

IgE and Mast Cells: The Endogenous Adjuvant

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Contents

1. Overview	2
2. Biology of Mast Cells and IgE	2
2.1 Mast Cell Ontogeny	2
2.2 Mast Cell Homeostasis	3
2.3 The Biology of IgE	4
2.4 Interdependence of IgE and Mast Cells	5
3. Mast Cell Activation Pathways	6
4. Immunomodulatory Functions of Mast Cells	7
4.1 Roles in Disease and Immunity	8
4.2 Mast Cells Functions in Allergic Inflammation	9
4.3 Findings from Novel Genetic Models of Mast Cell Deficiency	10
5. Mast Cells and IgE in Food Allergy	13
5.1 Prevalence and Immunology of Food Allergy	13
5.2 Effector Roles of IgE and Mast Cells in Food Allergy	13
5.3 Adjuvant Roles of Mast Cells and IgE	15
6. Immunomodulatory Roles of IgE Antibodies and Mast Cells	16
6.1 Effects on Th2 Responses	16
6.2 Mast Cell and Treg Interactions	19
7. Silencing of the IgE: Mast Cell Axis to Restore Immune Homeostasis	20
7.1 Immunomodulatory Effects of IgE-Targeted Approaches	22
7.2 Treg Control of Mast Cell Activation	26
7.3 IgG:FcγRIIb-Mediated Inhibition of Mast Cell Activation	29
8. Summary and Conclusions	37
Acknowledgment	38
References	38

Abstract

Mast cells and immunoglobulin E (IgE) are most familiar as the effectors of type I hypersensitivity reactions including anaphylaxis. It is becoming clear however that this pair has important immunomodulatory effects on innate and adaptive cells of the immune system. In this purview, they act as endogenous adjuvants to ignite evolving

immune responses, promote the transition of allergic disease into chronic illness, and disrupt the development of active mechanisms of tolerance to ingested foods. Suppression of IgE-mediated mast cell activation can be exerted by molecules targeting IgE, FcεRI, or signaling kinases including Syk, or by IgG antibodies acting via inhibitory Fcγ receptors. Recent reports indicate that such interventions have promise in the development of strategies to treat allergic disease.



1. OVERVIEW

In this review, we discuss the biology and functions of immunoglobulin E (IgE) and mast cells, two central players in allergic reactions. We set forth the evidence that IgE and mast cells serve to tune evolving immunological responses and that these actions can affect the course of immunity and allergic pathology. We cover recent advances in our understanding of food allergy and the mechanisms by which IgE-mediated activation of mast cells alters the development of both allergic effector mechanisms and immunological tolerance. Finally, we discuss various potential approaches to counteracting the effects of IgE and mast cells and the therapeutic implications of these manipulations for human disease.



2. BIOLOGY OF MAST CELLS AND IgE

2.1 Mast Cell Ontogeny

Mast cells are long-lived cells of the immune system that reside in tissues and rarely circulate in the bloodstream. They were named by Paul Ehrlich as fat-tening cells or “Mastzellen” due to their numerous granules, which he erroneously concluded were present to nurture the surrounding tissue, and their function as effectors of immediate hypersensitivity reactions including anaphylaxis was not known until the 1950s (Beaven, 2009; Blank, Falcone, & Nilsson, 2013). Mast cells derive from myeloid lineage progenitor cells originating in the bone marrow which circulate to tissues where they undergo maturation and tissue-specific differentiation (Gurish & Boyce, 2006). Mast cells are enriched in mucosal tissues where their proximity to the interface with the external environment makes them one of the first cells of the immune system to encounter foreign antigens. This placement along with their ability to store and rapidly release potent mediators renders them an effective line of defense against venoms (Metz et al., 2006) and has contributed to the idea that they shape the initial events in developing immune

responses (Abraham & St John, 2010; Bischoff, 2007; Burton & Oettgen, 2011; Galli, Grimaldeston, & Tsai, 2008; Galli et al., 2005; Galli & Tsai, 2012; Galli, Tsai, & Piliponsky, 2008; Rodewald & Feyerabend, 2012).

2.2 Mast Cell Homeostasis

Mast cells arise in tissues from circulating progenitors and depend fundamentally on the presence of stem cell factor (SCF) for their survival. This cytokine acts on its receptor, c-Kit, which is expressed on mast cells in both mice and humans. Kit-deficient mice lack mast cells entirely. Some additional cytokines promote mast cell growth and survival, notably interleukin (IL)-3, IL-4, and IL-9, which are synthesized during the course of parasite infections and in allergic disease. Others, such as IL-10 and transforming growth factor (TGF)- β , counteract mast cell growth and activation (Broide, Wasserman, Alvaro-Gracia, Zvaifler, & Firestein, 1989; Shelburne & Ryan, 2001; Toyota, Hashimoto, Matsuo, & Iizuka, 1995). IL-3 is a major contributor to mast cell expansion in murine helminth infection (Lantz et al., 1998), but human mast cells do not universally respond to IL-3 (Gebhardt et al., 2002; Valent et al., 1990). Although mouse and human mast cells are both highly granular, tissue-resident cells that express the high-affinity IgE receptor Fc ϵ RI, there are important differences in localization and phenotype between species. The phenotype of mast cells varies considerably depending on the tissue in which they are situated, with mast cells in the mucosal compartments of the airways or gastrointestinal tract generally being smaller and less densely granulated than their counterparts in the skin or musculature (Xing, Austen, Gurish, & Jones, 2011).

In addition to governing mast cell growth, cytokine signals regulate the granule content of mast cells. SCF concentrations increase granularity and lower the threshold for mast cell activation. IL-4 exerts similar functions, although it lacks the ability to directly trigger degranulation the way high levels of SCF can (Coleman, Holliday, Kimber, Zsebo, & Galli, 1993; Lorentz et al., 2005; Suzuki et al., 2000; Taylor, Galli, & Coleman, 1995). Whereas SCF is produced primarily by endothelial cells and fibroblasts, IL-4 derives from immune cells and can consequently dramatically increase during the course of T helper (Th) 2 immune responses, such as occur following parasite infestation or in food allergy. In these cases, IL-4 may drive exponential increases in intestinal mast cell numbers, acting through the IL-4 receptor alpha chain (IL-4R α) and the signaling molecule STAT6 (Madden et al., 1991, 2002; Takeda et al., 1996). IL-4R α signaling

is the key driver of intestinal mast cell expansion in food-allergic responses (Brandt et al., 2009; Burton et al., 2013).

2.3 The Biology of IgE

IgE and mast cells have a long-standing historical connection due to their involvement in immediate hypersensitivity. The search for IgE was triggered by the observation that an unknown substance in serum could transfer immediate hypersensitivity from allergen to a nonallergic subject (the famed Prausnitz–Küstner test). Although the identification of IgE and mast cells as the effectors of this response would take decades, it was their close relationship that allowed IgE, the last and rarest of the immunoglobulins, to be found (Blank et al., 2013). Mast cells had been reported as early as 1863, but their function eluded scientists for several decades. Eventually, the discoveries that histamine could reproduce symptoms of immediate hypersensitivity and that mast cells could be activated to release histamine by IgE led to an understanding of the roles of IgE and mast cells in allergy and anaphylaxis (Blank et al., 2013).

IgE antibodies have a standard immunoglobulin structure of paired heavy and light chains. The constant regions of these antibodies are comprised of four immunoglobulin domain repeats instead of three as found in IgG isotypes, with the fourth replacing the normal hinge region. IgE is heavily glycosylated, a posttranslational modification that may affect binding to cellular receptors. Two receptors account for the majority of IgE's activity: the high-affinity receptor, FcεRI, expressed on mast cells and basophils and also widely on dendritic cells and eosinophils in humans but not in mice; and the low-affinity receptor, CD23, expressed predominantly on B cells, dendritic cells, and epithelial cells. The exceptionally strong interaction between IgE and FcεRI (10^{-9} M K_d) accounts for the unusual ability of IgE to remain more or less permanently attached to cells and renders the receptor saturated at physiologic IgE concentrations (Kulczycki, Isersky, & Metzger, 1974). Interactions between IgE and CD23 occur with much lower affinity, but the formation of CD23 trimers allows for a compensatory increase in avidity. IgE has a markedly shorter half-life than IgG in the bloodstream, on the order of a day rather than 3 weeks (Haba, Ovary, & Nisonoff, 1985; Hirano, Hom, & Ovary, 1983; Kubo, Nakayama, Matsuoka, Yonekawa, & Karasuyama, 2003). This is due in part to rapid clearance by binding to cellular receptors (Cheng, Wang, & Locksley, 2010; Greer et al., 2014) and may also be influenced by the lack of recycling

via FcRn, which maintains serum IgG levels (Lencer & Blumberg, 2005; Roopenian & Akilesh, 2007). The tissue half-life of IgE (where it is fixed to mast cells) is thought to be much longer, weeks or month.

