural killer (NK) cell- and macrophage-mediated tumor cell cytotoxicity [antibody-dependent cellular cytotoxicity (ADCC)] [4, 26–28]. FcγRIII has also been reported to engender macrophage-mediated tumor cell phagocytosis [antibody-dependent cellular phagocytosis (ADCP)] [29]. The high-affinity IgG receptor, FcγRI or CD64, with affinity for IgG ($K_a = 10^8\text{ M}^{-1}$) two orders of magnitude lower than that measured for

the IgE–FcεRI complex [4], can mediate both ADCC and ADCP of tumor cells [30–33]. Since FcγRs confer anti-tumor responses, it is likely that the potency of engaging IgE with FcεRI in parasitic infections and allergies may also be harnessed to combat cancer cells. The high-affinity of IgE–FcεRI complexes implies that strategies aimed at engaging IgE–FcεRI against tumor cells may confer superior anti-tumor cell responses in tissues compared to those mediated by IgG and its receptors.

8.1.2 The Low-Affinity IgE Receptor (CD23)

The human low-affinity IgE receptor, CD23 a type II integral membrane protein, is different from all other immunoglobulin receptors in that it belongs to the C-type lectin superfamily. Its two splice variants, CD23a and CD23b, differ in their first six (CD23b) or seven (CD23a) amino acids at the cytoplasmic N-terminus and contain different signaling motifs that determine their functions [34]. Human CD23a is expressed only on antigen-activated B cells prior to differentiation into antibody-secreting plasma cells and is involved in IgE antibody-dependent antigen presentation and regulation of IgE synthesis. The expression of human CD23b is induced by IL-4 on various inflammatory cells, for example, T cells, eosinophils, platelets, monocytes, B cells, DC and epithelial cells [1, 35]. Engagement of CD23b by IgE–antigen complexes promotes monocyte/macrophage activation, induces nitric oxide synthase (iNOS) and generates pro-inflammatory cytokines [36, 37] (Fig. 8.1). These processes are thought to play a role in clearance of parasitic infections through the release of TNF-α and nitric oxide (NO) [38, 39]. A natural function for human CD23b is IgE antibody-dependent cellular ADCP of hapten-coated red cells by monocytes/macrophages [35, 40], suggesting that CD23 may mediate this function against tumor cells.

In humans, IgE binds to CD23 with relatively high affinity ($K_a = 10^7 - 10^8 \text{ M}^{-1}$), as high as that of IgG for its high-affinity receptor FcγRI [1, 4] (Table 8.1, Fig. 8.1). Numerous studies now describe the functions of CD23 inducing inflammatory and memory responses in response to allergens and parasitic infections. There are also reports that tumor-associated macrophages (TAMs) express CD23 in a number of human cancers [41]. Furthermore, the soluble fragments of CD23 (sCD23) have also been associated with blood-borne cancers. Increased sCD23 levels are associated with poorer patient survival in chronic lymphocytic leukemia [42]. It is conceivable that sCD23 may be elevated in certain malignancies, resulting in blocking the formation of IgE–FcεRII complexes on the surface of effector cells, thereby interfering with IgE-mediated anti-tumoral responses. However, a potential role for CD23 in tumor killing is only starting to emerge [43, 44]. The effector cell functions mediated by CD23 in the context of allergies and parasitic infections, coupled with its comparatively high affinity for IgE, may make it a potentially effective IgE receptor for mediating tumor cell targeting and killing.

8.1.3 *Galectin-3*

Galectin-3, also known as the beta-galactoside-binding lectin galectin-3, epsilon-binding protein (e-BP), IgE-binding protein or Mac-2, binds specifically to oligosaccharide structures with a terminal β -galactose through a lectin–carbohydrate interaction [45]. It recognizes both IgE and Fc ϵ RI and can activate immune responses [3, 46]. Due to its ability to form pentameric complexes with oligosaccharides, it can crosslink Fc ϵ RI or receptor-bound IgE on the surface of mast cells and basophils [47, 48]. Despite its potential to enhance mast cell activation and mediate the phagocytotic properties of monocytes, increased expression of galectin-3 has been associated with poor prognosis in thyroid and pancreatic cancers, whereas reduced levels of the protein were detected in colon, ovarian, breast, endometrium and skin cancers, compared to normal tissues [49]. Galectin-3 expression in malignancies may have a bearing on the immunogenic nature of the tumor, but this has not yet been fully explored. Although expressed in monocytic cells, at least in one in vitro study, galectin-3 did not appear to mediate tumor cell death [43]. To-date, there is no evidence linking galectin-3 to IgE antibody-dependent tumor cell killing although further research may shed more light on potential mechanisms that may link galectin-3 to IgE-mediated immune cell activation signals against cancer cells.

8.2 What is Known About IgE Effector Cells in Cancer? Missing Activation Signals May Tip the Balance in Favor of Tumor Growth

The relationship between tumor cells and the immune system is vastly complex. The dialog between tumor cells and the immune system in cancer results in inflammation and tumor regression but has also been associated with tumor growth, angiogenesis, invasion and metastasis. Patient prognoses for some cancers also appear to be dependent on the nature of the immune responses elicited in the tumor microenvironment; positive and negative correlations between immune infiltrate and clinical outcome have been reported. It is well established that solid tumors are associated with inflammatory responses resulting in infiltration of mast cells, B and T lymphocytes, neutrophils, NK cells, DC, macrophages and eosinophils [50]. These cells are recruited by a variety of cytokines and chemokines expressed by local inflammatory cells that bind to upregulated adhesion molecules and cell surface receptors in situ. Tumor cells also secrete cytokines and chemokines that contribute to this process; often, this promotes angiogenesis, tumor growth and metastasis [51].

Most of the inflammatory cell infiltrates are IgE effector cells. These cells have the potential to direct strong anti-tumor activities in situ that may tip the balance

against tumor growth and invasion. Herewith, we describe the relationship between these effector cells and cancer tissues and how effector cell activities are influenced by tumor cells.

8.2.1 Mast Cells and Basophils

Tryptase-positive mast cells infiltrate the invasive fronts in greater numbers than in any other area of malignant tumor lesions, but their contribution to cancer growth and spread has been controversial [52]. In breast cancer the presence of mast cells in the peritumoral areas has correlated with a favorable clinical prognosis [53, 54]. Hodgkin's lymphoma, endometrial cancer and melanoma patients with higher numbers of mast cells in tumor lesions have a worse prognosis, whereas mast cell numbers in lymph nodes do not correlate with clinical outcomes in Hodgkin's lymphoma [55–57]. Increased mast cell numbers in breast cancer lesions and lymph nodes with metastases have been reported compared to those from benign lesions and from lymph nodes clear of metastatic disease [58–60].

