

Review article

IgE-mediated enhancement of antibody responses: the beneficial function of IgE?

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Antigen in complex with antibodies elicits profoundly different antibody responses than does antigen alone (1). Small amounts of passively administered, as well as actively produced, antibodies can cause complete suppression or more than a 1000-fold enhancement of antibody responses to the antigens to which they bind. An important feature of feedback regulation is that it is antigen- but not epitope-specific. This means that when an animal is immunized with e.g. immunoglobulin (Ig)G anti-TNP (trinitrophenyl) and TNP-BSA (bovine serum albumin) and an unrelated antigen such as ovalbumin (OVA), responses to both TNP and BSA, but not to OVA, will be regulated. Although the antibody feedback regulation was described already over 100 years ago (2), the molecular mechanisms behind the phenomenon remain elusive. An immune complex is composed of antigen, antibody, and, when the antibody is of a complement-activating class, complement factors and can bind to B-cell receptors (BCR) as well as to Fc- and complement-receptors. Ligation and coligation of these receptors can affect cell activation, both negatively and positively. Cocrosslinking of BCR and Fc γ RIIB (CD32) leads to inhibition of B-cell activation through an ITIM (immuno-receptor tyrosine-based inhibitory motif) in the cytoplasmic tail of Fc γ RIIB (3). When an ITIM is brought in close contact with other receptors containing ITAMs (immuno-receptor tyrosine-based activation motifs), such as the BCR, T-cell receptor (TCR), Fc γ RI (CD64), Fc γ RIIA (CD32), Fc γ RIII (CD16) and Fc ϵ RI, it inhibits cell activation through the latter. Other explanations for antibody-mediated suppression may be that immune complexes are eliminated via Fc- or complement-receptor-mediated phagocytosis before

they are able to elicit an antibody response, or even more simple, that antibodies mask antigenic epitopes, thereby preventing B cells from recognizing and responding to the antigen. Feedback enhancement of antibody responses on the other hand, may take place by cocrosslinking BCR and the complement-receptor 2 (CD21)/CD19-complex, known to lower the threshold for B-cell activation more than 100-fold (4). Immune complexes are also endocytosed efficiently by antigen-presenting cells (APCs) and increased presentation of antigen to T-helper cells may indirectly facilitate antibody production. In addition, follicular dendritic cells (FDCs) can capture immune complexes and present them to B cells. Given this multitude of possibilities for antibodies to interfere with responses to antigen, it is not surprising that identification of the mechanisms involved has proven to be a difficult task.

Antibodies play an important role in the modulation of normal antibody responses and in the pathogenesis of allergic and autoimmune disease. In addition, the ability of small doses of syngeneic antibody to specifically up- or downregulate immune responses has therapeutic potential. One of the most successful clinical applications of specific immunoregulation utilizes the ability of IgG antibodies to suppress responses to erythrocytes. Rhesus D negative (RhD⁻) mothers may become immunized against transplacentally acquired RhD⁺ fetal erythrocytes, resulting in production of IgG anti-RhD which cross the placenta and damage fetal erythrocytes. Since the 1960s, small amounts of IgG anti-RhD are administered to RhD⁻ mothers shortly after delivery of their RhD⁺ babies (5). This treatment has dramatically decreased the incidence of hemolytic disease of the newborn (6). The mechanism behind the clinical effects of specific immunotherapy in the treatment of allergies may involve negative regulation of B cells via cocrosslinking of BCR and Fc γ RIIB by IgG/allergen complexes, generated after hyperimmunization with allergen

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extracts. Interestingly, cocrosslinking of Fc γ RIIB and Fc γ RIIA/C or Fc ϵ RI on human basophils led to inhibition of histamine release, another possible way for IgG to interfere with allergic inflammation (7, 8). Finally, intravenous gamma globulin (IVIG), used in the treatment of autoimmune disease, was recently shown to act by upregulation of Fc γ RIIB on macrophages (9).

Antibody feedback regulation

The present review will focus on the ability of IgE to upregulate *in vivo* antibody responses and the possible biological implications of this phenomenon. In order to put this into perspective, a short summary of classical feedback regulation will first be presented.