Synthesis of IgE is induced by IL-4-driven transcription at the C ϵ locus in IgM⁺ or IgG⁺ B cells, followed by class-switch recombination driven by costimulatory CD40:CD40L interactions (Gould & Sutton, 2008; Gould et al., 2003). Switching to IgE often takes longer than with other isotypes. This may be due in part to the occurrence of sequential switching, first to IgG and then to IgE. The IgG switch is favored at sites where T cells first encounter antigen in secondary lymphoid organs (which are not conducive to IgE switching), while the IgE switch is favored at mucosal sites. The importance of sequential switching and the relative contributions of common and stable IgG⁺ B cells versus rare and seemingly unstable IgE⁺ B cells to IgE memory have been addressed in a series of recent studies, reviewed by Gould and Ramadanii (2015).

2.4 Interdependence of IgE and Mast Cells

As a result of the tight adherence of IgE to Fc ϵ RI, circulating IgE levels are very low and tissue mast cells are the main reservoir for IgE. Once bound to a mast cell, IgE may persist for months if not the life of the cell, as has been documented in the case of solid-organ transplant recipients receiving tissue from allergic donors (Castells & Boyce, 1998; Legendre et al., 1997). This has clinical implications including the fact that several weeks of treatment with anti-IgE antibodies (which does not dislodge receptor-bound IgE) are required to eliminate IgE-mediated hypersensitivity.

Although we often think of IgE-mediated effects on mast cells as being antigen-dependent, occurring after polyvalent allergens cross-link Fc ϵ RI-bound IgE, there is accumulating evidence for the existence of antigen-independent Fc ϵ RI-mediated IgE effects on mast cells. Even in the absence of allergen, exposure of mast cells to IgE favors survival and in some cases can cause minor Fc ϵ RI aggregation, driving low-level production of cytokines that prevent apoptosis (Asai et al., 2001; Kalesnikoff et al., 2001; Kashiwakura et al., 2009). These findings were first described using *in vitro* culture of mouse bone marrow-derived mast cells (BMMCs). BMMCs require IL-3 and/or SCF in order to survive, but the addition of monoclonal IgE can stave off programmed cell death following growth factor removal. Some IgE clones are more effective than others due to their ability to trigger Fc ϵ RI signaling and secretion of cytokines such as IL-3

(Kawakami & Galli, 2002; Kawakami & Kitaura, 2005; Kitaura et al., 2003). One particularly potent IgE clone is the commonly used SPE-7, which carries a primary specificity for the hapten dinitrophenyl (DNP), but intriguingly also recognizes endogenous thioredoxin in an alternate conformation (James, Roversi, & Tawfik, 2003). It may be the case that IgE antibodies like SPE-7 that carry a low level of autoreactivity will create a constant mild stimulus for the mast cell, promoting survival. This relationship between IgE and mast cells' survival appears to favor the development of allergic disease (Burton & Oettgen, 2011). As would be predicted from the pro-survival effects of IgE antibodies on mast cells in culture, we have observed that mast cell numbers *in vivo* are tied to IgE production in mouse models of asthma, food allergy, and parasite infection (Burton, Noval Rivas, et al., 2014; Gurish et al., 2004; Mathias et al., 2009).

The presence of IgE also increases FcεRI expression, a positive feedback mechanism that automatically creates more anchoring sites for IgE (Furuichi, Rivera, & Isersky, 1985; Malveaux, Conroy, Adkinson, & Lichtenstein, 1978; Yamaguchi et al., 1997). This induction is clinically relevant; basophils from patients treated with omalizumab have lower surface FcεRI density and are therefore refractory to allergen challenge (Beck, Marcotte, MacGlashan, Togias, & Saini, 2004; MacGlashan, 2004; MacGlashan & Saini, 2013). It is now believed that many of the salutary effects of omalizumab are exerted by this receptor downregulation more than by the removal of circulating allergen-specific IgE. This may be particularly true in chronic urticaria, where omalizumab is quite effective.



3. MAST CELL ACTIVATION PATHWAYS

IgE and mast cells are known first and foremost for driving immediate hypersensitivity, the class of acute allergic reactions such as hives, wheezing, and anaphylaxis. These occur when allergen interacts with allergen-specific IgE bound to the mast cell via the high-affinity IgE receptor, FcεRI. Aggregation of FcεRI activates the key proximal kinase Syk initiating the formation of a signaling complex including the linker proteins LAT and SLP76 and recruitment of a number of enzymatically active signal transducers including PLCγ. PLCγ-dependent production of inositol triphosphate (IP₃) results in release of calcium stores in the endoplasmic reticulum into the cytosol, triggering granules to fuse with the plasma membrane and release preformed vasoactive mediators such as histamine and serotonin. Parallel activation of mitogen-activated protein (MAP) kinases, including p38,

ERK, AKT, JNK, and PI3K, leads to cytokine and chemokine gene transcription alongside induction of eicosanoid metabolism. Degranulation and eicosanoid mediator synthesis occur rapidly and act primarily on nonhematopoietic cells to elicit the acute allergic reaction, whereas production of cytokines requires hours and induces a secondary response through actions on cells of the immune system (Gilfillan & Tkaczyk, 2006).

IgE confers antigen specificity to mast cells, arming them to serve as effectors of the adaptive immune system and focusing their effector responses to molecules that have already initiated T and B cell (IgE) responses. Antigen-specific mast cell activation can similarly be mediated by IgG through activating Fc gamma receptors, such as FcγRIII. However, the low affinity of this interaction requires far higher levels of IgG and antigen for effective stimulation. Mast cells are also equipped with an array of innate receptors that enable them to react directly to pathogen triggers or endogenous danger signals. Among these, Toll-like receptors (TLRs), G-protein receptors, and complement receptors can also direct cytokine secretion and/or mediator release. Mast cells are often located adjacent to neuronal processes, and a variety of neural signaling molecules, including substance P and vasoactive intestinal peptide, have been shown to activate mast cells, suggesting that higher level neural processes can also trigger degranulation (Buhner & Schemann, 2012; MacQueen, Marshall, Perdue, Siegel, & Bienenstock, 1989; van Diest, Stanisor, Boeckxstaens, de Jonge, & van den Wijngaard, 2012).



4. IMMUNOMODULATORY FUNCTIONS OF MAST CELLS

The last couple of decades have seen the emergence of understanding of a range of previously unknown functions for mast cells, with accumulating evidence revealing that they serve not only as effectors of immediate hypersensitivity but also play perhaps even more important roles as immune regulators. The placement of mast cells at mucosal interfaces where foreign antigens are first encountered along with their ability to secrete significant quantities of cytokines positions them to enhance and direct early immune responses. This is particularly true in situations where antigen is limiting or there are few additional immune triggers (such as TLR activation) and the sensitive FcεRI signaling cascade mediates massive bioamplification of limiting antigenic signals. In this way, IgE and mast cells can exert an adjuvant-like effect, priming immune responses through effects on both innate and adaptive cell types.

Mast cells have been reported to influence a wide array of cell types *in vivo*, including vascular and epithelial tissues as well as hematopoietic lineages. Mast cells store some cytokines preformed for immediate release upon degranulation, and several other cytokines and chemokines are induced rapidly in response to IgE stimulation, driving recruitment, activation, and differentiation of innate and adaptive immune cell types. Tumor necrosis factor (TNF) α , histamine, and proteases are immediately available upon degranulation and act to recruit neutrophils, T cells, and B cells (Galli & Tsai, 2012). Rapid *de novo* synthesis of leukotrienes and prostaglandins supports chemotaxis of various cell types and also influences antigen-presenting cell (APC) function and CD4⁺ T cell proliferation and differentiation (Boniface et al., 2009; Chen et al., 2009; Kickler et al., 2012; Krause et al., 2009; Yao et al., 2013). Cytokines produced by mast cells have the potential to significantly influence developing immune responses, with TNF α , IL-1, IL-4, IL-5, IL-6, IL-10, IL-12, and IL-13 all being capable of altering B and/or CD4⁺ T cell differentiation (Galli, Grimbaldston, et al., 2008). Steady-state interactions of IgE with Fc ϵ RI in the absence of antigen underlie the immune priming functions of mast cells in the skin, and this constant low-level excitation governs the maturation and migration of dendritic cells upon initial encounters with antigen (Bryce et al., 2004; Dudeck et al., 2011; Suto et al., 2006).

4.1 Roles in Disease and Immunity

Functions for mast cells have been demonstrated in shaping protective immunity against bacteria, viruses, and multicellular parasites (Abraham & St John, 2010). Mast cell-derived TNF α plays an important role in recruiting cells to the draining lymph node during infection (McLachlan et al., 2003). Preformed stores are particularly important in creating a rapid innate response through maturation of dendritic cells, which carry pathogen antigens for presentation (Gordon & Galli, 1990; Suto et al., 2006). TNF α is important along with other factors in recruiting neutrophils to the site of bacterial infections, facilitating pathogen clearance (Malaviya, Ikeda, Ross, & Abraham, 1996; Sutherland, Olsen, McKinstry, Villalta, & Wolters, 2008).

