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Murine B cells regulate serum IgE levels in a CD23-dependent manner[1]

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Abstract

The manifestations of allergic disorders are closely tied to the biologic effects of IgE activation with antigen. In immediate hypersensitivity reactions, IgE effector function requires prior binding to innate immune cells, primarily mast cells and basophils, with the blood acting as a reservoir for unbound IgE. As the severity of allergic disease is proportional to the size of this unbound IgE pool, we hypothesized that cellular mechanisms exist to limit the size and/or enhance the clearance of free IgE molecules. We examined this in mice by engineering a reporter IgE molecule that allowed us to track the fate of IgE molecules *in vivo*. The absence of high-affinity IgE receptor (FcεRI) expressing cells did not affect serum IgE levels, but B cells regulated serum IgE by controlling the size of the free IgE pool. B cells captured IgE by direct binding mediated by the low-affinity IgE receptor, CD23. These data indicate a novel mechanism regulating serum IgE and additionally clarify the role of CD23 in this process.

INTRODUCTION

The clinical manifestations of allergic disease result from the effects of a complex network of cells and effector molecules (1). Among these, immunoglobulin E (IgE) plays a central role in mediating allergic disease. Acute allergic reactions, such as anaphylaxis, are primarily the result of IgE-mediated processes, though IgG can mediate similar effects (2–4). IgE also contributes to chronic allergic diseases, including asthma and atopic dermatitis. In these diseases, the serum levels of total or specific IgE correlate closely with disease severity (5, 6). Therefore, the regulation of IgE production and/or clearance is central to allergic disorders.

Among the soluble immunoglobulin isotypes, IgE exhibits several unique properties. First, the most prominent effects of IgE effector function, as seen in type I hypersensitivity reactions, require prior binding to the high-affinity IgE receptor, Fc epsilon receptor I (FcεRI). FcεRI is a heterotetrameric complex expressed primarily on the surface of mast cells and basophils. The complex is composed of an α chain (which binds IgE), a β chain (which amplifies signaling), and a dimer of γ chains (which is the primary signaling component) (7). Loading of IgE onto mast cells and basophils enables these cells to

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recognize and respond to specific antigens with immediate release of pre-formed molecules, synthesis of lipid mediators, and cytokine production (8–10). In humans, antigen presenting cells, including Langerhans cells, express a trimeric form of the FcεRI ($\alpha\gamma_2$), which play a role in antigen processing (11). Though FcεRI$^+$ cells are found throughout the body, mast cells are the most abundant with a cellular distribution weighted toward peripheral tissues, including the skin as well as the gastrointestinal and respiratory tracts (12).

A low-affinity IgE receptor (CD23 or FcεRII) is also present and exists as membrane-bound trimers as well as soluble monomers and oligomers (13, 14). In mice, CD23 is expressed primarily on B cells, though follicular dendritic cell expression has also been described (15, 16). Human CD23 has a broader expression pattern with the existence of two CD23 isoforms (17). Membrane-bound CD23 has been shown to both enhance and diminish IgE production by B cells, to facilitate the clearance of IgE immune complexes, and to mediate transport of IgE across the gut epithelium (18, 19). The soluble form has an order of magnitude lower affinity for IgE than the membrane-bound form and may indirectly enhance IgE production (9). CD23 also has a broader ligand binding profile than FcεRI, which may account for the disparate effects of CD23 on IgE production (20, 21).

The amount of free IgE available for binding to receptors is also unusual. Free IgE is found primarily in the blood and is the scarcest serum immunoglobulin. In normal children and adults, IgE is found at 100- to 10,000-fold lower levels than IgG (8, 9). This paucity of IgE reflects the rapid clearance of free IgE from the blood. While murine IgA, IgM, and IgG have serum half-lives on the order of a day to weeks, the half-life of free IgE is 5–12 hours (22, 23). The half-life of human IgE in normal individuals is longer than mice (approximately 2 days), but it is still the shortest among soluble immunoglobulins (24).

The rapid clearance of unbound IgE from the serum is in part related to the catabolism of serum IgE (24, 25). However, other mechanisms may also exist. In severely atopic individuals, IgE levels increase up to 1,000-fold, and the rate of serum IgE clearance in these individuals is inversely related to this rise in serum IgE (24, 25). This contrasts with IgM, IgG, and IgA, for which the clearance rate increases proportionally with increases in serum Ig levels. These data suggest that a saturable mechanism may exist to regulate serum IgE levels and/or alter IgE clearance. Given the presence of at least two IgE receptors and the relative abundance of IgE-binding cells, we hypothesized that a cellular mechanism may mediate IgE clearance. Our data indicate that CD23 expression on B cells, rather than FcεRI$^+$ mast cells or basophils, regulates free serum IgE levels. These data reveal a novel cellular mechanism for monomeric antibody clearance and suggest an additional function for circulating B cells.