Mast cell activation and degranulation promotes the destruction of local tissue architecture, tissue remodeling and angiogenesis, and this is thought to assist tumor cell growth and metastasis [61]. A number of factors may also contribute to the inability of resident mast cells to mount effective immune responses against tumors. One reason may be the location of mast cells in the invasive fronts, away from the core of solid tumors, suggesting that mast cells may respond to tumor cell stimuli and may be recruited to assist tumor growth and invasion. Another factor may be the production of tryptase, this may result in the loss of local tissue architecture that promotes tumor invasion but also in stimulating proliferation of vascular endothelial cells, promoting tumor-associated neovascularization. The production of angiogenic factors and cytokines by mast cells in response to tumors (VEGF, TGF- β , FGF-2, IL-8, TNF- α , MMP-9) appears to tip the balance in favor of tumor growth and metastasis [62–66]. It has been suggested that the intensity of mast cell activation/response in the absence of antigenic stimuli may be a contributing factor in this process [61, 67]. Since mast cells are potent IgE effector cells, it is therefore tempting to consider that treatment with tumor antigen-specific IgE antibodies may stimulate mast cells against cancer cells and help redress the balance in favor of immune activation and IgE-mediated anti-tumoral responses.

The role of basophils in cancer has not been extensively elucidated. One notable exception is perhaps blood malignancies such as chronic myeloid leukemia, where basophil numbers are increased in later stages of the disease and basophil markers are used for diagnostic but also prognostic evaluations [68]. Basophils have also been observed in the inflammatory infiltrate in experimental tumors. They may be recruited into tumor areas by in situ expression of cytokines such as IL-4 and in response to chemoattractant signals like VCAM-1 and MCP-1 [69] and are thought to be associated with tumor regression. Their potential IgE-mediated anti-tumor functions are yet to be explored.

8.2.2 *Monocytes/Macrophages*

TAMs are found in virtually all types of tumors and can comprise more than 50% of the total tumor mass in both primary and metastatic lesions [70]. Blood monocytes are constantly recruited to the tumor sites by chemokines and cytokines released by tumor cells (CSF-1, CC chemokines) and neighboring endothelial cells (VEGF). Evidence suggests that TAMs switch off anti-tumor immunity and are associated with poor clinical outcomes [71]. Once in tumor sites, TAMs can function by promoting tumor growth and metastasis, producing angiogenic factors and metalloproteinases [72, 73]. Macrophage infiltration of various tumors such as melanoma, stomach and colorectal cancers has been correlated with immunogenicity but also inversely correlated with metastatic potential, while also the opposite has been reported for cancers of the breast, prostate and endometrium.

Macrophages from normal and infected tissues have been shown to lyse tumor cells, present tumor-associated antigens to T cells and express co-stimulatory factors that enhance T cell and NK cell anti-tumor functions [74]. On the contrary, TAMs are generally thought to be immature in their phenotype and this may be partly due to their in situ exposure to tumor cell and immune cell-secreted cytokines such as IL-4, IL-10 TGF- β 1 and prostaglandin E2. It has been proposed that these signals polarize macrophages into the alternatively activated type II or M2 macrophage phenotype. M2 TAMs constitutively express high levels of IL-1 β and IL-6 and low levels of TNF- α . They are thought to have impaired antigen-presenting capacity, can suppress activation of T cells, induce T cell apoptosis, switch off DC maturation through secretion of IL-10 and engage in tissue repair and remodeling in favor of tumor growth, angiogenesis and invasion [73, 75].

TAMs accumulate in hypoxic tumor regions where they produce iNOS-derived NO, which can induce tumor cell apoptosis. However, it has been suggested that cytotoxic mediators, such as NOs that are produced by monocytes/macrophages in cancer tissues, are modulated by tumor cells. Arginase expression by TAM is thought to be a tumor-induced mechanism preventing formation of sufficient NO concentrations that are cytotoxic to tumors [76]. This may ensure that iNOS expression and/or activity are reduced, leading to lower levels of NO production than required for tumor cell cytotoxicity. Tumor-induced modulation of NO production is also potentially anti-apoptotic and/or pro-angiogenic. Thus, in tumors the balance of NO production appears tipped in favor of tumor growth [77, 78].

Although TAMs are known to be “conditioned” by tumor cells in situ to tumor growth, they also harbor the potential to become activated to kill tumor cells and release tumorigenic, pro-apoptotic and angiostatic compounds [79–82]. There are on-going studies evaluating targeted therapies aimed at exploring the plastic nature of these cells to this effect [83, 84].

So could TAMs be primed by IgE antibodies to turn against tumor cells? Immunohistochemical studies in breast cancer confirm the presence of CD23 on the surface of TAMs and the ability of this receptor to mediate expression of iNOS in human macrophages [36, 41, 85, 86]. Engaging CD23 by IgE and antigen on the surface of monocytic cells induces production of inflammatory mediators such

as TNF- α and iNOS in inflammatory responses in allergies and parasitic infections [85–88]. It is therefore conceivable that tumor antigen-specific IgE-mediated signals could target circulating monocytes and TAMs, enhance their tumor killing mechanisms and induce an allergy-like anti-tumor response in situ. Numerous findings now suggest that macrophages associated with tumors may be activated by IgE antibodies to kill tumors and that the pro-tumor growth signals may not be an inevitable fate of monocytes/macrophages. Future studies may help elucidate the potential of IgE antibodies in providing crucial signals that recondition TAMs to target immune responses against tumor cells.

8.2.3 *Eosinophils*

Infiltrating eosinophils are associated with a significantly better prognosis in some malignancies (esophageal squamous cell carcinoma, gastric cancer, head and neck cancer and colorectal carcinoma) [89–92]. Eosinophils contain cytotoxic granule proteins, such as major basic protein (MBP), eosinophil peroxidase, eosinophil cationic protein and eosinophil-derived neurotoxin, which are capable of inducing tissue damage and dysfunction [93], enhancing local inflammation and immune responses. Some, like eosinophil-derived neurotoxin, can induce tumor cell apoptosis and have been considered as potential treatments for cancer [94]. Eosinophil-derived cytotoxic mediators could therefore be harnessed to kill tumor cells. Several studies indicate that cytokines such as IL-4 or chemokines such as eotaxin-1 are associated with the inhibition of tumor cell growth and tumor cell killing [95, 96].