During the course of parasitic worm infections, IgE-dependent activation of mast cells may provide important protective effects relating to their ability to alter intestinal epithelial cell function, recruit other effector cells such as eosinophils, and secrete toxic molecules (Lawrence, Paterson, Wright, Knight, & Miller, 2004; Malaviya et al., 1994). IgE promotes mast

cell expansion and activation during the course of *Trichinella spiralis* infection, accelerating parasite clearance (Gurish et al., 2004; Urban et al., 2000), and mast cell-deficient mice exhibit delayed expulsion of hookworms during primary infection (Ohnmacht & Voehringer, 2010). Mast cell secretion of proteases has been shown to promote intestinal helminth expulsion by disrupting intestinal barrier function and larval killing of roundworm larvae through eosinophil recruitment (Lawrence et al., 2004; McDermott et al., 2003; Shin et al., 2008; Woodbury et al., 1984). As with most immunological functions, redundancies exist in antiparasite responses, and basophils provide complementary immunomodulatory and protective functions (Blankenhaus et al., 2014; Ohnmacht & Voehringer, 2010; Schwartz et al., 2014).

4.2 Mast Cells Functions in Allergic Inflammation

Rapid and copious production of proinflammatory cytokines by IgE-activated mast cells underlies their ability to incite late-phase responses to allergen and subsequently induce chronic allergic inflammation. Dissecting the specific *in vivo* contributions of mast cells to allergic pathology has been achieved using animals genetically deficient in mast cells or lacking molecules related to mast cell function, such as IgE. The discovery of mice with spontaneous mast cell deficiency due to c-Kit mutations led to a burst of research regarding the functions of mast cells. These Kit mutants, including the *Kit^{W-sh}* mouse, lack mast cells but exhibit additional phenotypes related to the lack of SCF signaling, including alterations in neutrophil homeostasis (Nigrovic et al., 2008). Because of this complexity in Kit function, alterations in allergic responses in Kit-mutant animals cannot be unambiguously attributed to mast cell function. A critical advance in the application of Kit mutants to the study of mast cell functions came about when it was demonstrated that Kit-mutant mice could be rendered replete with mast cells by injection of cultured mast cells generated *in vitro* from bone marrow progenitors (BMMCs). Restoration of a phenotype by this maneuver is considered strong evidence for a mast cell role. For many years, mast cell reconstitution of mast cell-deficient Kit-mutant mice remained the gold standard for investigating mast cell functions. Further confirmation of a mast cell-dependent effect could be obtained through genetic targeting of specific factors related to mast cells, such as IgE, FcεRI, or proteases, to confirm and valid findings obtained with Kit-mutant mice.

Important caveats still apply to the Kit-mutant mast cell reconstitution models, and these should be carefully considered when evaluating reports

based on such models. *In vitro*-generated mast cells are immature and do not fully replicate mature mast cells, which are phenotypically shaped by the surrounding tissue (Xing et al., 2011). It is not clear whether the cells take on appropriate specific phenotypes as they repopulate the tissues. Furthermore, while reconstitution of Kit-mutant mice with BMMCs generally restores significant numbers of mast cells, it does not necessarily recapitulate the normal distribution of these cells (Grimbaldeston et al., 2005; Wolters et al., 2005).

The application of Kit-mutant mice has permitted dissection of the immunomodulatory effects of mast cells *in vivo*. Work from Stephen Galli's group has established that mast cells can amplify the development of allergic airway and skin inflammation in settings where antigen is encountered in the absence of adjuvant (Galli & Tsai, 2012). Previous work in adjuvant-driven models had failed to show this mast cell effect. This suggests that mast cells act as amplifiers, substituting for artificial adjuvants in initial antigen encounters and promoting the induction of inflammation. In ovalbumin (OVA)-induced airway inflammation, IFN γ acting on mast cells drives chemokine expression, promoting the accumulation of eosinophils, neutrophils, and lymphocytes in the airways, which leads to tissue remodeling and disrupts normal breathing (Williams & Galli, 2000; Yu et al., 2011). Activation of mast cells through Fc ϵ RI is required to elicit robust inflammation in allergic skin inflammation and in delayed hypersensitivity responses to contact sensitizers (Ando et al., 2014; Bryce et al., 2004). These models require adaptive immune responses coordinated by CD4⁺ T cells, suggesting that mast cell-dependent alterations in antigen presentation or direct effects on T cell trafficking or phenotype are involved. Interestingly, mast cells have also been found to regulate the development of chronic skin inflammation through the production of IL-10 (Grimbaldeston, Metz, Yu, Tsai, & Galli, 2006; Grimbaldeston, Nakae, Kalesnikoff, Tsai, & Galli, 2007). In most of the examples reviewed here, findings from Kit-mutant models of mast cell deficiency have been corroborated by results obtained using other approaches to probing the IgE:mast cell axis, such as genetic targeting of IgE, Fc ϵ RI α , or Fc ϵ RI γ .

4.3 Findings from Novel Genetic Models of Mast Cell Deficiency

Several investigators have recently developed Kit-independent models of mast cell deficiency (Dudeck et al., 2011; Peschke, Dudeck, Rabenhorst, Hartmann, & Roers, 2015; Scholten et al., 2008; Voehringer, 2013).

Notable among these models are (1) the Cre-Master mouse, in which Cpa3-driven expression of Cre recombinase eliminates mast cells through genotoxic effects of Cre expression (Feyerabend et al., 2011); (2) Mas-TRECK mice, which enable diphtheria toxin-mediated depletion of mast cells through expression of the diphtheria toxin receptor (DTR) directed by an IL-4 promoter sequence unique to mast cells and basophils (Sawaguchi et al., 2012); (3) Cpa3^{Cre} or “Hello Kitty” mice wherein the Cpa3 promoter directs Cre expression without killing mast cells (Lilla et al., 2011); and (4) Mcpt5^{Cre} mice, in which Cre expression is specifically targeted to connective tissue mast cells (Scholten et al., 2008). In an interesting development that has introduced some controversy into the field, results obtained using some of the newer mutants appear to contradict long established understandings regarding immunomodulatory functions of mast cells previously derived from studies using Kit-based models (Rodewald & Feyerabend, 2012). It is important to recognize, however, that each of the new mast cell-targeted approaches has proven to have its own limitations: basophils are reduced or absent in Cre-Master, Cpa3^{Cre}, and Mas-TRECK mice; mucosal mast cells are not affected under steady-state conditions in Mcpt5^{Cre} mice; and the Cpa3 locus displays low-level activity in several types of leukocytes apart from mast cells (Feyerabend et al., 2009; Lilla et al., 2011; Reber, Marichal, & Galli, 2012; Rodewald & Feyerabend, 2012). In light of this complexity, it will be challenging to resolve some of the conflicting results.

In one example of the disparity of observations obtained using different models, studies in Kit mutants implicating mast cells in autoimmunity have been called into question by results obtained using mast cell-deficient Cre-Master mice (Feyerabend et al., 2011; Gutierrez et al., 2014). A previous work with Kit-deficient mice (as well as BMMC-reconstituted animals) by Brown, Syed, and colleagues had established functions for mast cells in inducing experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis. Their work convincingly showed mast cell-dependent priming effects on the induction of autoreactive T cells as well as end-stage effector roles for mast cells in antibody-mediated pathology (Gregory, Raju, Winandy, & Brown, 2006; Robbie-Ryan, Tanzola, Secor, & Brown, 2003; Secor, Secor, Gutekunst, & Brown, 2000). In conflicting studies, done more recently using Cre-Master mice, Feyerabend et al. were unable to replicate the reduced EAE susceptibility of Kit-mutant mice (Feyerabend et al., 2011). It was suggested that the distinctive white coat color that accompanies Kit mutations in mice might have prevented unbiased observation by

researchers (Rodewald & Feyerabend, 2012). It remains problematic that two distinct Kit-mutant mouse lines display highly divergent responses in EAE models, but at this point, the preponderance of studies does not suggest an obligate role for mast cells in this pathology (Li et al., 2011; Piconese et al., 2011).

Similarly, in investigations of immune complex-induced arthritis, mast cells had also been reported to influence disease progression through interactions with T cells and autoreactive antibodies. The role of mast cells in antibody-mediated pathology in arthritis had already been the subject of some controversy since different types of Kit-mutant mice exhibited variable susceptibility to joint inflammation (Elliott et al., 2011; Zhou, Xing, Friend, Austen, & Katz, 2007). Some of the newer Kit-independent mutants have recently been applied to arthritis models in an effort to bring clarity to this issue. While Cre-Master mice show no alterations in disease progression in the K/BxN serum transfer model of arthritis (Feyerabend et al., 2011), a gain-of-function mutation directed by $Mcpt5^{Cre}$ in mast cells exacerbated collagen-induced arthritis (Heger et al., 2014). Perhaps most interestingly, a comparison of results obtained using models of autoimmune arthritis suggests that mast cells may be more critical in priming the autoreactive T cell response but might be dispensable in antibody-mediated pathology, where neutrophils can substitute as effector cells (Schubert et al., 2014). The importance of mast cells in shaping evolving T cell responses, either directly or through effects on dendritic cells, will depend a great deal on the strength of the stimulus used and, in rodent models, whether potent adjuvants are used that circumvent immunological priming requirements. In this respect, the K/BxN serum transfer model, which completely bypasses T cell-driven immunity to elicit autoimmune pathology, might be least likely to involve mast cells.