MATERIALS AND METHODS

Mice

BALB/c, C57BL/6 and C57BL/6 FcεRI$^{-/-}$ mice were from Jackson Laboratories (Bar Harbor, ME) (26). μMT mice were from Jackson and backcrossed to BALB/c mice for 10 generations (27). *Stat6*$^{-/-}$ and *IL4/13*$^{-/-}$ mice have been described (28, 29). Mast cell-deficient W^{sh}/W^{sh} mice on a C57BL/6 background were provided by G. Caughey (UCSF) (30). *4get* BALB/c and *4get Rag2*$^{-/-}$ mice have been described (27). Mice were housed in Specific Pathogen Free facilities. Experimental mice were 8–12 weeks old. Animal use was governed by and in accordance with approved protocols overseen by the Laboratory Animal Resource Center (LARC) and Institutional Animal Care and Use Committee (IACUC) at UCSF.

Antibodies and flow cytometry

We used the following antibodies and clones: anti-c-kit (2B8, Biolegend, San Diego, CA), anti-CD49b (DX5, ebioscience, San Diego, CA), anti-mouse IgE (23G3, Southern Biotech, Birmingham, AL), B220 (RA3-6B2, BD Biosciences, San Jose, CA), and CD19 (1D3, BD Biosciences). Hemagglutinin tags were detected with a monoclonal antibody conjugate (GG8-1F3.3.1, Miltenyi Biotec, Auburn, CA). We used an LSRII flow cytometer (Becton-Dickinson, San Jose, CA) for cell acquisition and analysis. For intracellular HA staining, fixed splenocytes were treated for 5 minutes with 0.5 Units per ml of Liberase CI (Roche, Indianapolis IN) at 37°C in an orbital shaker. The reaction was immediately quenched with PBS containing 2% FCS. Cells were then permeabilized in PBS supplemented with 2% FCS and 0.5% saponin. We performed post-acquisition analysis using FlowJo software (Treestar, Ashland, OR).

Reverse passive cutaneous anaphylaxis

We performed local hypersensitivity challenges following passive sensitization similar to published protocols (31). In brief, we sensitized mice with varying amounts of anti-TNP IgE antibody (C38-2) of the IgE^a allotype (BD Biosciences). Two days later, we loaded the mice with 200 μl of 1% Evans Blue dye in PBS and challenged the mice with a 1 μg intradermal ear injection of TNP_{13}-ovalbumin (Biosearch Technologies, Novato, CA) in PBS. One hour after challenge, we isolated the ears, weighed the tissue, and placed the tissue in formamide overnight at 55°C. We then filtered the supernatant and measured the absorbance at 620 nm (A620). The absorbance was corrected for background values and then divided by the weight of the tissue.

Construction of hemagglutinin-tagged Fcε (HA-Fcε)

We isolated IgE cDNA from an IgE secreting hybridoma, IGEL4.1a (kindly provided by M. Wabl), which encodes the *a* allotype of the IgE heavy chain sequence, Cε1-Cε4, using RT-PCR. This fragment was sub-cloned using ZeroBlunt Topo (Invitrogen, Carlsbad, CA) and sequence verified. The Cε2–4 cDNA, which includes two disulfide bonds required for homodimerization, was amplified using the following primer pair: 5′-agatctgttcgacctgtcaacatcac-3′ and 5′-gttcgtcgacgggcccg-3′. We cloned this amplicon in-frame using a Bgl2/Sal1 digestion into an expression vector derived from pcDNA-3, resulting in the addition of a V_{kappa} signal sequence and hemagglutinin epitope tag on the N-terminus of the protein. To enhance expression of the HA-Fcε, we subcloned the cDNA to an expression vector containing the EF-1α promoter, pShooter (Invitrogen). We then transfected the hybridoma fusion partner SpAg14 by electroporation with 10 μg of ScaI linearized plasmid DNA. We selected stable clones with 2 mg/ml of G418 and verified protein production by ELISA.

ELISA

For native IgE and HA-Fcε capture, we used anti-IgE clone RME-1 (ebioscience). IgE detection was performed using biotinylated anti-IgE (EM95), followed by streptavidin conjugated alkaline phosphatase (BD Biosciences). HA detection was performed using an anti-HA horseradish peroxidase (HRP) conjugate (Miltenyi Biotec).

CD23 blockade

Similar to published protocols (32, 33), we infused 100 μg of B3B4 (Biolegend) or isotype control rat IgG2a antibody into the tail vein, followed by the indicated treatments.