The most well known regulatory effects of antibodies is probably the ability of IgG to suppress responses to erythrocytes. Cocrosslinking of BCR and Fc γ RIIB by IgG/erythrocyte complexes has been a widely accepted explanation for how IgG-mediated suppression operates (10). However, recent data surprisingly showed that IgG suppressed equally well in mice lacking Fc γ RIIB, or the other known Fc γ Rs, as in wildtype mice (11, 12). Although IgG is the most well characterized immunosuppressor (13), both IgM (14) and IgE (11, 15) can inhibit responses to erythrocytes. The model that best explains available data on *in vivo* feedback suppression is that antibodies act by masking of epitopes followed by Fc- and complement-independent elimination of the antigen (12, 16). This would prevent B cells from recognizing and responding to antigenic epitopes, but would not inhibit the uptake of antigen by APCs and subsequent presentation of peptides to T cells. In line with this, T-cell priming was shown to occur normally even when antibody responses were completely suppressed (11). The mechanism behind IgG-mediated suppression is still controversial and a thorough discussion has recently been published elsewhere (17–20).

The same hapten-specific monoclonal IgG that suppresses carrier-responses when administered with haptenated SRBC, enhances carrier-responses when administered with haptenated proteins in salt solutions (21, 22). IgG1 and IgG2a enhance antibody responses in the absence of CD35/CD21 (complement receptor 1 and 2) and also in situations when the antibodies are unable to activate complement (23, 24). In contrast, IgG1 and IgG2a lost their enhancing effect in mice lacking Fc γ RI and Fc γ RIII, but not in mice selectively lacking Fc γ RIII, pointing to an important role of Fc γ RI (25). *In vitro*, IgG facilitates Fc γ R-mediated endocytosis of antigen and subsequent presentation of antigenic peptides to T cells by macrophages and dendritic cells (26–28). It is likely that similar mechanisms explain the enhancing effects of IgG1 and IgG2a on antibody responses *in vivo*.

Animals and humans lacking complement factors 1–4

or CD35/CD21 have severely impaired antibody responses to suboptimal, but not to optimal, doses of antigen (29–36). Classical (31–33) but not alternative (37) pathway activation is required, suggesting that antibodies play an important role. Because IgG-mediated enhancement operates in the absence of a complement (23, 24), IgM is implicated. In agreement with this, IgM-mediated enhancement functions only with suboptimal antigen doses (13), requires that IgM can activate complement (38) and that CD35/CD21 are present (24). Because complement is necessary for primary responses, a puzzling question has been how naive animals, who have not yet encountered the antigen, can have specific IgM antibodies ready to activate the classical pathway. A resolution was offered by the observation that antibody responses were impaired in mice lacking natural IgM (39). Taken together, available data suggest that early primary antibody responses are initiated when natural IgM recognizes antigen, activates complement and the resulting IgM/antigen/complement complexes cocrosslink CD21/CD19 and BCR, thereby facilitating B-cell activation (4, 40). This would lead to the production of specific IgM, which in a positive feedback loop would further enhance the antibody response. Noteworthy is that this pathway will only be operative when the antigen is large enough to allow the IgM to bind with its five arms and assume the conformation change required for complement activation. In agreement with this, IgM-mediated enhancement has only been described with erythrocytes, malaria parasites and keyhole limpet hemocyanine (13, 21, 41), all of which are larger than IgM, whereas no effect is seen with BSA-TNP or OVA-TNP (Heyman, unpublished observations).

IgE-mediated feedback enhancement

In the early 1990s, feedback regulation had been ascribed to IgM and IgG and in one report also to IgA (42), whereas the effects of IgE had not been studied. It was hypothesized that a physiological function of IgE was to capture antigens and enhance their presentation to T cells via IgE-receptors expressed on various APCs (43). I decided to test the idea experimentally and from Mathias Wabl acquired a panel of B-cell hybridomas producing monoclonal TNP-specific IgE (44). Mice were immunized intravenously in physiological salt solutions with BSA-TNP and OVA (as a specificity control) alone or together with TNP-specific monoclonal IgE. Analysis of serum samples demonstrated that all three monoclonal IgE-antibodies tested enhanced the production of primary BSA-specific IgG (both IgG1 and IgG2a) and IgE, sometimes by more than 100-fold (45, 46). No effect on OVA-specific responses was seen, demonstrating that the enhancement was antigen-specific (45, 46). In order