Not all findings from models using genetic targeting of mast cells have conflicted with the canon of data developed using Kit mutants. Investigations using $Mcpt5^{Cre}$ mice confirmed the requirement for mast cells in initiating contact sensitivity following hapten exposure on the skin (Dudeck et al., 2011). As had been demonstrated with Kit-mutant and IgE-deficient mice, in the absence of mast cell activation, dendritic cell migration is impaired and T cell-dependent sensitivity to the hapten is impaired (Bryce et al., 2004; Suto et al., 2006). Depletion of mast cells in Mas-TRECK mice reduced the development of chronic allergic inflammation and decreased IL-4 production in an adjuvant-independent model of airway hyperresponsiveness, findings that parallel those previously obtained with

Kit-mutant mice (Sawaguchi et al., 2012). Many additional functions attributed to mast cells have yet to be addressed with the new genetic systems. We anticipate that accumulating data obtained using various experimental models of mast cell depletion will demonstrate that mast cells have a general ability to tune developing immune responses by enhancing the maturation of dendritic cells and the priming of T cells, but that these effects are best observed in the absence of potent innate immune stimuli (such as adjuvants) and when immunity arises following chronic low-level exposure (as in allergy).



5. MAST CELLS AND IgE IN FOOD ALLERGY

5.1 Prevalence and Immunology of Food Allergy

Food allergy is quickly becoming one of the major health issues facing developed countries with prevalence rates in the United States having increased over the last two decades to as high as 6% in children and 3% in adults (Branum & Lukacs, 2008, 2009; Eigenmann et al., 2008; Turner et al., 2014). Severe food reactions are the leading cause of hospital visits for anaphylaxis in the pediatric population (Turner et al., 2014). In addition to the risk of reactions, children with food allergies suffer dietary restrictions and a reduced quality of life at home and in school (Lieberman & Sicherer, 2011).

Immunologically, food allergies are characterized by a failure to develop tolerogenic regulatory T cell (Treg) responses and instead inappropriate Th2 responses to ingested proteins. T cell production of IL-4 encourages B cell class switching to IgE and growth of mast cells, setting the stage for immediate hypersensitivity responses that produce characteristic symptoms including hives, itching, vomiting, and diarrhea. In some individuals, allergen ingestion can result in systemic anaphylaxis, wherein mast cell mediators act on the vascular tissue, opening the spaces between endothelial cells to allow plasma extravasation, shock, and occasionally cardiovascular collapse and death (Finkelman, 2007; Simons, 2010).

5.2 Effector Roles of IgE and Mast Cells in Food Allergy

A major strategy for the analysis of IgE and mast cell functions in immune sensitization and effector responses in food allergy has been to develop animal models. Reproducing food allergy in mice has been difficult since mice, like most humans, normally develop sustained immunological tolerance to ingested antigens. Several models exist in which coadministration of allergen

with bacterial toxins as adjuvant can be used to create a modest disruption in oral tolerance (Ganeshan et al., 2009; Li et al., 2000). Alternatively, parenteral priming with allergen and adjuvant followed by repeated gavage feedings with allergen can provoke some feature of food allergy (Brandt et al., 2003; Kweon, Yamamoto, Kajiki, Takahashi, & Kiyono, 2000). These models are typically associated with Th2 recall responses to the allergen, production of allergen-specific IgE, and intestinal mast cell expansion, but symptoms elicited by allergen challenge are usually mild and systemic anaphylaxis can only be reliably achieved in response to parenteral injection. Disruption of the skin barrier as occurs in atopic dermatitis, a condition associated with a high risk of food allergy in infants, leads to the development of anaphylactic food allergy in mouse models (Bartnikas et al., 2013). Together, these models have established clear requirements for IgE and mast cells in the acute allergic responses to food allergens (Bartnikas et al., 2013; Brandt et al., 2003; Reber et al., 2013).

In collaboration with Talal Chatila, we characterized a mouse model of food allergy based on a genetic approach. Research into the etiology of asthma led to the discovery of associations between human asthma susceptibility and polymorphisms in the IL-4 receptor alpha chain (IL-4R α) that enhanced signaling (Tachdjian et al., 2009). IL-4R α signals are required for many aspects of allergic disease and had been clearly implicated in established murine models of food allergy (Brandt et al., 2003). Capitalizing on the effects of a known modest mutation of IL-4R α , a new strain of inherently atopic F709 mice was generated in which mutation of the IL-4R α immunoreceptor tyrosine-based inhibitory motif (ITIM) rendered IL-4 receptor signaling disinhibited (Tachdjian et al., 2010). In addition to being generally more susceptible to allergy, these mice develop allergic sensitization upon introduction of a new food into their diet (Mathias et al., 2011). Initially, these studies were conducted with the model allergen OVA, commonly used in such models, but have since successfully adapted to the clinically relevant food allergen, peanut. In this model, allergen is delivered by gavage feedings weekly for 4–9 weeks to evoke sensitization. Mice are then subjected to an enteral challenge with a higher dose of allergen, which provokes anaphylactic shock. The course and severity of the anaphylaxis are measured using subcutaneously implanted microchips that measure core body temperature, which decreases in response to vasodilation and peripheral cooling. Unlike other models of food allergy in mice, no adjuvant or nonphysiologic parenteral priming is required to sensitize these F709 mice to ingested food allergens, and robust anaphylaxis is observed without

systemic injection of allergen. They also exhibit some spontaneous sensitization to standard chow components such as wheat and soy (Burton, Noval Rivas, et al., 2014).

We used the inherently atopic F709 mice to examine the contributions of IgE and mast cells to food allergen-induced anaphylaxis. In other models of murine food allergy, anaphylaxis can be evoked following parenteral injection of allergen, a nonphysiologic means of allergen exposure. In this setting, cells other than mast cells and molecules other than IgE have been shown to contribute to the severity of the anaphylaxis (Oettgen et al., 1994; Khodoun et al., 2009; Reber et al., 2013). In our ingestion challenge model, however, both anaphylaxis and other measures of mast cell activation are abrogated in the absence of IgE or FcεRI (Burton, Noval Rivas, et al., 2014; Mathias et al., 2011). F709 mice exhibit completely mast cell-driven anaphylactic responses, as evidenced by crosses with mast cell-deficient *Kit^{W-sh}* mice or mast cell ablation in F709 *Mcpt5^{Cre}* iDTR mice (Burton, Noval Rivas, et al., 2014). Consistent with a purely IgE-driven anaphylactic response, acute pharmacological inhibition of the key kinase downstream of FcεRI, Syk, blocks anaphylaxis and mast cell degranulation in allergic F709 mice (Burton, Noval Rivas, et al., 2014). These findings established mast cells and IgE as the key effectors of food allergen-induced anaphylactic reactions following exposure through the oral route.

5.3 Adjuvant Roles of Mast Cells and IgE

Application of the novel F709 genetic model of food allergy led to the unveiling of a previously unknown role for mast cells and IgE in disrupting the establishment of oral tolerance (Burton, Noval Rivas, et al., 2014). The lack of a requirement for adjuvant in this model allowed for the interrogation of physiologic immune sensitization pathways that occur in the gastrointestinal mucosa under atopic conditions.

Surprisingly, in addition to their effector functions in the end-stage allergic reaction, IgE and mast cells were also required to achieve immune sensitization. Mast cells and IgE regulated the crucial balance between tolerance and allergy by disrupting allergen-specific Treg induction, favoring Th2 induction. Upon feeding of either the relevant food allergen peanut or the model allergen OVA, F709 mice exhibited vastly reduced frequencies of Foxp3⁺ Tregs among allergen-specific CD4⁺ T cells relative to their wild-type counterparts. In the absence of IgE, F709 *IgE^{-/-}* mice developed near normal levels of Treg. The requirement for IgE for Treg suppression in

food allergy suggested that mast cells and their products might inhibit Treg formation. We attempted to test the contributions of mast cells using both Kit-dependent and Kit-independent models of mast cell deficiency. F709 mice carrying the *Kit*^{W^{-sh}} mutation or depleted of mast cells by Mcpt5^{Cre}-targeted ablation regained peanut-specific Treg induction, suggesting that IgE-dependent activation of mast cells was responsible for the loss of Treg. This interpretation was confirmed by reconstituting F709 *Kit*^{W^{-sh}} mice with mast cells, which resulted in reduced Treg generation. Similarly, specifically disrupting IgE receptor signaling in mast cells by Cre-lox-mediated excision of *Syk* restored Treg to near wild-type levels (Burton, Noval Rivas, et al., 2014).

In the same manner that mast cells and IgE counteracted Treg induction, they facilitated allergic sensitization by enhancing allergen-specific CD4⁺ T cell production of IL-4 and B cell class switching to IgE. In the absence of IgE, Th2 responses were dramatically reduced. Ablation of mast cells or genetic targeting of *Syk* in mast cells largely abolished IgE production and reduced IL-4 secretion by memory T cells. Normal but not IL-4-deficient mast cells were able to elicit allergic sensitization in reconstituted *Kit*^{W^{-sh}} mice, suggesting that mast cells might directly favor the conversion of naïve T cells to Th2 and simultaneously inhibit Treg conversion through the production of IL-4. Without mast cells as a cellular source of IL-4, oral tolerance was maintained and allergic sensitization did not proceed (Burton, Noval Rivas, et al., 2014).