RESULTS

IgE levels correlate with severity of the allergic response

Clinical data suggest a direct correlation between the amount of antigen-specific serum IgE and the degree of local hypersensitivity. Therefore, we used a reverse passive cutaneous anaphylaxis (rPCA) assay to determine the extent of IgE loading on mast cells in peripheral tissues following IV infusion with increasing amounts of antigen-specific IgE. We infused BALB/c mice with 0.25 to 5 μg of trinitrophenol (TNP) specific IgE, and two days later, we administered Evans blue dye by intravenous injection, followed by a challenge with 1 μg of TNP-OVA into the ear by intradermal injection. One hour after challenge, we harvested ear tissue and extracted Evans blue dye. The rPCA procedure caused some dye to extravasate even in the absence of IgE. However, the degree of dye extravasation was directly proportional to the amount of IgE with a maximal response at the highest dose of IgE administered (Figure S1). Thus, these data indicate that the amount of serum IgE is proportional to the subsequent local hypersensitivity response.

Construction and characterization of a surrogate IgE molecule

We next sought to develop a system to track the disposition of serum IgE in IgE-sufficient animals. To accomplish this, we engineered hemagglutinin-tagged IgE molecules (HA-Fcε) by fusing an HA tag N-terminal to the Cε2–4 domains of the IgE heavy chain (Figure 1A). The crystal structure of the Fcε was based upon a similar molecule and prior chimeric molecules have been similarly engineered (34, 35). Though HA-Fcε would be predicted to have a similar structure to native IgE molecules, we wanted to ensure that HA-Fcε displayed similar clearance kinetics as native IgE. Thus, we infused IgE-deficient *Rag2*$^{-/-}$ mice (which lack detectable serum IgE and lack cell surface IgE on basophils or mast cells) with 1 μg of IgE or HA-Fcε. We serially bled the mice and assayed for serum IgE levels at 0, 1, 7.5, and 24 hours (Figure 1B). Just prior to the infusion, we found no detectable IgE in these mice. IgE levels were highest at one hour post-infusion and rapidly declined by 7.5 hours. By 24 hours, no serum IgE was detectable in either group. The levels of serum IgE at each time point were nearly identical between the two groups.

We next compared the capacity of HA-Fcε and native IgE to bind to FcεRI^{+} basophils. To facilitate the detection of FcεRI^{+} splenic basophils, we used *Rag2*$^{-/-}$ mice on a *4get* background (27). These mice (*4getRag2*$^{-/-}$) contain the coding sequence of the enhanced green fluorescent protein (GFP) under the translational control of an internal ribosomal entry site (IRES) at the 3′ end of the last exon of the IL-4 gene. Basophils, mast cells, and eosinophils from such mice constitutively express GFP (36). To examine binding of HA-Fcε to basophils, IgE-deficient *4getRag2*$^{-/-}$ mice received 1 μg of HA-Fcε or control IgE by tail vein injection. Twenty-four hours later, we analyzed splenic basophils for surface IgE levels and the presence of HA-Fcε. As expected in the absence of serum IgE, splenic basophils from *4getRag2*$^{-/-}$ mice showed no detectable IgE staining at baseline. After infusion, IgE was readily detected on splenic basophils (Figure 1C), with similar mean fluorescence intensity of IgE staining in IgE-and HA-Fcε-infused animals. By contrast, only splenic basophils from HA-Fcε-infused animals had detectable HA staining (Figure 1C and 1D).

We next addressed whether HA-Fcε was capable of binding to basophils and mast cells in IgE-sufficient hosts. In addition, we examined the amount of HA-Fcε sufficient to uniformly load both basophils and peripheral mast cells with IgE. Therefore, we administered increasing doses of HA-Fcε from 0.1 to 10 μg to IgE-sufficient *4get* mice on a Balb/c background. Twenty-four hours after infusion, both basophils and mast cells readily accumulated cell surface HA-Fcε, and basophils showed detectable staining even at the lowest infusion dose (Figure 2A and B). Presumably, peritoneal mast cells required higher

infusion doses owing to differential delivery of HA-Fcε in blood as compared to the peritoneum. At the 2.5 μg dose, both peritoneal mast cells and basophils demonstrated uniform acquisition of HA-Fcε. Taken together, HA-Fcε demonstrates similar kinetic and biochemical properties to native IgE.

B cells control the set point for serum IgE levels

Given the relative abundance of IgE receptor-bearing cells, we hypothesized a cellular control mechanism for serum IgE levels. Though FcεRI^{+} cells have previously been shown to have little bearing on the regulation of serum IgE (26), we wanted to verify these findings using our reporter IgE molecule. Therefore, we administered 2.5 μg of HA-Fcε to *FcerI*$^{-/-}$ mice or wild-type C57BL/6 controls and performed serial bleeds at 1, 3, 7.5, and approximately 24 hours post-infusion (Figure 3A). The peak HA-Fcε level in the blood at 1 hour was nearly identical between the two groups of mice, and there were no significant differences between the two groups at the remaining time points. Experiments in mast cell-deficient Sash mice (W^{sh}/W^{sh}) yielded similar results (*data not shown*).