for the IgE to have an effect, the antibodies had to be administered within a few hours before or after the antigen (46). No significant enhancement was observed with IgE doses lower than 10 µg or antigen doses lower than 2 µg/mouse (46, 47). IgE enhances antibody responses not only to BSA-TNP, but also to other TNP-conjugated proteins (OVA, diphtheria toxoid and tetanus toxoid) whereas responses to TNP-sheep erythrocytes (SRBC) or TNP-keyhole limpet hemocyanine (KLH) are not enhanced (46, 47). The epitope density on the antigen played an important role: responses to BSA with more than 27 TNP residues or OVA with more than 1 TNP residue/molecule could not be upregulated (48). The effect of IgE has mainly been studied during primary antibody responses, but the induction of immunological memory is also enhanced (24–144-fold) (46). In contrast, no upregulation of delayed type hypersensitivity was seen (49). The effect of the administration route was investigated by Westman et al. (48). Intravenous and intraperitoneal administration of IgE and antigen were equally efficient. When antibody was given intravenously and antigen subcutaneously, the early response was enhanced by IgE, but after 5 weeks the levels in controls had reached those of IgE-immunized animals. IgE did not upregulate responses to antigen administered in complete Freund's adjuvant. Thus, effects of IgE can only be detected when antigen is administered during 'suboptimal' conditions, resembling the effects of complement and IgM on antibody responses.

The kinetics of IgE-mediated enhancement is interesting. Already 6 days after immunization, the number of B cells secreting BSA-specific IgG was 500-fold higher in animals given IgE and antigen than in animals given antigen alone (48). This remarkably early IgG-peak was also seen when measuring serum levels of IgG (45–48). Equally interesting is that the serum level of specific antibody remains high for many weeks (45, 46). The unusually rapid response to a fairly mild immunization with antigen in physiological solutions, prompted us to ask whether IgE/antigen complexes could activate B cells without the need for T-cell help. However, IgE did not enhance in T-cell deficient nude mice (46).

IgE upregulates antibody responses in all conventional mouse strains tested except in those with the MHC-haplotype b (47). Neither C57BL/6 nor 129/Sv mice (both H-2^b) could respond to immunization with IgE/BSA-TNP, whereas they mounted a strong response to BSA-TNP administered in Freund's complete adjuvant (47). Not only responses to IgE/BSA-TNP, but also to IgE/OVA-TNP, IgE/tetanus toxoid-TNP and IgE/diphtheria toxoid-TNP were low in H-2^b mice. Further genetic mapping, using intra-MHC recombinant mouse strains, showed that low responsiveness was linked to the MHC class II A^b region (47). A^b-linked low-responsiveness was also observed when

mice were immunized with IgG/BSA-TNP and is therefore not exclusive for responses to IgE-immune complexes (50). The A^b region, as defined by intra-MHC recombinant mouse strains, encompasses more than just the genes encoding the A^b molecule (51). This, together with the unlikely event that none of all possible BSA- (or OVA-, tetanus toxoid-, or diphtheria-toxoid-) peptides would be able to bind in the peptide-binding groove of A^b, made us predict that another gene product than A^b was responsible for the low responsiveness. In order to test this, we used C57BL/6 mice which carried transgenes encoding a responder A molecule (A^k). These mice expressed high levels of A^k and could respond to A^k-restricted control peptides. Surprisingly, the transgenic mice were still low-responders to IgE/BSA-TNP (47). This suggests that a nonclassical MHC (non-Ab) molecule is required for IgE-mediated enhancement and that the b-haplotype of this factor functions poorly. Candidate molecules are H2-O and H2-M, both encoded in the A region and both involved in intracellular transport and processing of antigen (52–54).

Exclusive role for CD23 on bone marrow-derived cells in IgE-mediated enhancement

It seemed reasonable to hypothesize that the IgE-mediated enhancement was dependent on the low affinity receptor for IgE (FcεRII or CD23), shown *in vitro* to increase antigen presentation to T cells (55, 56). Mice were injected with a CD23-specific rat monoclonal antibody, produced in Daniel Conrad's laboratory (57), before immunization with IgE and antigen. Pretreatment with anti-CD23 completely abrogated the ability of IgE to enhance, demonstrating for the first time an *in vivo* function of CD23 (45, 46). These observations were subsequently confirmed in CD23-deficient mice (58, 59) and also supported by a slightly different experimental approach where immunization with antigen covalently coupled to anti-CD23 resulted in higher antibody responses than immunization with antigen coupled to an irrelevant antibody (60). The complete lack of responsiveness in the absence of functional CD23 showed that none of the other receptors binding murine IgE (FcεRI, FcγRIII and FcγRIIB) were required. This conclusion was confirmed by the observation that IgE-mediated enhancement was normal in mice lacking FcεRI, FcγRIII and FcγRIIB (25). IL-4 is known to upregulate CD23 on B cells (61) and the level of CD23 on B cells in IL-4-deficient mice is five-fold lower than in wildtype controls (62). However, IgE enhanced equally well in these two strains, showing that basal levels of CD23 are sufficient (62). CD35/CD21 are crucial for normal antibody responses (34–36, 40) and human CD21 is a ligand for CD23 (63). It was therefore of interest to study the importance of these receptors in