These findings confirm a long-held theory that the IgE:mast cell axis can act as a feedback loop, amplifying Th2 responses. This immunomodulatory effect is essential to the development of allergic pathology in a model that does not require adjuvant. In our hands, sensitization with adjuvant obscures many of the contributions of mast cells and IgE to allergic sensitization, presumably because the adjuvant performs many of the same functions that the mast cell would normally accomplish.



6. IMMUNOMODULATORY ROLES OF IgE ANTIBODIES AND MAST CELLS

6.1 Effects on Th2 Responses

There is accumulating evidence that antigen presentation functions, cognate mast cell-T cell interactions, and secreted cytokines mediate the augmentation of Th2 responses by mast cells. Mast cells are known to produce substantial quantities of Th2-associated cytokines upon FcεRI engagement.

The ability of mast cells to make IL-4 and thus provide an early innate priming of Th2 induction remains somewhat controversial. The *Il4* locus is constitutively active in mast cells and basophils, resulting in GFP expression in 4get reporter mice (Fig. 1A) (Gessner, Mohrs, & Mohrs, 2005). IgE: FcεRI-dependent activation of BMMCs results in rapid and impressive induction of transcripts for both IL-4 and IL-13, but IL-4 protein is often

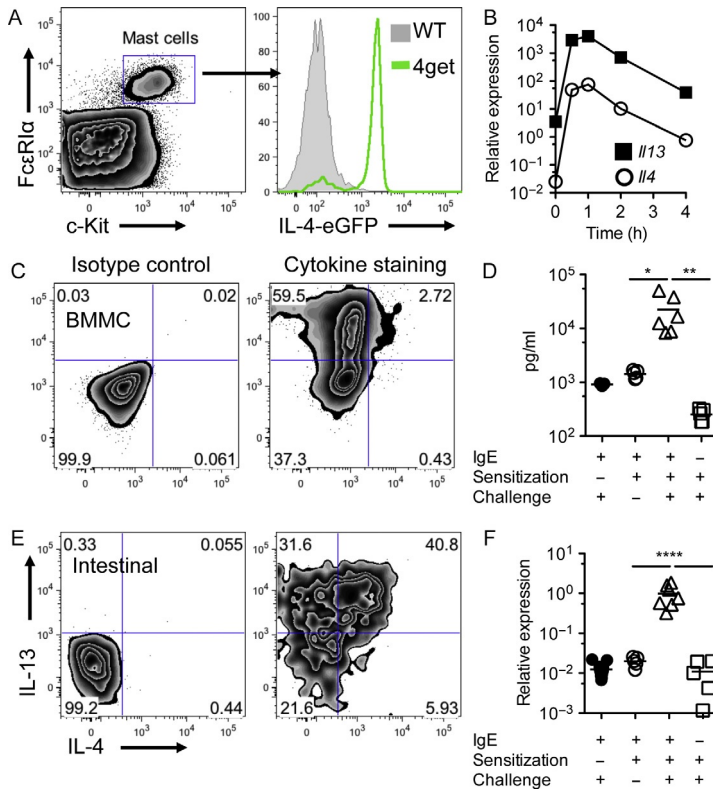


Figure 1 Mast cells as an innate IL-4 source. (A) Mast cells are GFP⁺ in 4get IL-4 reporter mice. Sample flow plots showing gating strategy for jejunal mast cells gated on viable CD45⁺ cells. (B) qPCR reveals rapid upregulation of both *Il4* and *Il13* in IgE anti-TNP-sensitized BMMCs stimulated with TNP-OVA. (C) Intracellular staining of BMMCs and peritoneal mast cells demonstrates abundant IL-13 but minimal IL-4 protein. Cells were stimulated with phorbol myristate acetate (PMA), ionomycin, and brefeldin A for 4 h. (D) *In vivo* cytokine capture assay shows elevated IL-4 levels in serum 4 h subsequent to gavage food challenge in allergic IgE-sufficient mice. (E) Intracellular cytokine staining in jejunal mast cells from OVA-challenged, OVA-allergic F709 mice show abundant IL-4 protein production. (F) qPCR for *Il4* in the jejunum 1 h after food challenge. Data were normalized to the housekeeping gene *Hprt1*. **P*<0.05, ***P*<0.01, *****P*<0.0001 by Bonferroni post-test on ANOVA of log-transformed values.

difficult to detect while IL-13 is abundant (Fig. 1B and C). Using a sensitive *in vivo* cytokine capture assay developed by Finkelman, Morris, Orekhova, and Sehy (2003), IL-4 levels were observed to rise approximately 10-fold in the serum of food-allergic mice following enteral challenge in an IgE-dependent manner. A similar pattern was seen in *Il4* transcripts in the small intestine (Fig. 1D and E). These findings suggested an FcεRI⁺ cell as the source, and basophils do not appear to be present in the intestinal lamina propria (unpublished observations). Intracellular cytokine staining on intestinal mast cells from food-allergic mice revealed substantial production of IL-4 protein (Fig. 1F). Indeed, among intestinal leukocytes in these mice, mast cells are second only to CD4⁺ T cells as the primary source of IL-4 (data not shown). These findings suggest that when appropriately stimulated, mast cells can markedly alter the total levels of IL-4 systemically, and thus would not need to be in direct contact with the T cells undergoing differentiation to promote Th2 polarization.

Direct interactions between mast cells and T cells have also been suggested to support Th2 induction. Mast cells do not normally express major histocompatibility complex (MHC) II and are not professional antigen-presenting cells. Nevertheless, it has been reported that MHC II expression can be induced and that mouse and human mast cells will then present antigen *in vitro* (Kambayashi et al., 2009; Poncet, Arock, & David, 1999). Regardless, if the T cell receptor (TCR) is activated by a professional antigen-presenting cell, neighboring mast cells can provide accessory costimulation through cytokine secretion and direct mast cell:T cell contacts via cell surface proteins (Nakae et al., 2006). Mast cell-expressed TNFα and OX40L are important for these interactions, with both factors promoting T cell proliferation (Nakae et al., 2006). OX40L has gained some notice for its role in promoting Th2-driven allergic disease by dendritic cells, but it does not exclusively support Th2 polarization (Arestides et al., 2002; Blazquez & Berin, 2008; Ito et al., 2005). In any event, the contributions of mast cell-expressed OX40L to T cell polarization *in vivo* remain to be assessed, as is the case for most mast cell costimulatory molecules.

Mast cells may also indirectly support polarization of naïve T cells toward Th2 by conditioning dendritic cells. Mast cells and dendritic cells often reside in close proximity at environmental interfaces such as mucosal surfaces and in the skin. Activation of mast cells through FcεRI has been shown to alter dendritic cell phenotype through histamine and other mediators, resulting in an increased ability to support Th2 induction and reduced secretion of Th1-promoting IL-12 (Kitawaki et al., 2006; Mazzoni, Siraganian,

Leifer, & Segal, 2006). The importance of mast cell-derived stimuli for dendritic cells maturation has been documented *in vivo*. Mast cells and IgE are required for optimal sensitization to haptens in the skin, and this effect depends on the low-level cytokine production that occurs from IgE interacting with FcεRI in the absence of antigen (Bryce et al., 2004; Dudeck et al., 2011; Suto et al., 2006).

6.2 Mast Cell and Treg Interactions

There is accumulating evidence that direct interactions between mast cells and Treg may occur, and that these interactions may affect the development of allergy and tolerance. This area of research is relatively new, and there are some reports that mast cells can support Treg function as well as interfering with it. The majority of evidence, however, demonstrates that activation or degranulation of mast cells leads to a loss of suppressive functions in Treg and disrupts the generation of new Treg during immune responses. Mast cells make an array of cytokines, notably IL-1, IL-4, IL-6, and TNFα, which are known to potently suppress Treg induction and function (Dardalhon et al., 2008; Veldhoen, Hocking, Atkins, Locksley, & Stockinger, 2006). Mast cell-derived IL-6 has been shown to destabilize Treg expression of Foxp3, trigger IL-17 production, and prevent control of proliferating effector T cells (Piconese et al., 2009). Direct cellular contact mediated by mast cell OX40L binding to OX40 on the Treg disrupts Treg suppressive functions (Frossi et al., 2011; Piconese et al., 2009). Interestingly, histamine also appears to disrupt Treg suppression through its actions on the H1 histamine receptor (Forward, Furlong, Yang, Lin, & Hoskin, 2009).