We next examined whether B cells, which, in mice, are the primary CD23-expressing cells, contribute to regulation of serum IgE (8, 13). B cell-deficient μMT$^{-/-}$ mice received 2.5 μg of HA-Fcε by tail vein injection. Serial assessment of HA-Fcε levels revealed a 2-fold increase in the peak IgE level at one hour compared to wild-type controls (Figure 3B). Whereas B cells affected the serum IgE level, the absence of B cells had no impact on the overall clearance kinetics of HA-Fcε as B cell-deficient and -sufficient mice had similar half-lives of 5.9 and 5.2 hours, respectively. We also observed similar results in *Rag2*$^{-/-}$ mice (*data not shown)*. To ensure that the differences were solely due to the absence of B cells (rather than IgE), we performed similar experiments in *Stat6*- and *IL-4/13*-deficient animals. Both of these animals have B cells but no serum IgE or detectable surface IgE on FcεRI^{+} cells. Despite the absence of IgE, wild-type, *Stat6*$^{-/-}$, and *IL4/13*$^{-/-}$ mice had overlapping peak serum IgE levels and clearance kinetics (Figure 3C). Together, these data suggested a role for B cells in controlling the serum IgE level but not the rate of IgE clearance.

CD23 blockade is sufficient to inhibit B cell regulation of IgE levels

We next examined whether CD23 expression on B cells was responsible for the B cell-dependent regulation of serum IgE levels. CD23 is thought to have multiple functions on B cells, and the anti-CD23 monoclonal antibody, B3B4, antagonizes IgE binding to CD23 (32). We therefore administered 100 μg of B3B4 or isotype control antibody to groups of mice and, one day later, examined clearance of a 2.5 μg HA-Fcε challenge (Figure 4A). At 1 hour post-infusion, B3B4-treated mice exhibited a 1.8-fold increase in serum IgE as compared to isotype controls. This increase was similar to the differences seen in B cell-deficient animals as compared to controls. CD23 blockade also did not affect the overall clearance of HA-Fcε as the curves showed parallel declines in IgE with nearly complete absence of HA-Fcε by 24 hours in both groups of animals. Binding of HA-Fcε to peritoneal mast cells similarly reflected the increased serum IgE levels as B3B4-treated mice demonstrated a 7-fold increase in mean fluorescence intensity (MFI) on mast cells as compared to isotype controls (Figure 4B). The effect on splenic basophils was similar but more modest (Figure 4C).

Taken together, our data indicate that CD23 expression by B cells regulates serum IgE levels. CD23 can, however, exist in both soluble and membrane-bound forms, and our data do not preclude a role for soluble CD23 in IgE clearance. To investigate whether B cells directly bind IgE, we first verified CD23 expression on splenic B cells (Fig. 5A). The majority of B cells were CD23^{+}, similar to published data (37). We then examined IgE

binding to B cells by infusing BALB/c mice with 2.5 μg HA-Fcε. Two or twenty-four hours after infusion, we generated single cell suspensions and immediately fixed the cells with 4% paraformaldehyde, which enhanced detection of cell surface HA-Fcε on B cells. We then analyzed $CD19^+B220^+$ B cells for HA-Fcε capture by flow cytometry. B cells from mice that had received PBS demonstrated no HA staining two hours after infusion. By contrast, B cells from mice, which had received HA-Fcε two hours prior, demonstrated uptake of HA-Fcε (Figure 5B). This binding of HA-Fcε was short-lived and directly proportional to the serum HA-Fcε level, as no HA-Fcε was detectable on B cells twenty-four hours after infusion. To ensure that the fixation did not result in non-specific binding to cells, we verified that neither $CD4^+$ nor $CD8^+$ T cells showed HA-Fcε binding after fixation (*data not shown*).

We next examined whether HA-Fcε binding to B cells was CD23 dependent. Groups of mice received pretreatment with B3B4 (or isotype control) followed by 2.5 μg of HA-Fcε the next day. Administration of B3B4 completely blocked HA-Fcε binding to B cells two hours after treatment with HA-Fcε (Figure 5C).

Following binding to CD23, IgE molecules can be internalized and degraded (38). Thus, we examined whether splenic B cells internalized HA-Fcε after binding. BALB/c mice received either PBS or HA-Fcε, and 2 hours later, we harvested spleens and fixed single cell suspensions as above. To differentiate between extra- and intra-cellular HA-Fcε, the samples were split into two groups. One group was stained for CD19, B220, and HA. A second group was treated briefly with Liberase CI to remove extracellular HA-Fcε. As before, B cells had cell surface HA-Fcε two hours after infusion (Figure 5D), and these molecules were almost completely stripped off after brief exposure to Liberase CI. Subsequent permeabilization of the cells led to an increase in HA staining. Together, these data demonstrate that IgE binds directly to B cells in a CD23-dependent manner and that bound IgE is subsequently internalized.