A number of experimental models have demonstrated that eosinophil infiltration in solid tumors is correlated with tumor eradication and evidence suggests that eosinophils participate in tumor cell killing in vitro and also in anti-tumor immune surveillance in vivo [97, 98]. It is also of great importance that eosinophils can exert potent anti-tumor activities in Th2 cytokine-rich environments, normally associated with immune tolerance in cancer. In a recent study of mouse melanoma, eosinophils were seen to be concentrated in necrotic areas and capsule regions of the tumors, where evidence of eosinophil MBP suggested extensive degranulation and eosinophil participation in tumor necrosis [99]. Eosinophil recruitment was a dynamic and an on-going process that occurred independently of Th2-dependent immune responses, implying that these cells can perform potent anti-tumoral functions in situ. In a separate study of CTL-resistant mouse melanoma, regression of tumor metastases was directly associated with tumor-associated eosinophil activation via STAT6, degranulation and secretion of eotaxin-1 and clearance of lung and visceral metastases [100].

Although eosinophils express exceedingly small numbers of IgE receptors on their cell surfaces [7, 101, 102], engagement of receptors with IgE has been shown to activate these cells to kill tumor cells [44]. The role of these cells in protecting against parasitic infections by ADCC mechanisms has also been reported

[103]. Eosinophils may also co-operate with other IgE effector cells such as monocytes/macrophages in tumor clearance, and this has been demonstrated in a number of in vivo studies [97, 104, 105]. The anti-tumoral potential of these potent IgE effector cells is yet to be fully realized. It certainly appears that the cells possess tumor-killing potential in situ, which may further be enhanced by implementation of IgE antibody therapies for cancer.

8.2.4 Natural Killer Cells

As essential effector cells of the innate response, NK cells have been associated with successful defenses in vivo, tumor cell killing capacity ex vivo and better patient prognosis in a number of cancers such as multiple myelomas, breast and non-small cell lung cancers [106–108]. NK cell anti-tumoral activities are also thought to form part of the clinical benefits conferred by therapeutic antibodies such as trastuzumab in breast cancer patients [109]. Fc ϵ RI expression has not been detected in NK cells, and IgE-mediated anti-tumoral activities may not be mediated via this receptor. However, NK cells are able to mediate ADCC through engagement of Fc receptors by antibodies; indeed, it has been demonstrated that FcR gamma subunit-deficient mice exhibit loss of phagocytic and ADCC functions, suggesting the potential for antibodies to activate NK cells against cancer cells [110]. NK cells in breast cancer tissues can secrete enhanced levels of IL-4, compared to those secreted by NK cells from patient blood. Increased levels of tumor-associated IL-4 would upregulate CD23b on resident and tumor-infiltrating monocytes/macrophages [111], possibly enhancing their tumor-targeting and killing potential. The potential effects of tumor antigen-specific IgE antibodies on NK cell anti-tumor activities still remain to be elucidated.

8.2.5 Antigen-Presenting Cells

APC such as B lymphocytes, monocytes/macrophages, Langerhans cells and DC in tumor infiltrates express CD23 and/or Fc ϵ RI. It has been proposed that one of the immune escape mechanisms of tumor cells is the pathway inducing defective differentiation and maturation of APC, thereby reducing adaptive immune responses against tumor antigens. IL-6 secreted by tumor cells and resident TAMs in tumor microenvironments is thought to inhibit the differentiation of CD34+ cells into mature DC and thus reduce their antigen-presenting capacity. It has also been demonstrated that immature DC can be activated by locally secreted lymphokines in the tumor microenvironments, such as IL-4, IL-13 or IFN- γ ; these have been shown to rescue maturation of DCs known to be blocked by tumors [112, 113].

Engagement of IgE with the high-affinity IgE receptor Fc ϵ RI on the surface of DC increases the efficiency of antigen uptake and presentation by 100- to 1,000-fold [114]. This may be significant in the context of IgE therapy against tumors,

since even small amounts of IgE on the surface of DCs may be sufficient to lead to an efficient activation of autologous T-cells and thus provoke powerful anti-tumoral adaptive immunity. Furthermore, expression of CD23 has been detected at different stages of DC maturation and CD23 expression has also been detected, albeit at lower levels, under immunosuppressive conditions induced by tumor cells [113]. This suggests that DCs harbor the potential to engage IgE via CD23, leading to antigen presentation. Although the outcome of the antigen presentation depends on the local environment, tumor antigen-specific IgE antibodies may contribute to enhance maturation of DC as well as stimulate effective adaptive immune responses against cancers [115–118].

The role of B lymphocytes in cancer has been controversial. In some in vivo models of colon carcinoma and melanoma, B cells possess tumor growth-promoting functions, possibly through secretion of immunomodulatory cytokines such as IL-6 and IL-10 [119–121]. It is conceivable however, that even within the immunosuppressive environments of tumors, tumor antigen-specific IgE antibodies may tip the balance against tumor growth by exploiting the potent mechanisms of B-cell activation, antigen presentation and induction of adaptive immunity, associated thus far with IgE in allergic diseases and immune responses against parasites.

8.3 Evidence for IgE and Effector Cell-Mediated Anti-Tumoral Responses

Is IgE a possible missing link with potential to “re-educate” immune effector cells against cancer? The benefits of IgE-mediated effector functions against tumor cells have been emerging in a number of in vivo and in vitro systems. Mounting evidence also points at the importance and pivotal role of IgE effector cells in mediating IgE inflammatory responses against tumors and a possible role for IgE in promoting adaptive immunity. Exciting insights into mechanisms of effector cell activation and tumor cell targeting and killing are also being elucidated.

8.3.1 Evidence for PBMC and Monocyte Activities in IgE-Mediated Anti-Tumoral Responses

Tumor killing effector functions mediated by antibodies have been previously evaluated in ADCC assays using human peripheral blood mononuclear cells (PBMC) to target tumor cells. IgE-mediated anti-tumoral effector cell functions have also been studied in similar assays and in some cases in direct comparison to corresponding IgG antibodies. Evidence now suggests that primary monocytes/macrophages and monocytic cells engaged with tumor antigen-specific IgE have the potential to kill tumor cells. We describe what has been learned about PBMC and monocyte/macrophage anti-tumoral activities mediated by IgE.