In certain settings, mast cells have been reported to have immunosuppressive effects that support Treg generation. Contrary to general thinking of mast cells as effector cells and producers of inflammatory mediators, Noelle and colleagues have shown that mast cells are in fact required for certain aspects of immunological tolerance (de Vries, Pino-Lagos, Elgueta, & Noelle, 2009; Lu et al., 2006). It is worth noting that mast cell chymase can activate latent TGFβ (Lindstedt et al., 2001), which is key to peripheral tolerance and prevention of graft rejection (Lu et al., 2006). Even in this model, however, triggering mast cell degranulation abrogated tolerance and interfered with Treg responses (de Vries et al., 2009). It is not clear what function the mast cells are performing in this setting, but it may relate to recruitment of Treg. Tunis et al. found that the absence of mast cells did not significantly alter the development of oral tolerance (Tunis, Dawicki, Carson, Wang, & Marshall, 2012). These findings have yet to be addressed

with new mast cell-targeting approaches. Recent evidence obtained from *in vivo* studies of the development of tolerance or sensitization to ingested allergen in atopic mice demonstrates the ability of mast cells, IgE, and IgE receptor signaling to impair the generation of Treg. In this setting, mast cell activation disrupts Foxp3⁺ Treg generation in response to ingested allergens, leading to a breakdown of oral tolerance and sensitization to food. We have used cell culture systems to test whether this is a direct of mast cells on T cells and whether specific mast cell-associated factors might mediate the suppression. We observed that TGFβ-dependent generation of Foxp3⁺ Treg from naïve CD4⁺ T cells stimulated by plate-bound anti-CD3 and anti-CD28 is greatly inhibited by mast cells, provided that the mast cells are activated through FcεRI (Fig. 2A) (Burton, Noval Rivas, et al., 2014). The reduction in Treg in these cultures is accompanied by a modest increase in proliferation, likely due to the costimulatory effects of mast cell-derived cytokines. The contribution of mast cell-derived IL-4 to this effect is minor (data not shown), perhaps due to the low levels of IL-4 that are secreted by *in vitro*-generated mast cells (Fig. 1). Neutralization of IL-6 or inhibition of CD40 costimulation restores Treg induction in this simple system indicating a suppressive role of these two factors in Treg differentiation (Fig. 2B). While direct inhibition of Treg generation occurs *in vitro* by FcεRI-activated mast cells and suppression of Treg responses can be observed *in vivo* due to mast cell-derived IL-4, the effects of IL-4 *in vivo* may be through indirect mechanisms such as favoring Th2 induction or autocrine effects on mast cell growth (Burton, Noval Rivas, et al., 2014; Burton et al., 2013).



7. SILENCING OF THE IgE: MAST CELL AXIS TO RESTORE IMMUNE HOMEOSTASIS

By the time patients arrive in an allergist's office for evaluation, their allergies are well established with active Th2 and IgE responses underway free to expand in the setting of a weak Treg counterresponse. The twenty-first century challenge for clinicians will be the development of strategies that reverse established Th2 responses while bolstering Treg. We suggest that therapies designed to suppress the proallergic immunomodulatory effects of IgE and mast cells may be successful. One advantage of targeting IgE or mast cells in therapies developed to reprogram the Th2/Treg balance is the benefit that IgE blockade, in addition to its immunomodulatory effects, can block acute allergic reactions. Omalizumab, a humanized mouse

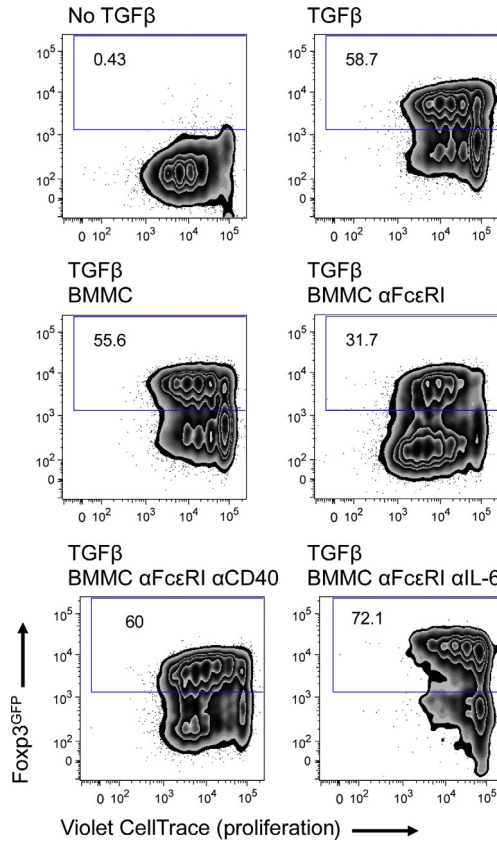


Figure 2 Mast cells suppress Treg induction. Naïve CD4⁺ T cells were isolated by immunomagnetic selection from DO11.10 Rag^{-/-} Foxp3^{GFP} splenocytes. CD4⁺ T cells labeled with Violet CellTrace were cultured for 5 days on plate-bound anti-CD3 (1 µg/ml) and anti-CD28 (5 µg/ml), and Foxp3 expression and cellular division in CD4⁺ TCRβ⁺ cells were measured by flow cytometry. Treg induction in the presence of recombinant human TGFβ1 (2 ng/ml) is suppressed when BMMCs are stimulated by plate-bound anti-FcεRI (5 µg/ml). Blocking CD40:CD40L interactions with anti-CD40 or antibody neutralization of IL-6 (10 µg/ml) reverses the suppressive effect of BMMCs.

monoclonal anti-IgE antibody, was originally developed for the treatment of allergic asthma (Busse et al., 2001; Soler et al., 2001). In recent years, it has been applied in other settings including as an adjunctive therapy in patients undergoing oral immunotherapy (OIT) for food allergy and emerging evidence indicates that omalizumab may indeed have immunomodulatory effects.

In healthy individuals, exposure to food proteins through the oral route results in the generation of Treg and a potent suppression of effector

immune responses against those proteins. OIT attempts to exploit the mechanisms of oral tolerance induction to restore a nonreactive state in sensitized patients. In OIT, food-allergic subjects receive incrementally increasing doses of the foods to which they are sensitive over many months, eventually developing a desensitized state in which they can ingest the offending food protein without reaction. Because food allergen ingestion during dose escalation frequently elicits allergic reactions in these patients, OIT protocols are not easily tolerated. In strategies developed to enhance both the safety and tolerability of OIT, several groups have added omalizumab treatment to standard OIT. These protocols have established that omalizumab-treated subjects experience fewer symptoms and more rapidly reach the target allergen dose (Nadeau, Schneider, Hoyte, Borras, & Umetsu, 2011; Rafi et al., 2010; Sampson et al., 2011).

The IgE:FcεRI interaction is the central focus of interest in the development of novel therapeutics for allergic disorders, and a variety of approaches have been established to interfere with this pathway. Peptide-based mimicry of IgE or FcεRI components can disrupt the interaction between IgE and its receptor, but the bioavailability and pharmacokinetic characteristics of peptides are not optimal and they are some ways from clinical testing (Eggel et al., 2014; Zhou et al., 2013). Monoclonal antibodies, including omalizumab, have shown promise (Logsdon & Oettgen, 2015). Omalizumab blocks both immediate- and late-phase hypersensitivity reactions (Fahy et al., 1997) in allergen-exposed human subjects and has received FDA approval for the treatment of asthma (Busse et al., 2001; Soler et al., 2001) and chronic urticaria (Maurer, Rosen, & Hsieh, 2013; Maurer, Rosen, Hsieh, Saini, et al., 2013).

7.1 Immunomodulatory Effects of IgE-Targeted Approaches

7.1.1 Blockade of IgE or the IgE Receptor

In addition to blocking acute allergic reactions, antibody-mediated neutralization of IgE has been documented to suppress the development of allergic sensitization in mice (Tumas et al., 2001). Studies on animals treated with anti-FcεRI in several models of allergic disease have revealed FcεRI-dependent Th2 response induction (Perrigoue et al., 2009; Poddighe et al., 2014; Sokol, Barton, Farr, & Medzhitov, 2008; Sokol et al., 2009; Yoshimoto et al., 2009). While this was initially attributed to the depletion of basophils that is induced by these antibodies, it is now clear that mast cells and FcεRI⁺ dendritic cells are also affected by this treatment (Denzel et al., 2008; Hammad et al., 2010). Findings from studies of peanut allergy in mice

predict that paralysis of the IgE:mast cell axis by omalizumab in the course of OIT will also suppress or reverse established Th2 and IgE responses while enhancing the emergence of Treg. Studies to test this hypothesis are in an early phase and discussed below.

Modeling anti-IgE therapies has posed some challenges. Unlike the anti-human IgE, omalizumab, which is “nonanaphylactogenic,” binding only to free IgE and incapable of interacting with FcεRI-bound IgE, all available antimouse IgE antibodies bind to IgE both in solution and when anchored to FcεRI, invariably resulting in some degree of mast cell activation following administration. In order to circumvent this side effect, Khodoun and colleagues applied rush desensitization techniques to develop an incremental dosing strategy for both anti-IgE and anti-FcεRI that effectively prevents most symptoms while neutralizing the IgE:FcεRI axis (Khodoun et al., 2013). Anti-IgE treatment using such a strategy was applied to a model of peanut allergy. In this model, inherently atopic F709 mice (see Section 5.3) were subjected to gavage feeding with commercial peanut butter (22 mg protein equivalent) once a week for 4–8 weeks, a protocol which both induced potent peanut-specific Th2 and IgE responses and rendered them susceptible to intense anaphylactic reactions upon enteral challenge with a high dose of peanut butter (100 mg protein equivalent). IgE neutralization with a monoclonal antimouse IgE in the course of peanut feeding dampened Th2 responses, enhanced Treg induction, and protected mice from developing anaphylaxis. Extending this approach to mice with already established allergy, we found that anti-IgE treatment in combination with high-dose allergen feeding caused a reduction in allergen-specific IgE and Th2 responses along with an increase in allergen-specific Treg numbers and complete resistance to peanut-induced anaphylaxis (Burton, Noval Rivas, et al., 2014). These findings suggest that recurrent mast cell activation during repeated food ingestion prevents the establishment of long-term tolerance in food allergy, and that anti-IgE treatment facilitates tolerance induction, an effect that is now being investigated using omalizumab in OIT protocols.