CD23 blockade enhances IgE-mediated hypersensitivity reactions

As CD23 blockade increases the peak serum IgE level, we next examined whether this increase leads to greater delivery of IgE to tissue mast cells and enhanced local hypersensitivity reactions after antigen challenge. To address this, we blocked CD23 in BALB/c mice with 100 μg B3B4 on day 0. On day 1, the mice received 0.5 μg of anti-TNP IgE by tail vein injection. We chose this dose as it is on the linear portion of the dose-response curve (Figure S1). On day 3, we administered Evans blue and challenged the mice with 1 μg TNP-OVA in the rPCA assay. As compared to mice that lacked specific IgE, isotype control-treated mice had a significant increase in Evans blue dye extravasation 1 hour after challenge (Figure 6). In the presence of B3B4, this effect was further enhanced with a 40% increase in Evans Blue after challenge. These data indicate that blockade of CD23-dependent IgE binding significantly increases the severity of IgE-mediated reactions.

CD23 blockade leads to a rapid increase in total serum IgE

Our data suggest that cell surface bound IgE has a rapid turnover (Figure 5B), and we therefore expected blockade of CD23 to lead to an increase in total serum IgE soon after administration. To examine this, we administered B3B4 (or isotype control) to groups of BALB/c mice and performed serial bleeds 1, 4, and 7 days after infusion to monitor total serum IgE levels. Consistent with a role for CD23 in regulating serum IgE levels and rapid turnover of IgE bound to B cells, the serum IgE level nearly doubled within 24 hours of CD23 blockade (Figure 7). This effect began to wane by 4 days and was nearly absent by 7 days post-infusion. These data indicate that CD23 blockade not only has effects on passively infused IgE but also the native IgE pool.

DISCUSSION

Our data reveal an unproven *in vivo* role for murine B cells in the regulation of serum IgE levels *in vivo*. B cells act as a sink to eliminate excess serum IgE by binding to IgE in a CD23-dependent manner. Though B cells eliminated excess serum IgE, the overall rate of serum IgE catabolism remained largely unchanged.

The lack of contribution by FcεRI^{+} cells to the clearance of IgE has previously been reported (26). These observations are particularly striking given the affinity of the FcεRI for IgE (sub-nanomolar range) (9). One possible explanation is that only a limited number of unoccupied receptors exist on the surface of basophils and mast cells. Although quantifying the numbers of unoccupied receptors is difficult (39), it was clear that both basophils and mast cells had sufficient unoccupied receptors to bind exogenous IgE (Figure 2). Indeed, the regulation of surface FcεRI is thought to occur mainly through stabilization of unoccupied receptors after IgE binding with a dynamic balance between *de novo* IgE production and endocytosis of unoccupied receptors (40). The number of target cells available may also have been a factor. Basophils are the least represented hematopoietic cell in the peripheral blood, found at levels approximately 10% of the numbers of B cells in normal mice and humans. Further, B cells are heavily represented in secondary lymphoid organs such as the spleen and lymph nodes, while basophils are more rarely observed in these tissues (41, 42). Basophils undergo rapid turnover and could thereby regulate serum IgE levels (43); however, these effects would need to be in an FcεRI-independent manner. By contrast, mast cells are widely distributed in peripheral tissues, including the skin, intestines, and respiratory tract (44). Traditionally, IgE loading of tissue mast cells has been thought to occur through a passive diffusion model. If so, we would have expected mast cells to play some role in IgE clearance. However, mast cell-deficient mice showed overlapping peak IgE levels and rates of clearance as compared to wild-type mice (*data not shown*). Therefore, while similar logic regarding a limited number of unoccupied receptors is certainly possible, we speculate that tissue mast cells may have limited access to serum IgE by virtue of the endothelial barrier. If such limitations in access existed, mast cells might then be expected to contribute little to serum IgE clearance, unless local changes in delivery or vasopermeability occurred. These possibilities require further study.

The absence of B cells (or blockade of CD23) led to an approximately 2-fold increase in the peak serum IgE concentration (Figure 3B and 4A). Despite this increase in peak serum IgE concentration, we observed no clear change in the overall rate of IgE clearance from the serum (Figure 3B). This lack of change in IgE clearance is in agreement with published findings in CD23$^{-/-}$ mice (45). However, in these CD23-deficient mice, no difference in peak IgE was observed after intravenous infusion. This likely reflects the 30–40 fold larger amount of infused IgE (~75–100 μg). The peak IgE level after such infusions is in the 30–40 μg range, which may exceed physiologic limits that might have been detected using the amounts we used (13). Additionally, the use of the epitope-tagged IgE molecule allowed us to isolate the kinetics of an individual group of IgE molecules independent of the native IgE pool.