8.3.2 Chimeric Ovarian Carcinoma Antigen-Specific MOv18 IgE – Efficacy and Effector Cells

The potency of effector functions activated by tumor antigen-specific IgE has been explored in a series of studies on chimeric antibodies (with human constant regions and mouse variable regions) MOv18 IgE and MOv18 IgG1. These antibodies were engineered from the original MOv18 clone against a tumor-specific antigen, folate-binding protein (FBP), expressed in 80% of ovarian cancers. IgE and IgG were compared in two disparate human xenograft models of ovarian carcinoma grown in immunodeficient mice. Treatment with IgE afforded superior protection than the equivalent IgG1 antibodies in both systems [122, 123].

In one human ovarian carcinoma xenograft model, grown subcutaneously in severe combined immunodeficiency (SCID) mice, intravenous treatment of the mice with MOv18 IgE combined with human PBMC, added as effector cells, had a superior and longer-lasting effect in restricting tumor growth than the same treatment with IgG1 [122]. IgE-mediated effects on tumor growth were only observed when antibodies were combined with human PBMC, and as the human Fc ϵ region of the chimeric IgE is not recognized by mouse Fc ϵ receptors, it became apparent that the human IgE effector cells were necessary for the anti-tumoral functions of IgE. Histological examination of tumor sections from mice treated with MOv18 IgE exhibited larger areas of tumor necrosis, compared to controls, further suggesting that anti-tumoral effector cell functions were activated in response to IgE treatment. This study clearly pointed at the potentially superior effector functions of IgE as a cancer therapeutic.

Subsequent studies in a patient-derived human ovarian carcinoma model, grown orthotopically (intraperitoneally) in nude mice, demonstrated that intraperitoneal administration with MOv18 IgE and human PBMC gave significantly greater protection than PBMC alone, while MOv18 IgG1 with human PBMC offered no survival advantage (Fig. 8.2A) [123]. Immunohistochemical studies of tumor sections showed infiltration of human monocytes/macrophages (CD68+) into tumor lesions, while no human macrophage infiltrates were found in tumors from mice treated with human PBMC alone (Fig. 8.3). Monocyte/macrophage infiltration correlated with significantly enhanced survival of mice given tumor antigen-specific IgE. Immunohistochemical studies examining the presence of other human PBMC effector cells in xenograft tumor areas failed to identify any infiltrating (CD3+) T cell, (CD19+ and CD20+) B cell or (CD56+) NK cell populations associated with tumors. These observations suggested that human macrophages may be important IgE effector cells that are recruited by tumor antigen-specific IgE in tumor areas and their presence may contribute to tumor growth arrest in this *in vivo* model.

More recent studies utilizing the ovarian carcinoma xenograft model confirm a vital role of monocytes as IgE effector cells in anti-folate receptor IgE antibody responses *in vivo* [43, 44]. Depletion of monocytes from PBMC abrogated the survival advantage conferred by MOv18 IgE, while reconstitution of monocytes in the PBMC treatments restored IgE-mediated enhanced survival of mice (Fig. 8.2B,

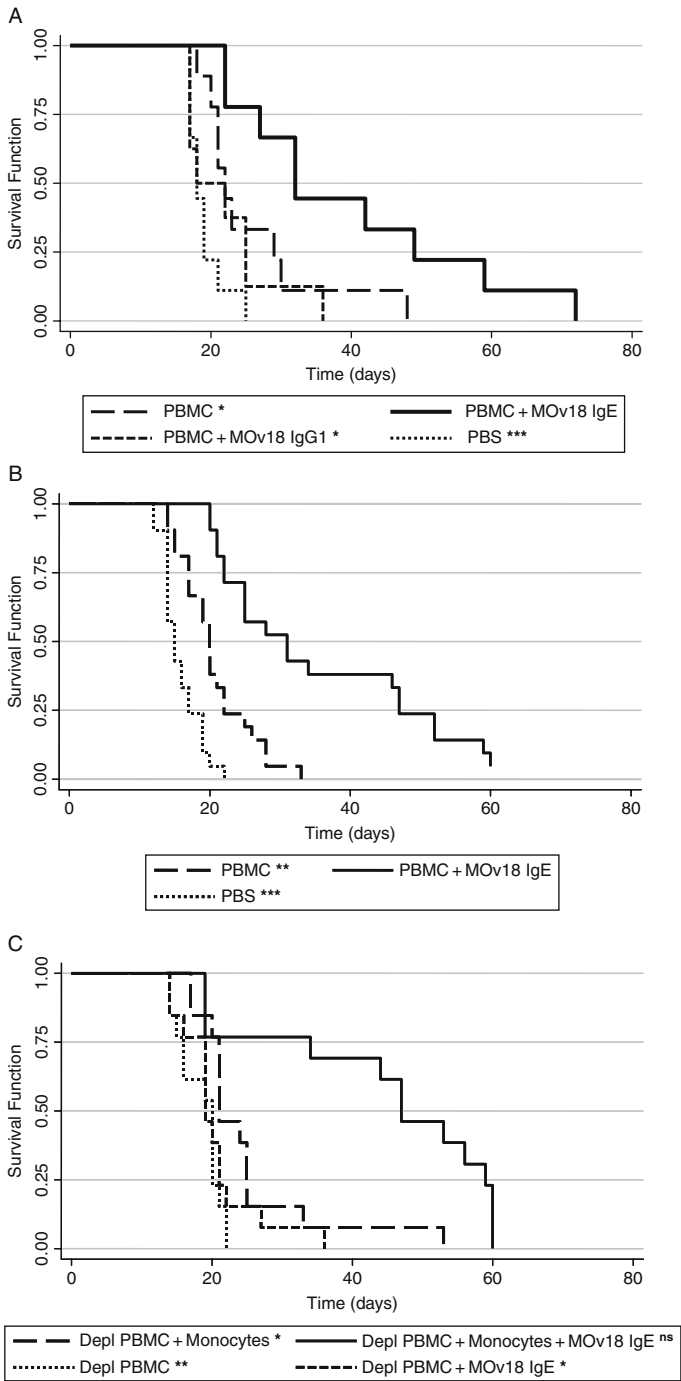


Fig. 8.2 (continued)

8.2C) [44]. In a separate study, U937 monocytic cells, which express Fc ϵ RI and low levels of CD23, when administered in combination with MOv18 IgE prolonged the survival of ovarian carcinoma-bearing mice compared to controls [43]. These findings confirmed that monocytes are important effector cells in tumor antigen-specific IgE-mediated responses *in vivo*.

8.3.3 Lessons on the MOv18 IgE-Mediated Tumor Killing Mechanisms – ADCC

The above data demonstrating tumor infiltration by monocytes and their significant involvement in prolonging survival of tumor-bearing mice were complemented by *in vitro* assays examining the interactions between monocytes and tumor cells mediated by IgE. A number of these studies not only implicated monocytes as IgE effector cells but also gave valuable insights into the mechanisms of IgE-mediated tumor targeting and killing.