IgE-mediated regulation. Interestingly, CD35/CD21-deficient and wildtype mice responded equally well to IgE/antigen, suggesting that these complexes evade normal regulatory pathways (24). The results also show that CD21, although a ligand for CD23 in man, is not required for IgE/CD23-mediated enhancement in murine *in vivo* systems.

Murine B cells and FDCs constitutively express CD23 (57, 64) and it is easy to envisage how both, or either one of them, could be involved in IgE-mediated enhancement. FDCs capture immune complexes, hold them on their surface for long periods of time and present the antigen to B cells in the form of iccosomes (65) but cannot endocytose antigen and therefore do not present peptides on MHC class II to T cells. The contribution of FDCs to an immune response can be assayed in adoptive transfer experiments. These cells are irradiation resistant and in lethally irradiated mice, adoptively transferred with bone marrow from a genetically different donor, FDCs will be of recipient type and leukocytes of donor type (66, 67). Irradiated CD23⁺ mice, reconstituted with CD23⁻ bone marrow, were unable to respond to IgE/antigen complexes (59) while irradiated CD23⁻ mice, reconstituted with CD23⁺ bone marrow, responded normally (59). This experiment demonstrates that a bone marrow-derived cell type must express CD23 in order for IgE-mediated enhancement to occur. Because B cells are the only bone marrow-derived cells shown to express CD23 constitutively, these data strongly suggest that B cells are the effector cells.

Possible mechanisms for IgE-mediated enhancement

Assuming that B cells are indeed the effector cells, at least two different mechanisms for how they could up-regulate antibody responses via CD23 can be postulated. IgE/antigen could increase the B cell signal transduction or the B cells could endocytose and present IgE/antigen to T-helper cells. A mechanism involving enhanced signalling following cocrosslinking of CD23 and BCR would nicely explain the strict antigen specificity of enhancement. Arguing against this model are the findings that such coligation results in the inhibition of murine (68, 69), and human (70), B cell activation. However, only high degrees of CD23/BCR cocrosslinking induced negative signalling in murine B cells (68) whereas the IgE-mediated enhancement operates efficiently during conditions where a high degree of cocrosslinking is unlikely, such as in IL-4-deficient mice (62) and when antigens with low density of TNP are used (48). Therefore, it remains a possibility that IgE-mediated enhancement is induced by cocrosslinking of BCR and CD23 when conditions are 'suboptimal', but that the B cell is turned off when a high density of CD23 is reached. Ana-

Table 1. CD23-dependent IgE-mediated enhancement

Mice	Immunization	IgG anti-BSA (ng/ml serum)
CD23 ^{+/+}	BSA-TNP	24
CD23 ^{+/+}	IgE anti-TNP + BSA-TNP	10 072
CD23 ^{-/-}	BSA-TNP	42
CD23 ^{-/-}	IgE anti-TNP + BSA-TNP	150

CD23-deficient (CD23^{-/-}) mice (58) were backcrossed 5 generations to CBA/J mice. Wildtype (CD23^{+/+}) and CD23-deficient mice were immunized intravenously with 20 µg TNP-BSA with or without 50 µg purified monoclonal IgE anti-TNP (44). IgG anti-BSA levels were measured in ELISA 14 days after immunizations.

lysis of whether IgE can enhance secondary responses or in mice overexpressing CD23 would elucidate this question.