The similar rate of IgE clearance in B cell-deficient mice suggests that IgE homeostasis is regulated on several levels. While B cells limit the overall size of the IgE pool by rapidly binding free IgE molecules, other mechanisms exist to determine the catabolism and clearance of IgE. IgE-binding factors have long been postulated to contribute to the clearance of IgE (8, 21), but a detailed understanding is lacking. The regulation of IgE levels by CD23 has focused primarily on alterations in IgE production. In particular, the first published CD23-deficient mouse had a moderate 2-fold increase in total serum IgE, along with an up to 10-fold increase in antigen-specific IgE production following immunization

(37). Subsequent mouse strains had a mixture of results including normal baseline serum IgE levels, no alteration in antigen-specific IgE production, and defects in antigen focusing following hapten treatment in mice pre-sensitized with antigen-specific IgE (45, 46). These data supplemented previous work suggesting defective IgE production in anti-CD23 treated rodents (47). Many of these differences likely relate to the inter-strain variation, genetic backgrounds, and different experimental model systems, as well as to the inherent complexity of CD23 biology, including the binding characteristics of the IgE-CD23 interaction and subsequent effects on B cell survival (8). In humans, the biology is further complicated by interactions between CD23 and CD21, which enhance IgE production (21, 48, 49).

Anti-CD23 has been considered as a biotherapeutic in human allergic disease. The impact of anti-CD23 treatment on allergic disease remains unclear, but early data pointed to a rapid drop in IgE levels following anti-CD23 treatment (50, 51). Though these data would appear to counter ours, the antibody targets as well as differences in human and mouse CD23 biology, including an approximately 10-fold lower affinity of human CD23 for IgE, may account for these outcomes (13, 17, 52).

CD23 exists in both a membrane-bound and soluble form. Our data indicate that B cells can directly bind to serum IgE, although the low-affinity of the interaction required us to perform fixation to detect this binding. While our data do not exclude a role for soluble CD23 in the regulation of IgE levels, previous work with isolated overexpression of each form of CD23 has suggested that the membrane-bound form has a much greater impact on IgE biology than does the soluble form (53). The lower affinity of soluble CD23 for IgE, and an inability of mouse CD23 to interact with CD21, further diminishes the likelihood of a significant role for soluble CD23 in regulating serum IgE levels or IgE production (49).

The amount of CD23-associated IgE on B cells was directly proportional to the serum IgE level and showed rapid turnover (Figure 5A). Though it is possible that ADAM10-mediated CD23 shedding increases with ligand binding (54, 55), no data yet exist to demonstrate such a correlation, and ligand binding may in fact decrease CD23 shedding (13). As our data indicate, IgE molecules are internalized and likely degraded. This receptor-mediated endocytosis has also been demonstrated by *in vitro* experiments using murine B cell hybridomas expressing CD23, Epstein-Barr virus (EBV)-transformed human B cells, and additional cell lines (38, 56, 57).

The size of the free serum IgE pool is a function of production, clearance of IgE molecules, and capture of IgE by peripheral $Fc\varepsilon RI^{+}$ cells. Our data indicate that the latter appears to make little contribution to the regulation of the free IgE pool and that B cells, through a low-affinity receptor, are a primary regulator of the IgE pool. Binding of monomeric IgE by B cells likely plays at least two important roles in the expression of allergic inflammation. First, CD23 binding of monomeric IgE serves to enhance antigen presentation and antigen focusing as has been previously described (46). Serum IgE levels can be detected within the first 5–8 days of an immune response (58, 59), and these antibodies could facilitate antigen capture and presentation by B cells. Second, B cell capture of serum IgE levels decreases the availability of IgE molecules for loading onto mast cells in peripheral tissue. As loss of this binding capacity enhances hypersensitivity responses (Figure 6), we speculate that B cells act as a checkpoint to guard against the potentially fatal consequences of inappropriate mast cell activation by IgE and antigen. This system biases mast cells toward loading with IgE specificities that are highly represented in the serum. Presumably, these specificities would be the most relevant for host defense, though in the modern age, allergic manifestations are the more common result. Further work in humans to examine if human CD23 plays a similar regulatory role as murine CD23 and to clarify the precise role of specific domains of CD23

as well as the cell types involved will give us a better perspective on the role of CD23 in allergic inflammation and novel insights to target CD23 function in disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations used

HA	hemagglutinin
TNP	trinitrophenol
OVA	ovalbumin

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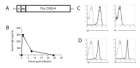

Figure 1.

Construction and characterization of HA-Fcε. A) Schematic of the HA-Fcε construct. The Cε2–4 domains of the IgE heavy chain are positioned C-terminal of a V_{kappa} signal sequence and the HA epitope tag. B) Groups of 2–3 IgE-deficient *4getRag2*$^{-/-}$ mice received 1 μg of native IgE (■) or HA-Fcε (▲) at time 0. We then serially bled the mice to follow the fate of infused IgE. Error bars represent the standard error of the mean (SEM). In C) and D), *4getRag2*$^{-/-}$ mice received 1 μg of native IgE (C) or HA-Fcε (D) by tail vein injection. Twenty-four hours later, we analyzed splenic basophils (GFP^{+} CD49b^{+} SSC^{lo}) for the presence of both total IgE and HA (solid black lines). The gray histograms indicate the staining of negative control IgE-deficient mice. Data are representative of at least 3 independent experiments.

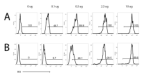

Figure 2.