While adjunctive omalizumab is currently being applied in OIT trials to reduce reaction rates as discussed above, immunological analyses of its effects on cellular effector and regulatory responses are in an early phase (Fried & Oettgen, 2010; Logsdon & Oettgen, 2015). In uncontrolled trials in allergic asthmatics, removal of omalizumab was followed by a decrease in Foxp3 expression (Pace, Ferraro, Bruno, Bousquet, & Gjomarkaj, 2010), but no Treg induction change was found following the initiation of therapy in patients with cow's milk allergy (Bedoret et al., 2012). Ongoing mechanistic

studies in placebo-controlled studies of omalizumab in food allergy will hopefully provide more definitive information regarding the effects of IgE blockade on effector and regulatory mechanisms in human food allergy. There are several differences between peanut allergy studies performed in IgE^{-/-} mice or animals treated with anti-IgE and human OIT trials with omalizumab. In the murine models of IgE inhibition, IgE is completely absent (IgE^{-/-}) or neutralized. In contrast, the inability of omalizumab (compared with antimurine IgE reagents) to interact with receptor-associated IgE probably results in a substantial pool of allergen-specific IgE remaining bound to mast cells, and as a result, mast cell activation may not be entirely prevented. Moreover, the levels of IgE in some allergic subjects, particularly those with coexisting atopic dermatitis (common in food-allergic patients), are so high enough that omalizumab may not completely neutralize all the IgE in circulation. Clinical studies of omalizumab in asthma revealed that patients with IgE levels above 700 IU/mL get less antiallergic benefit than those with lower levels (Casale et al., 1997). An additional consideration is that the compressed time frame of sensitization and/or desensitization with peanut in mouse models of food allergy (which occurs over weeks) does not reflect the kinetics of Th2 and Treg modulation during OIT in patients, which is performed over months or years. A recent study by Syed and colleagues demonstrates that allergen-specific Treg induction during OIT may be restricted to patients who become tolerant, and that this response develops gradually over the course of therapy (Syed et al., 2014). Thus, although a number of lines of evidence in animal work predict a potential immunomodulatory effect of omalizumab in food allergy, careful analyses of its effects in placebo-controlled human OIT studies, now underway, will be necessary to test whether such mechanisms are operative in humans.

7.1.2 Inhibition of FcεRI Signaling Intermediates

Current pharmacologic options for treating allergy are largely restricted to reducing the symptoms caused by mast cell mediators such as histamine and leukotrienes using small-molecule blockers of their respective receptors or on a global suppression of proinflammatory cytokines using glucocorticoids. Compounds designed to specifically interfere with FcεRI signaling would theoretically provide a more focused attack along with a greater benefit since release of all mast cell and basophil small-molecule mediators would be reduced while also suppressing cytokine production. As many of the molecules mediating IgE receptor signaling are shared by other

immune signaling pathways, e.g., B cell receptor (BCR) and TCR activation, this approach would not without challenges. In the case of food allergy, however, exposure to the allergen can be controlled, which means that short half-life signaling inhibitors could be applied briefly and intermittently to block FcεRI activation at the time of ingestion. Such a strategy might be particularly applicable in clinical trials for OIT.

Drugs initially approved to fight BCR–Abl fusion cancers have found secondary success in treating mastocytosis due to their inhibitory effects on c-Kit, which synergizes with FcεRI to amplify degranulation (Scheinfeld, 2006; Schittenhelm et al., 2006). In the FcεRI signaling pathway, inhibition of the Src family kinases, Syk and Btk, with small-molecule protein kinase inhibitors has been shown to reduce phosphorylation of downstream kinases, limiting cytokine secretion and the immediate release of mediators including histamine and serotonin (Hantschel et al., 2007; Matsubara et al., 2006). Syk is a particularly attractive target due to its position at the start of the signaling cascade where it drives downstream events leading to both degranulation and gene transcription. Compounds such as R406 and piceatannol that block Syk have been shown to inhibit IgE-mediated activation of mast cells and the development of airway hyperreactivity and eosinophilia in rodent models of allergic asthma (Matsubara et al., 2006; Seow, Chue, & Wong, 2002). Since these molecules lack specificity and likely interfere with many other signaling intermediates, it is unclear whether these observed effects were due to suppression of Syk *per se* (Braselmann et al., 2006; Matsubara et al., 2006; Miura, Lavens-Phillips, & MacGlashan, 2001). In our hands, R406 administration did not result in observable reductions in the severity of IgE-mediated anaphylaxis (unpublished observations), possibly due to the off-target effects of this molecule on the inhibitory kinase Lyn.

We have recently used the F709 peanut allergy model to examine the utility of Syk inhibition in food allergy using a novel highly specific inhibitor, Syki (Moy et al., 2013). Syki is orally bioavailable and displays a dose-dependent ability to block IgE-mediated passive systemic anaphylaxis and at the therapeutic dose completely abrogated core temperature loss, symptoms, and mortality in acute food allergen challenge of sensitized mice. Since Syki potently blocked immediate anaphylactic reactions, we reasoned that food-allergic mice could be subjected to an OIT regimen under Syki-covered gavage feedings with doses of allergen that would otherwise be fatal. We predicted that pharmacologic Syk inhibition would have the same effects on peanut-specific T cell responses (reduction of Th2 and induction of Treg

cells) we had previously observed in experiments with IgE blockade (discussed in [Section 7.1.1](#)).

This prediction was indeed borne out. Analysis of T cell phenotype after Syki-facilitated OIT revealed marked increases in allergen-specific Treg coinciding with reductions in Th2 cells ([Burton, Noval Rivas, et al., 2014](#)). The same effects were observed using OVA as the sensitizing allergen. Antigen-specific T cell tracking experiments using adoptively transferred DO11.10 OVA-specific T cells showed *de novo* generation of Foxp3⁺ Treg *in vivo* in allergic mice treated with OVA and Syki, whereas IL-4-expressing Th2 cells were induced in animals treated with vehicle ([Fig. 3](#)).

Induction of high levels of allergen-specific IgG is considered a hallmark of successful desensitization in OIT. Syki-treated mice still exhibited the marked induction of allergen-specific IgG ([Fig. 4A](#)), perhaps because plasma cells do not strictly require Syk ([Turner et al., 1997](#)) or because intermittent Syk inhibition had no net effect on B cells and plasma cells. Intermittent blockade may be crucial in permitting B cell function and the development of protective IgG antibody responses. Critically, Syki-desensitized mice remained largely unresponsive to allergen challenge long after the discontinuation of Syki therapy ([Fig. 4C](#)), suggesting that tolerance had been restored ([Burton, Noval Rivas, et al., 2014](#)).

7.2 Treg Control of Mast Cell Activation

Tolerance to ingested antigens requires the continuing presence of Treg, as evidenced by experiments in which depletion of Treg in mice unleashes allergic responses ([Hadis et al., 2011](#)), and the rapid development of multiple severe food allergies in patients and mice with Foxp3 mutations ([Bennett et al., 2001](#); [Chatila et al., 2000](#); [Fontenot, Gavin, & Rudensky, 2003](#); [Hori, Nomura, & Sakaguchi, 2003](#); [Lin et al., 2005](#)). Moreover, increases in allergen-specific Treg have been associated with the natural loss of cow's milk allergy that occurs in many children around the age of 3–5 years old ([Karlsson, Rugtveit, & Brandtzaeg, 2004](#); [Shreffler, Wanich, Moloney, Nowak-Wegrzyn, & Sampson, 2009](#); [Sletten, Halvorsen, Egaas, & Halstensen, 2007](#)). Adoptive Treg therapy has shown promise in graft-versus-host disease, and strategies that expand Treg or increase their suppressive capacity are under consideration for a variety of autoimmune diseases ([Sakaguchi, Miyara, Costantino, & Hafler, 2010](#)). Here, we explore the possibilities and implications of Tregs as a therapeutic option for food allergy.

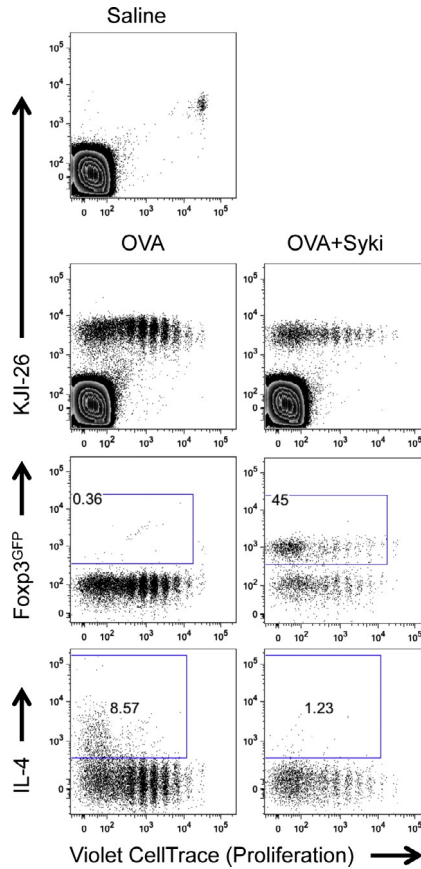


Figure 3 Mast cell inactivation with Syk inhibitor alters T cell phenotype acquisition during OIT. Violet CellTrace-labeled naïve DO11.10 Rag^{-/-} Foxp3^{GFP} CD4⁺ T cells were adoptive transferred into OVA-sensitized F709 mice prior to OIT. After 2 weeks of daily gavage feedings of OVA in combination with Syki or vehicle control, T cells were recovered from the mesenteric lymph nodes and analyzed for Foxp3^{GFP} expression and intracellular IL-4 production by flow cytometry. Representative plots are shown gating on viable CD4⁺ KJ1-26 TCR⁺ cells. A mouse that did not receive OVA (saline control) is shown to demonstrate the antigen specificity of the DO11.10 T cell proliferation.