In preliminary *in vitro* ^{51}Cr -release (ADCC) assays examining MOv18 IgE-mediated effector functions, MOv18 IgG and human PBMC conferred superior ADCC of FBP-expressing IGROV1 ovarian tumor cells compared to those of MOv18 IgE and PBMC [122]. In a subsequent study, using two ADCC assay systems, levels of IgE-mediated ADCC of tumor cells by PBMC were 50% lower compared to those measured with the corresponding IgG1 [123]. These data were in stark contrast to the clear superiority of MOv18 IgE over IgG1 in two human ovarian carcinoma xenograft models *in vivo*. A number of important findings and insights arose from these observations:

- a) Long retention of IgE–Fc ϵ RI complexes in tissues: The intrinsic affinity of IgE–Fc ϵ RI is at least 10^2 higher than that of IgG–Fc γ R complexes, reflected in prolonged half-life of IgE (~14 days) in tissues compared to IgG (~2–3 days). This may be translated to recruitment and retention of antibody in tumor areas and may contribute to the superior efficacy of IgE *in vivo*. The opposite is true



Fig. 8.2 (A) Survival of ovarian carcinoma human xenograft-bearing nude mice: survival (days) of nude mice injected with a patient-derived intraperitoneal xenograft is enhanced following treatment with chimeric folate receptor-specific antibody MOv18 IgE together with human PBMC (solid line). Survival is plotted for each of nine animals treated as shown. Data from Karagiannis et al. [123]. Copyright Wiley-VCH Verlag GmbH, and Co. KGaA. Reproduced with permission. (B) Effect of monocytes and MOv18 IgE antibody on survival of human ovarian carcinoma xenograft-bearing mice. Survival is superior in animals treated with the combination of MOv18 IgE and human PBMC. Survival is plotted for each of 13 animals treated as shown. (C) Impaired efficacy using PBMC depleted of monocytes can be salvaged by reconstituting PBMC with monocytes, implying that monocytes are necessary and sufficient for therapeutic IgE-mediated folate receptor targeting. Survival is plotted for each of 21 animals in each group, studied in the same experiment shown in (B). Data reproduced with permission from Karagiannis et al. [44]. Significance of values compared with samples given PBMC + MOv18 IgE by the Student's *t* test. n/s, $p > 0.05$; * $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$

- for IgE in circulation (half-life ~2 days) compared to IgG (~20 days) and may be reflected in the lower tumor killing capacity of IgE compared to IgG in vitro.
- b) Effects of Fc ϵ receptor density on tumor killing by monocytes: The nature and density of Fc receptors expressed on the surface of human peripheral blood monocytes were examined. No CD23 expression was detected and the abundance of Fc ϵ RI on monocytes (~2,900 molecules/cell) is about 100 times lower than that on mast cells or basophils and 50–100 times lower than that of Fc γ R receptors on monocytes (~200,000 molecules/cell). Fc γ RIII is the IgG receptor with the closest homology to Fc ϵ RI and is also the Fc γ receptor implicated in IgG ADCC. Despite the 50-fold reduction in abundance of Fc ϵ RI than Fc γ RIII on monocytes, MOv18 IgE was half as active in ADCC as MOv18 IgG1 in vitro [123], and this is most likely due to the high affinity of the IgE–Fc ϵ RI interaction.
 - c) Effect of Fc ϵ receptor occupancy on tumor killing by monocytes: Another significant observation was that human PBMC from allergic donors were less effective than those from non-allergic individuals in mediating IgE ADCC. Studies on occupancy of IgE receptors on monocytes demonstrated that levels of ADCC were proportional to the numbers of free IgE receptors on the surface of monocytes. These findings implicated Fc ϵ RI in the mechanism of IgE-mediated tumor killing by ADCC.
 - d) Role of Fc ϵ RI in tumor cell ADCC by monocytes: Purified human monocytes were able to kill tumor cells by ADCC in vitro. Since freshly purified blood monocytes were found to express low levels of Fc ϵ RI, but no CD23, this further implicated the high-affinity receptor in IgE tumor death by ADCC.

8.3.4 MOv18 IgE-Mediated Tumor Killing Mechanisms – ADCP

Early microscopical observations of effector–tumor cell interactions gave the first clue of another potential tumor killing mechanism not detected by ADCC assays [123]. Time course examinations of IgE-mediated effects of PBMC–tumor cell contact revealed that tumor cells and monocytes, but not T cells, B cells or NK cells

Fig. 8.3 (continued) sections of nude mice following i.p. tumor transplant and treatment with MOv18 IgE and human PBMC. Horizontal bars indicate the mean values. **(B)** Immunohistological examination of human and mouse macrophage infiltrates in sections of ovarian carcinoma xenografts grown in nude mice treated with MOv18 IgE and human PBMC. Human macrophages were rarely observed in ovarian carcinoma xenografts from mice treated with PBMC alone **(a)** compared with those treated with PBMC and MOv18 IgE **(b)**. Human macrophages are mostly seen in stromal areas adjacent to tumor islands **(b)**. Mouse macrophages are abundant in stromal areas surrounding tumor as well as in tumor islands whether mice were treated with PBMC alone **(c)** or PBMC and MOv18 IgE **(d)**. Figure from Karagiannis et al. [123], copyright Wiley-VCH Verlag GmbH, and Co. KGaA. Reproduced with permission

from PBMC, engaged in physical contact in the presence of MOv18 IgE. Monocyte–tumor cell contact and tumor cell death were observed as early as 20 min in culture and contact was maintained up to 3 h. ADCP of tumor cells by monocytes was evident from 90 min in cultures and always associated with loss of viability and/or membrane integrity of tumor cells.