Uptake of HA-Fcε by splenic basophils and peritoneal mast cells. We infused *4get* BALB/c mice with increasing amounts of HA-Fcε as listed at the top of the figure. Twenty-four hours later, we analyzed $GFP^{+}CD49b^{+}SSC^{lo}$ splenic basophils (A) or $GFP^{+}c\text{-}kit^{+}$ peritoneal mast cells (B) for HA-Fcε using an anti-HA antibody. The percent of HA-$Fc\varepsilon^{+}$ cells is depicted with each histogram. Data are representative of 2 independent experiments.

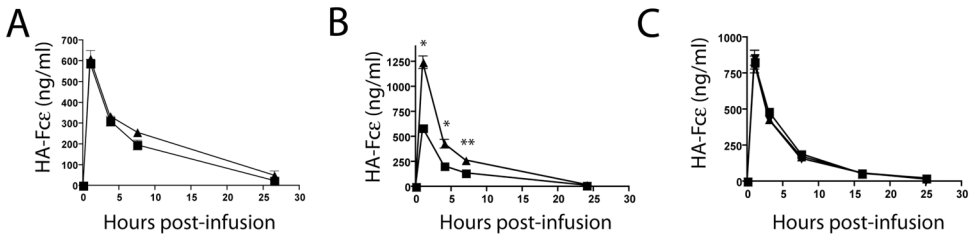

Figure 3.

B cells regulate serum IgE levels. A) Wild-type (■) or FcεRI$^{-/-}$ (▲) mice received 2.5 μg of HA-Fcε by tail vein injection. We then analyzed HA-Fcε levels at the indicated time points. B) We analyzed wild-type (■) or μMT$^{-/-}$ (▲) animals for HA-Fcε clearance at the indicated time points. C) Wild-type (■), Stat6$^{-/-}$ (▲), or IL-4/13$^{-/-}$ (▼) mice received 2.5 μg of HA-Fcε and were analyzed at the indicated time points. Data are representative of at least 2 independent experiments each. Wild-type and FcεRI$^{-/-}$ groups had 3 mice each. μMT and Stat6$^{-/-}$ groups had 4 mice each. The IL-4/13$^{-/-}$ group had 2 animals. Error bars represent the SEM. *p<0.001, **p<0.007

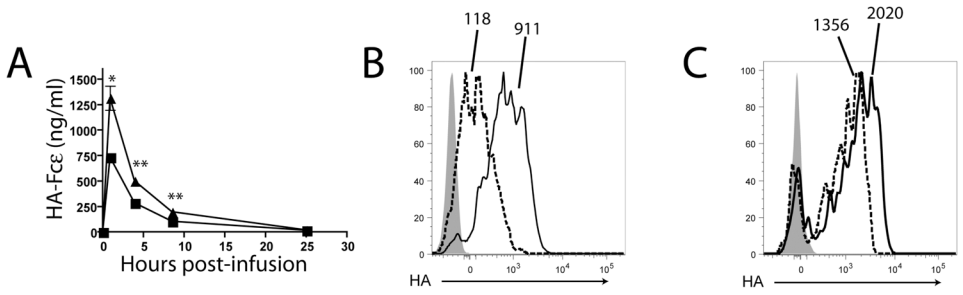

Figure 4.

Anti-CD23 blockade increases peak serum IgE levels and enhances IgE loading. A) BALB/c mice received either 100 μg of B3B4 (▲) or isotype (■) control antibody by tail vein injection. One day later, the mice received 2.5 μg of HA-Fcε and were serially bled to examine HA-Fcε serum levels. There were 5 mice in each group with error bars representing the SEM. *$p<0.015$, **$p<0.005$ In B) and C), the mice received B3B4 (solid black line) or isotype (dashed line) as above and were given 0.5 μg of HA-Fcε the next day. Surface HA-Fcε capture was assessed on B) Peritoneal mast cells and C) Splenic basophils twenty-four hours later. The mean fluorescence intensity is noted above the histograms. The shaded histograms represent the negative control. The data are representative of 2 independent experiments.

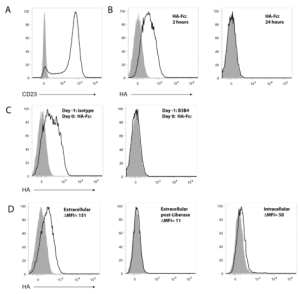

Figure 5.