In contrast to the hypothesis about enhanced B-cell signalling, which has little direct experimental support, there is ample evidence from *in vitro* studies that IgE/CD23 is involved in antigen presentation. Both human and murine B cells, cultured with IgE/antigen complexes and tested for their ability to activate antigen-specific T cells *in vitro*, are much more efficient in doing so than B cells cultured with antigen alone (55, 56, 71–73). The effect is dependent on CD23 and presumably caused by endocytosis of IgE/antigen complexes via CD23 and presentation of antigenic peptides on MHC class II molecules to specific T cells (74). Antigen presentation via CD23 *in vitro* is mediated by B cells that are not specific for the antigen, such as EBV-transformed human B cells or total spleen cell populations (55, 56, 71, 72), notably IgE-mediated enhancement *in vivo* is strictly antigen specific (45, 46, 59). The majority of CD23⁺ splenic B cells, regardless of specificity, bind IgE/antigen complexes via CD23 *ex vivo* (50), a finding probably reflecting the *in vivo* situation. Therefore, a mechanism preventing unspecific B cells from differentiating into antibody-producing B cells, although they present antigen via CD23 to specific T cells, must exist. It is known that T and B cells must recognize epitopes on the same antigen in order for the B cells to get sufficient T-cell help for antibody production and a reasonable interpretation of the experimental findings is that although all CD23⁺ B cells present antigen, only B cells which in addition can present antigen via BCR-mediated uptake will be activated and allowed to produce antibodies. In this scenario, CD23⁺ B cells act as nonspecific APCs in a similar manner as macrophages and dendritic cells. The advantage of this pathway would be to ensure rapid expansion of specific T cells in situations where the antigen elicits an IgE-response. There are indications that B cell-mediated antigen presentation induces Th2 cells (75, 76) and it remains to be seen whether this T-cell lineage is preferentially activated by IgE/antigen. Whether B cells *in vivo* can activate naive T cells or not has been a subject of debate (76–79). Should the mechanism for IgE-mediated enhancement of antibody responses prove

to be antigen presentation, this would be an example of a situation where B cells indeed are able to activate naive T cells *in vivo*.

Concluding remarks

To summarize, IgE forming complexes with small protein antigens administered in physiological solutions upregulates the production of specific IgM, IgG (both IgG1 and IgG2a) as well as IgE whereas no effect on polyclonal antibody responses is seen. The phenomenon is exclusively dependent on CD23, and this receptor must be expressed on a bone marrow-derived cell, presumably the B cell. IgE-mediated enhancement of antibody responses has some remarkable features. The response to IgE/antigen can be more than 1000-fold higher than the response to antigen alone and IgG-levels peak already 6 days after immunization, mimicking a secondary antibody response. IgE/antigen induces equally high antibody responses in CD21-deficient as in normal mice, although CD21/CD19 is required for antibody responses to suboptimal doses of most antigens. Despite the ability of IgE/antigen complexes to circumvent some immunoregulatory pathways, the induced responses are strictly antigen specific and dependent on T-cell help. The most likely mechanism behind the phenomenon is that B cells, via CD23, endocytose IgE/antigen and present peptides to specific T-helper cells.

Are these extraordinary effects of IgE laboratory findings only, or do they play a role in normal antibody responses when antigens are administered without IgE? The mere existence of this potent regulatory system *in vivo* suggests that it has a biological function, and the ability to upregulate responses during suboptimal conditions indicates a physiological role in early responses. However, there is so far no experimental data directly supporting this hypothesis. Neither IgE- nor CD23-deficient mice suffer from apparent disease, the animals do not die prematurely and lymphocyte development is normal (58, 80–82). IgE-deficient mice (80) do not show significantly lower antibody responses than wildtype controls (H. C. Oettgen, personal communication) and, with the exception of responses to IgE-complexed antigen, neither do CD23-deficient mice. The latter respond normally to SRBC or KLH administered in salt solutions (46), to proteins in Freund's complete adjuvant (58), to *Nippostrongylus brasiliensis* (58, 81, 82), and, in one out of two tested CD23-deficient mouse strains, also to antigens administered in alum (58). In contrast, higher production of specific IgE and IgG1 as well as polyclonal IgE was observed in one CD23-deficient strain (81). In line with this, impaired IgE- and IgG1-responses were found in mice overexpressing CD23, either as transgenes (83–85) or owing to treatment with an inhibitor of proteolytic

cleavage of CD23 (86). It is not known whether IgE or another CD23-ligand is involved in CD23-mediated negative regulation. Unlike in IgE/CD23-mediated enhancement of antibody responses, inhibition of antibody production via CD23 is mediated by non-lymphoid cells, probably FDCs (85). The mechanism behind the *in vivo* inhibitory effects of CD23 are not known (reviewed in (87)).