These observations prompted a series of experiments aimed at measuring ADCP as well as elucidating the mechanisms underlying IgE-mediated ADCP of tumor cells. A new three-color flow cytometric (ADCC/ADCP) assay was developed to simultaneously measure cytotoxicity and phagocytosis of tumor cells (Fig. 8.4) [124]. There were already indications that the high-affinity IgE receptor, FcεRI, mediated ADCC of tumor cells, and further experiments confirmed this function (Fig. 8.5A). There were also enough data to suggest that the low-affinity IgE receptor, CD23b, which can be expressed in response to IL-4 on the surface of monocytes/macrophages, is capable of mediating ADCP and that it mediates IgE effector functions in allergic diseases and parasitic infections. Stimulation of monocytes by IL-4 induces expression of CD23. Expression of CD23 by monocytes enhanced their tumor killing capacity (from 25 to 30% without CD23 to up to 70% tumor cell death in vitro), and this increase in tumor cell death was attributed to tumor cell ADCP (Fig. 8.5B). Microscopical observations confirmed these findings and demonstrated that induction of CD23 expression resulted in enhanced monocyte–tumor cell contact and extensive phagocytosis of tumor cell material, which validates the findings of the ADCC/ADCP assays and the role of CD23 in tumor cell killing by ADCP [44]. A further hint of an anti-tumoral role for CD23 in vivo was provided by evidence that enhanced CD23 expression on the surface of monocytic cells in combination with MOv18 IgE was associated with increased

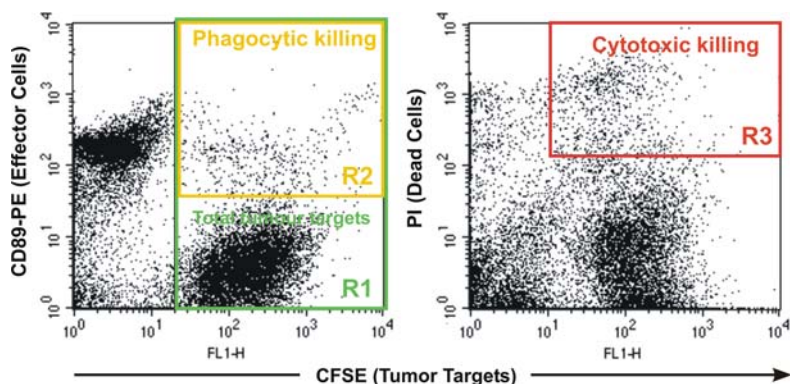


Fig. 8.4 A three-color flow cytometric assay can simultaneously measure ADCC and ADCP of tumor cells by effector cells. Dot plots of mixed monocyte effector and IGROV1 ovarian tumor target cells from which calculations were made. Region 1 (R1) represents total CFSE⁺ tumor cell targets. Region 2 (R2) depicts the CFSE⁺ tumor cells present within PE-stained monocytes (CFSE⁺/PE⁺), depicting ADCP. Region 3 (R3) contains tumor cells killed externally by effector cells (ADCC) (CFSE⁺/PI⁺). Data reproduced with permission from Karagiannis et al. [44].

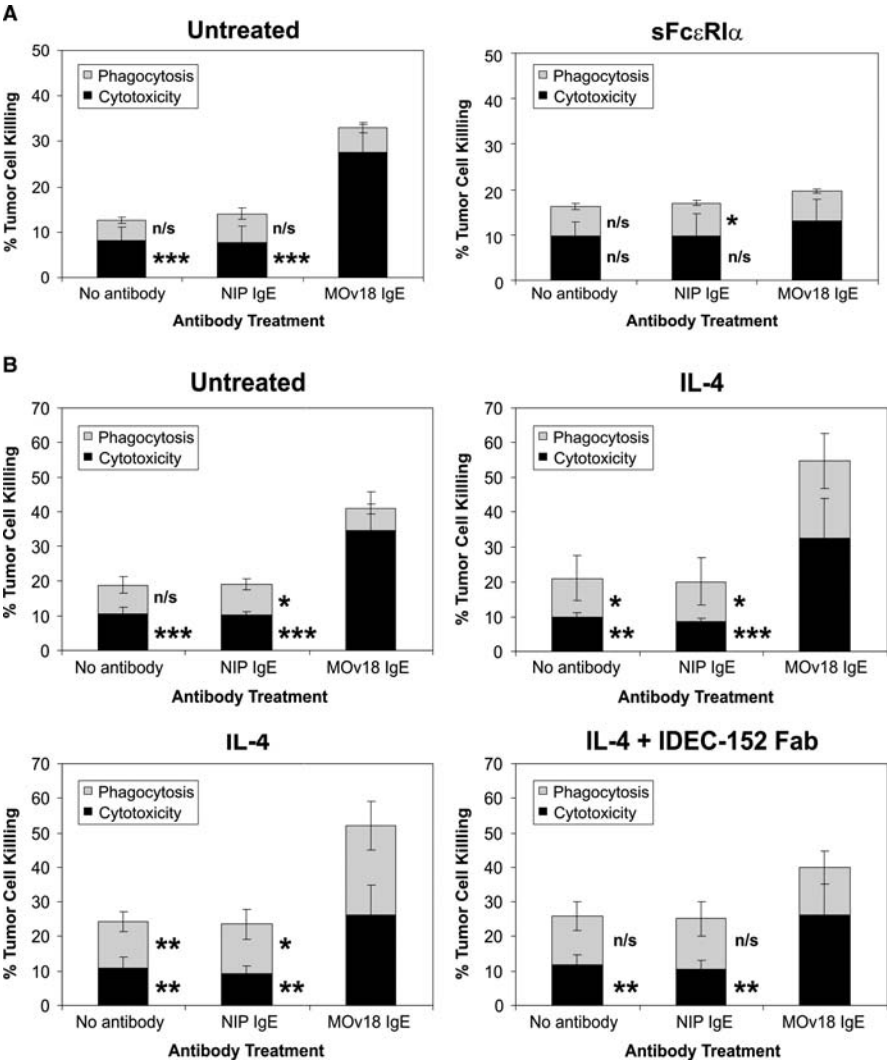


Fig. 8.5 (A) Monocytes can be activated by MOv18 IgE to kill tumor cells in vitro. The addition of MOv18 IgE to monocytes and IGROV1 tumor cells results in increased ADCC, compared to background levels measured with hapten-specific antibody (NIP IgE) or with no antibody controls (*left*). Blocking of MOv18 IgE binding to the IgE receptor, FcεRI, with soluble IgE receptor, correlated with loss of IgE-mediated killing of tumor cells (*right*). (B) CD23 role in MOv18 IgE-mediated ADCC of ovarian tumor cells. MOv18 IgE-mediated killing of IGROV1 tumor cells by untreated (*top left*) monocytes results in cytotoxic killing of tumor cells. Stimulation of monocytes with IL-4 enhanced the expression of CD23 (*top right*) and resulted in enhanced tumor killing capacity and killing by ADCP. ADCP killing of tumor cells by IL-4-stimulated primary monocytes (*bottom left*) was blocked by an anti-CD23 antibody Fab (*bottom right*), confirming a role for CD23 in tumor cell killing by ADCP. ADCP, black bars; ADCC, gray bars. Results shown are means $\pm$ SD of six independent experiments. Significance of values compared with samples given MOv18 IgE by the Student's *t* test. n/s, $p > 0.05$; * $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$. Reproduced with permission from Karagiannis et al. [44].

survival of human ovarian carcinoma xenograft-bearing mice compared to treatment with CD23-dim monocytic cells and MOv18 IgE .