B cells bind HA-Fcε in a CD23-dependent manner. A) $CD19^+$ splenocytes from BALB/c mice were stained with B3B4 (solid line) or isotype control (shaded histogram). B) BALB/c mice received either PBS or 2.5 μg of HA-Fcε. We harvested splenocytes 2 or 24 hours after HA-Fcε infusion and fixed the cells in 4% paraformaldehyde immediately. The timepoint at harvest is indicated in each of the histograms. We stained single cell suspensions with CD19, B220, and anti-HA antibodies. The solid lines in the each of the panels represent cell surface HA-Fcε on $CD19^+B220^+$ cells. The shaded histograms represent staining on B cells from mice that received PBS. C) Two hours after receiving 2.5 μg HA-Fcε, fixed B cells from mice that had received 100 μg of isotype control (left panel) or B3B4 (right panel) one day prior were examined as above. D) Two hours after receiving 2.5 μg HA-Fcε, fixed B cells from mice were stained for extracellular HA-Fcε (left panel) or intracellular HA-Fcε (right panel). The middle panel depicts extracellular HA-Fcε staining after collagenase digestion. Shaded histograms represent B cells from PBS infused animals. Data are representative of at least 2 independent experiments.

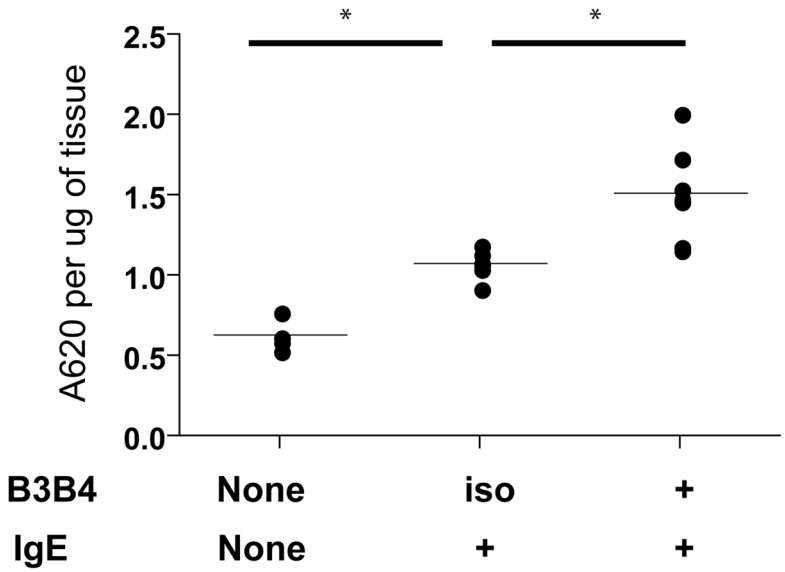

Figure 6.

CD23 blockade enhances local hypersensitivity reactions. Mice received either 100 μg isotype control or B3B4 antibody on Day 0. On Day 1, the mice were loaded with 0.5 μg of anti-TNP IgE. On Day 3, the mice received 200 ul of 1% Evans Blue dye by tail vein injection and then a 1 μg intradermal injection of TNP_{13}-OVA into the ear. A non-IgE loaded group of mice served as a background control. Average values were determined from the ears from 2 mice in the non-loaded group, 3 mice in the isotype treated group, and 4 mice from the B3B4 treated group. The data are representative of 2 independent experiments. *$p<0.01$

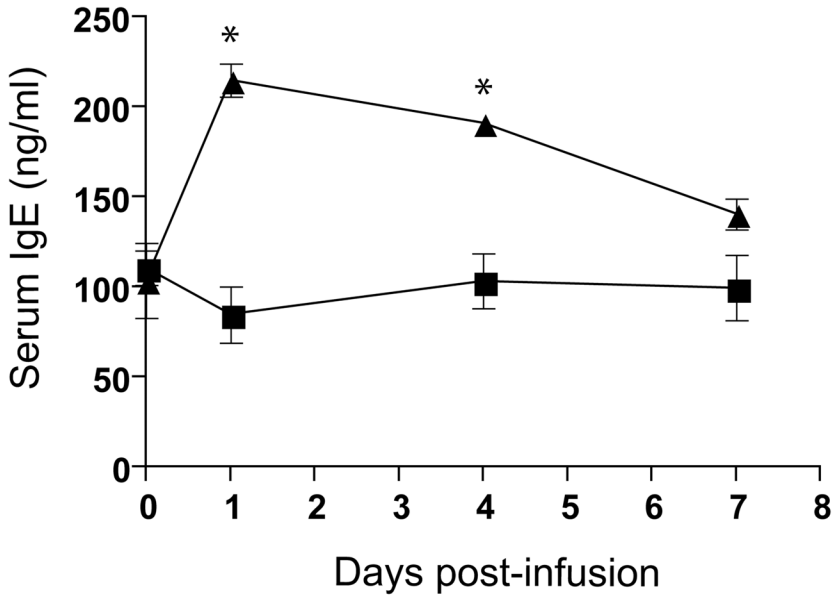

Figure 7.
CD23 blockade leads to an increase in the serum IgE pool. Groups of 3 BALB/c mice received 100 μg of either isotype control (■) or B3B4 (▲) antibody on Day 0. One, four, and seven days later, serum was collected from individual animals and serum IgE levels examined by ELISA. Error bars for each timepoint represent the SEM. Data are representative of 2 independent experiments. *$p<0.01$