Lack of evidence that CD23 plays a role in facilitating antibody responses to antigens not coadministered with preformed IgE, does not preclude a biological role for IgE-mediated enhancement. One has to bear in mind that with the exception of *Nippostrongylus brasiliensis*, immune responses in CD23- and IgE-deficient animals have only been studied using a limited number of noninfectious antigens, administered intravenously or in potent adjuvants, neither of which are very physiological. Laboratory mice are usually kept under strictly controlled health conditions, and would not be expected to encounter many spontaneous infections. The most obvious situation where IgE, via CD23, could serve to enhance immune responses would be when a pathogen has already elicited an IgE-response. Whether IgE also plays a role in the initiation of primary antibody responses is an interesting question. Serum concentrations of IgE are in the range of 0.3 µg/ml whereas IgE-mediated feedback enhancement requires administration of at least 10 µg specific IgE to a mouse with an estimated blood volume of 2 ml. However, local IgE concentrations may well be higher than serum concentrations and possibly sufficient for IgE-mediated enhancement. IgE-producing B cells primarily reside in gut-associated lymphoid tissue (88) and perhaps this would be a site where IgE plays a role in enhancing early primary antibody responses. An interesting observation is that IgM augments primary responses to large particulate antigens, but has no effect on responses to small proteins, whereas IgE-mediated enhancement shows the opposite pattern. These findings are compatible with the idea that these systems complement each other in facilitating early antibody responses. Clearly, the biological role, if any, of IgE in antibody responses is at present highly speculative, and more solid data is required to elucidate this question.

How relevant are studies in murine systems for the understanding of the role of human CD23-mediated immune regulation? Although there are many similarities between CD23 of the two species, there are also some important differences (reviewed in (89–92)). Murine CD23 is a 49-kDa and human a 45-kDa single-chain glycoprotein, which can form oligomers on the cell surface (93, 94). Both murine and human CD23 also exist in soluble forms (sCD23), produced by proteolytic cleavage of the membrane form. Human sCD23 binds IgE, whereas the affinity of murine sCD23 for IgE is 100-fold lower than that of the membrane form,

probably because murine sCD23 is unable to oligomerize (95). Human CD23 exists in two isoforms, a and b, which differ in their cytoplasmic region. CD23a is expressed constitutively on B cells and possibly FDCs whereas CD23b requires IL-4 for its expression and is distributed on a wide variety of cells such as T cells, eosinophils, dendritic cells and mast cells. Murine CD23a is constitutively expressed only on B cells (57) and FDC (64) and whether a murine CD23b isoform exists is controversial (96, 97). Because CD23 is the only Fc-receptor that does not belong to the superimmunoglobulin family, it has been proposed that its binding to IgE is fortuitous, and that its 'real' ligand is something else. Human CD23 binds CD21 (63) as well as the adhesion molecules CD11b/CD18 (CR3) and CD11c/CD18 (CR4) (98)). Murine CD23 also binds CD11b/CD18 (99) whereas no binding to CD21 has been demonstrated (100). The differences between mouse and human CD23 obviously makes it difficult to know whether findings in one species are representative for the other species. Given that mouse and human immune systems previously have been found to be very similar, it is likely that studies of mice will be of interest also in understanding the function of IgE and CD23 in man. In addition to the possibility of studying *in vivo* functions, the murine system offers the advantage of reductionist studies, limiting observations to the function of membrane CD23a expressed on B cells or FDCs.

It has been discussed whether CD23 plays a role in human allergic disease and the pros and cons have been reviewed elsewhere (92). Because human CD23 exists in two isoforms, is expressed on many celltypes, has a multitude of ligands and is able to bind IgE both in its membrane and soluble form, there are certainly many hypothetical possibilities for modulation of allergic inflammation. More relevant for the focus of this review, the ability of IgE to augment antigen-presentation to T cells has been a consistent finding in a number of different laboratories using human B cells (56, 71–73). An indication that this plays a role in IgE-mediated disease (101) comes from elegant experiments by van der Heijden et al. (73). Using an autologous system where B and T cells were derived from the same atopic dermatitis patient, it was shown that patient serum containing IgE anti-*Der p* II (*Dermatophagoides pteronyssinus*), a major house dust mite, facilitated EBV-B cell-mediated antigen presentation to T-helper cells *in vitro* (73).

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