From the volume of data showing that CD23-expressing monocytic cells accumulate in tumors where IL-4 is expressed, it is tempting to speculate that the “missing signal” in the form of a tumor antigen-specific IgE antibody may activate these cells to kill tumor cells and help reverse the tumor growth-promoting role of TAMs.

8.3.5 In Vitro Evidence from a Trastuzumab IgE Study

Further evidence that IgE antibodies are capable of activating monocytic cells to target and kill tumor cells was presented in a recent study examining the functional properties of an engineered IgE homologue to trastuzumab (trastuzumab-like IgE) against the human epidermal growth factor receptor 2 (HER2/neu), a tumor-associated antigen expressed in approximately 30% of breast carcinomas [125]. Trastuzumab (Herceptin[®]), a humanized IgG1, is the main antibody in clinical use for the treatment of early and metastatic breast cancers. Based on previous research on the effects of the chimeric MOv18 IgE in activating effector cells to kill tumors, and the clinical efficacy of trastuzumab, the potential anti-tumoral properties of a corresponding trastuzumab IgE merited examination. Trastuzumab (IgG1) and trastuzumab IgE were therefore compared in functional assays.

Trastuzumab-like IgE retained the well-characterized tumor cell growth arrest properties of trastuzumab (IgG1), independently of effector cells. Both trastuzumab and trastuzumab-like IgE could activate monocytic cells to kill tumor cells, and the levels of tumor death mediated by each antibody were comparable. However, they operated by different mechanisms: trastuzumab mediated tumor cell ADCP, whereas trastuzumab IgE killed tumor cells by ADCC after 2.5 h in culture. These distinct mechanisms of killing were confirmed by microscopic observations of monocytic cell–tumor cell interactions. Addition of trastuzumab and trastuzumab IgE induced enhanced monocytic-tumor cell contact, and ADCP of tumor cells was evident with trastuzumab. The antibody concentrations required to achieve maximum tumor cell killing by ADCC/ADCP were the same for trastuzumab (IgG1) and trastuzumab-like IgE in these assays. Trastuzumab-like IgE concentrations required for killing of breast cancer cells by effector cells were also 10-fold lower than the optimum found for MOv18 IgE killing of ovarian tumor cells in the same in vitro assay system. This implies that lower levels of trastuzumab-like IgE, compared to MOv18 IgE, may be required to achieve in vivo tumor growth arrest. In vitro tumor cell viability assays confirmed that like trastuzumab, trastuzumab-like IgE without effector cells can also mediate tumor growth arrest after 24 and 48 h in culture, suggesting that trastuzumab-like IgE retains the ability to block vital tumor cell growth signals through engagement of the HER2/neu receptor.

As trastuzumab-like IgE functioned with similar potency, but through mechanisms different from those of trastuzumab (IgG1), in vivo studies in clinically

relevant tumor models may help further elucidate efficacy and effector cell functions of IgE therapies against breast cancer. Furthermore, in light of different effector cell mechanisms potentiated by each antibody, examining whether a combination of trastuzumab and trastuzumab IgE could have potential synergistic anti-tumor effects is warranted.

8.3.6 Experimental Evidence Supporting Stimulation of Mast Cells, Basophils and Eosinophils by IgE

Mast cells are well-known activators and perpetrators of IgE responses in allergic diseases. Emerging evidence suggests that these powerful immunostimulatory effector cells can also become activated in response to tumor antigen-specific IgE. Human peripheral blood basophils release histamine in response to engagement of surface FcεRI with a chimeric antibody MOv18 IgE against the ovarian carcinoma antigen FBP when cross-linked by either polyclonal antibodies or FBP-expressing tumor cells [122]. In vitro microscopical studies of human PBMC incubated with FBP-expressing ovarian carcinoma cells and MOv18 IgE demonstrated basophils in frequent contact with tumor cells, engaging in targeted exocytosis of basophil granule contents [123]. This observation was significant as basophils are rare (less than 2%) in PBMC populations and the expected ratio of basophil effector: tumor target cell would be less than 1:1. This suggested that tumor antigen-specific IgE can recruit and activate basophils to contact and target antigen-expressing tumor cells. Recent studies of an engineered trastuzumab-like IgE antibody against the human breast cancer antigen HER2/neu demonstrated that trastuzumab IgE–FcεRI complexes on the surface of mast cells can be cross-linked by HER2/neu-expressing tumor cells to trigger mast cell degranulation in an antigen-specific manner [125].

These data are in agreement with growing evidence in favor of possible anti-tumoral functions of mast cells and basophils in vivo [126, 127] (see Noelle R., Chapter 6). It is tempting to speculate that these powerful IgE effector cells can be primed by tumor antigen-specific IgE antibodies to activate downstream immune responses against cancer cells; these responses may resemble inflammatory mechanisms well known in allergic diseases and parasitic infections.

A role for eosinophils as effector cells in IgE-mediated tumor targeting and killing has also been demonstrated [44, 128] (see Parish C., Chapter 11). In an in vitro assay examining the functions of the chimeric MOv18 IgE antibody specific for the ovarian carcinoma antigen FBP (MOv18 IgE), it was demonstrated that IgE could activate blood eosinophils to specifically kill tumor cells by ADCC in vitro; light and fluorescent microscopical observations revealed degranulating eosinophils associated with tumor cells in response to treatment with MOv18 IgE leading to tumor cell death (Fig. 8.6) [44]. In agreement, evidence by ADCC and proliferation studies using spleen-derived T lymphocytes from IgE-inoculated mice as effector cells confirmed the tumor cell killing (ADCC) capacity of cytotoxic T cells and

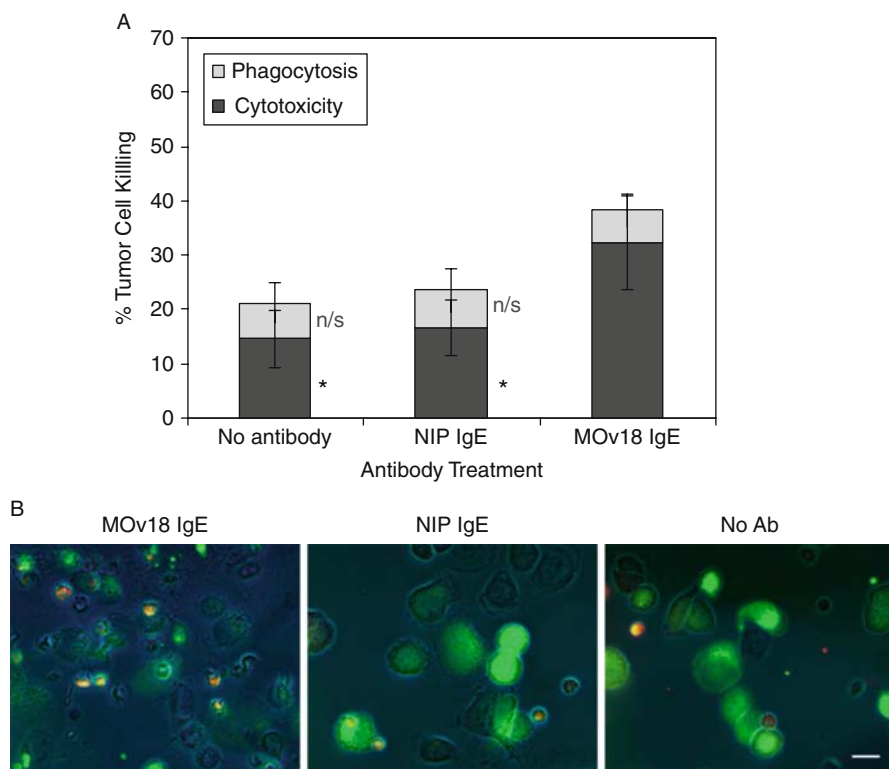


Fig. 8.6 Anti-folate-receptor antibody MOv18 IgE can activate human eosinophils to kill tumor cells. **(A)** MOv18 IgE-mediated ovarian carcinoma IGROV1 cell killing of tumor cells by primary eosinophils. ADCC, black bars; ADCP, gray bars. Results are means $\pm$ SD of four independent experiments. Significance was compared with samples given MOv18 IgE by the Student's *t* test. n/s, $p > 0.05$; * $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$. **(B)** Superimposed bright-field and fluorescent images of IGROV1–eosinophil interactions. CFSE-stained IGROV1 tumor cells (green) and CD49d-PE-labeled eosinophils (red) were incubated with IgE antibodies for 2.5 h. Treatment with MOv18 IgE resulted in frequent contact between eosinophils and tumor cells; tumor cell destruction was evident (*left*). Eosinophil-IGROV1 cell contact was observed less frequently in samples given control NIP IgE (*middle*) or no antibody (*right*). Original magnification, $\times 400$. Scale bars, 20 μ m. Reproduced with permission from Karagiannis et al. [44]

proliferative capacity of spleen-derived CD4⁺ T cells following antigenic tumor peptide stimuli. Eosinophil depletion eliminated IgE-mediated tumor growth arrest, suggesting induction of IgE-induced anti-tumoral immunological and inflammatory responses [128] (see Vangelista L. and Siccardi A., Chapter 9).

It appears, therefore, that the ingredients for the induction of an IgE-mediated response characteristic of allergic or inflammatory diseases are in place in human malignant diseases. These come in the form of resident and infiltrating mast cells and eosinophils in tumor regions and their capacity to induce potent inflammatory responses by IgE-mediated engagement of tumor cells *in situ*.

8.3.7 IgEs Against Pancreatic Cancer – A Case for Patient-Derived IgE Antibodies and Their Effector Functions

The presence of IgE antibodies in patients with pancreatic cancer and their tumor cell killing potential was investigated in a recent report, giving value to IgE as a potential therapeutic approach and confirming that naturally expressed IgE antibodies possess the ability to stimulate effector cells to kill cancer cells [129]. Fu et al. demonstrated that IgE and levels of soluble CD23 were uniquely elevated in pancreatic cancer patient sera, while levels of other immunoglobulin classes did not differ between patient and control groups. Pancreatic patient serum IgEs, but not IgEs from normal blood sera, could recognize a 50-kDa unidentified tumor antigen in pancreatic tumor cell lysates. Importantly, IgEs purified from patient sera killed pancreatic tumor cells only when combined with human PBMC in ADCC assays.

The presence of sCD23 in patient sera was not further examined in this report and levels of IgE and sCD23 were not correlated to clinical outcomes for the patients, but further examination of the role of sCD23 in IgE-mediated tumor cell killing may provide vital clues of how IgE effector functions are modulated in cancer patients. Finally, this study raises the potential that patient-derived tumor antigen-specific IgEs can direct inflammatory and immune cells against tumor cells and may be considered as future cancer therapeutics.

8.4 Conclusion

The mechanisms by which IgE antibodies exert their anti-tumor effects against cancer cells are now starting to emerge. IgE antibodies can engage both cell surface IgE receptors, FcεRI and CD23, and activate several lines of effector cells against tumor cells in vitro and in vivo employing a range of anti-tumoral activities. Research into the anti-tumoral functions of IgE antibodies demonstrate that the tumor-promoting roles of inflammatory cells in cancer may not be their only anticipated fate and that the fine balance between tumor growth-inducing inflammation and tumor targeting may be redressed in cancer therapy by IgE antibodies. Monocytes/macrophages, eosinophils, mast cells and basophils, either resident in tumor areas or recruited from the circulation, may be directed by tumor antigen-specific IgE antibodies to contribute to potent anti-tumoral responses. IgE may also activate APC to stimulate adaptive immune responses; these mechanisms still remain to be elucidated.

A number of biological/immunological questions facing the research community in the cancer immunology arena remain: (a) What are the immunological signals that down-regulate effective IgE immune responses against tumor cells in cancer and which parts of these networks may be amenable to therapeutic interventions? (b) Could IgE antibodies, and their Fcε receptors, either in their membrane-bound or soluble forms (such as various fragments of sCD23) be used as biomarkers in malignancies and could their presence have diagnostic or prognostic value? (c) What

are the effector cell mechanisms/pathways that will determine/predict the efficacy of IgE antibodies as cancer therapeutics? (d) What are the pivotal model systems that will predict the safety of cancer-specific IgE antibodies for clinical applications? (e) IgEs against tumor antigens have shown considerable promise for cancer therapy in various in vitro and in vivo systems. The question still remains whether antibodies of the IgE class can fulfill their potential in translational medicine and help us win the battle against cancer.

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