In addition to their functions in preventing dendritic cell maturation and T cell proliferation, Tregs have been reported to directly suppress mast cells through the cell surface receptor, OX40 (Ganeshan & Bryce, 2012; Gri et al., 2008). Interestingly, this inhibitory effect appears to be restricted to degranulation of the mast cell and the release of preformed mediators. Mast cell cytokine production appears to be unchanged or, in the case of IL-6,

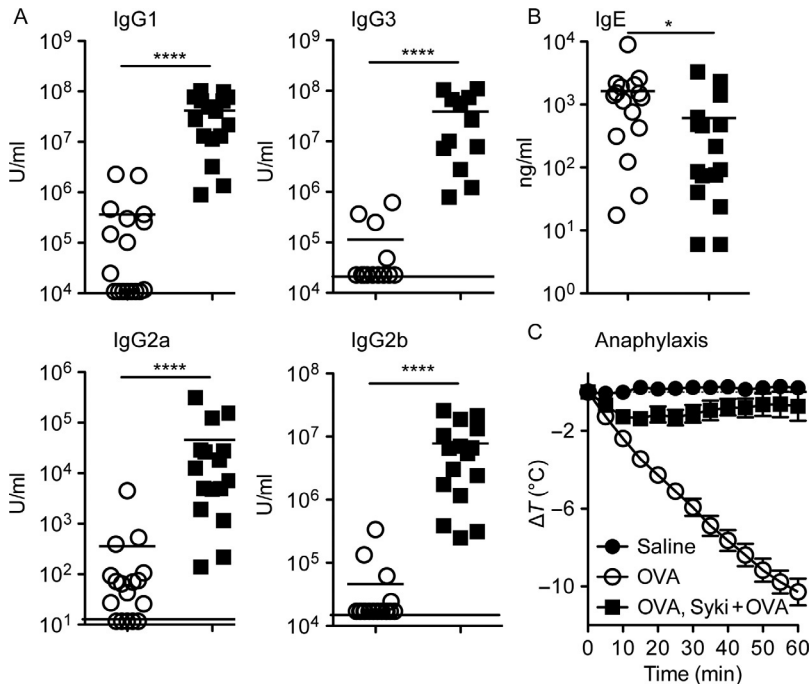


Figure 4 (A) Serum levels of allergen-specific IgG increase markedly following Syki-facilitated desensitization of OVA-allergic F709 mice. Open circles: OVA-sensitized F709 mice. Solid squares: mice after 2 weeks of daily Syki+OVA OIT. (B) OVA-specific IgE levels remain elevated but do not increase after OIT. * $P < 0.05$, **** $P < 0.0001$. P values in (A) and (B) derived from unpaired t tests on log-transformed values. (C) Anaphylaxis (core body temperature loss) upon enteral food challenge is greatly reduced following Syki+OVA treatment. A 10-day washout period was observed between the discontinuation of therapy and the acute challenge. $P < 0.0001$ by repeated measures two-way ANOVA between OVA and OVA, Syki+OVA groups.

even increased (Frossi et al., 2011; Ganeshan & Bryce, 2012). It now appears that OX40L ligation on mast cells suppresses Fyn activation without interfering with Lyn–Syk–LAT, resulting in decreased PI3K and Akt activation with enhanced SH2 domain-containing inositol-5'-phosphatase (SHIP) phosphorylation. Overall, this leads to a loss of PIP₃ production and diminished calcium flux, a key regulator of granule fusion (Sibilano et al., 2012). Treg:MC synapse formation has been observed for both mouse and human cells (Frossi et al., 2011). In addition to the effects of OX40L ligation at the cell surface, Tregs might exert effects on mast cells via soluble cytokines. The Treg-associated cytokines IL-10 and TGFβ are known to inhibit mast cell degranulation, cytokine production, and proliferation and also reduce

expression of FcεRI and c-Kit (Gillespie et al., 2004; Gomez et al., 2005; Macey et al., 2010; Mirmonsef, Shelburne, Fitzhugh Yeatman, Chong, & Ryan, 1999; Shelburne & Ryan, 2001; Zhao, Gomez, Yu, Ryan, & Schwartz, 2008).

The *in vivo* relevance of these OX40L- and/or cytokine-mediated mechanisms of mast cell suppression by Treg observed in culture studies is the subject of ongoing studies (Kashyap et al., 2008). There is emerging evidence however that supports the relevance of such interactions. It is not clear that the Treg is acting directly on mast cells and may instead be conditioning dendritic cells or T cells. Going forward, the use of cell-specific gene deletion, especially with Mcpt5^{Cre} and Cpa3^{Cre} models targeting the production of *xyz* factors exclusively in mast cells, may provide a handle to address the *in vivo* relevance of the Treg:mast cell interaction.

7.3 IgG:FcγRIIb-Mediated Inhibition of Mast Cell Activation

It is well established that IgG can inhibit IgE-mediated activation of mast cells and basophils. As far back as 1935, Cooke and colleagues demonstrated that patients undergoing therapy for ragweed allergy developed a transferable inhibitory antibody that suppressed skin reactions in the Prausnitz-Küstner test even in the continued presence of the sensitizing agent “reagin” (Cooke, Barnard, Hebal, & Stull, 1935). Inhibition of IgE-mediated allergy by IgG requires that the antibodies of both isotypes share specificity for the relevant allergen. Suppressive effects of IgG antibodies on IgE:FcεRI-mediated mast cell activation can result either from their masking the epitopes recognized by IgE (blocking) or through activation of the inhibitory Fc receptor, FcγRIIb. In clinical studies of subcutaneous aeroallergen immunotherapy for allergic rhinitis, increases in specific IgG “blocking” antibodies are strongly correlated with effective desensitization (Akdis, Barlan, Bahceciler, & Akdis, 2006; James et al., 2011; Lichtenstein, Holtzman, & Burnett, 1968). Similar relationships between antigen-specific IgG induction and efficacy of desensitization have been observed in OIT trials for food allergy (Bedoret et al., 2012; Caubet et al., 2012; Jones et al., 2009; Kim et al., 2011; Skripak et al., 2008; Vickery et al., 2013, 2014). As previously observed for aeroallergen immunotherapy, induction of the IgG4 isotype has emerged as the best clinical correlate for desensitization.

It has been suggested that IgG4's unique propensity to reassort immunoglobulin heavy chains might create antibodies with dual specificities that

cannot aggregate allergen molecules (Aalberse, van der Gaag, & van Leeuwen, 1983; van der Neut Kolfshoten et al., 2007; van der Zee, van Swieten, & Aalberse, 1986). This combined with IgG4's extremely low affinity for Fc γ receptors has led to the prevailing hypothesis that the IgG acts primarily as a "blocking" antibody, neutralizing allergen before it can engage IgE bound to Fc ϵ RI (Aalberse & Schuurman, 2002; Akdis et al., 2006; Smith & Clatworthy, 2010). However, there is more direct evidence, including the studies of Daeron and colleagues, for the alternative hypothesis that IgG binding to the inhibitory Fc receptor Fc γ RIIb delivers suppressive signals to mouse and human mast cells and basophils, dampening Fc ϵ RI-driven activation via direct effector cell interaction and intracellular signaling pathways (Cassard, Jonsson, Arnaud, & Daeron, 2012; Daeron, Malbec, Latour, Arock, & Fridman, 1995). Both mechanisms of action have been shown to block systemic anaphylaxis in mice, although inhibition of diarrhea in an experimental food allergy model by monoclonal IgG1 appears to be Fc γ RIIb independent (Kucuk et al., 2012; Strait, Morris, & Finkelman, 2006).

7.3.1 IgG and Fc γ RIIb in Food Allergy

Our own studies of the inhibitory mechanisms of IgG that is induced following OIT using serum from human patients and mice are most consistent with a receptor-mediated inhibitory mechanism of IgG (Burton, Logsdon, et al., 2014). We have used serum from food-allergic mice to sensitize BMDCs for degranulation to allergen, measured by upregulation of LAMP-1 (CD107a) using flow cytometry. The addition of post-OIT serum markedly reduces BMDC activation, unless the IgG is depleted from the serum using protein G. In this setting, Fc γ RIIb is required for the inhibitory effect of peanut-specific IgG antibodies, implicating a cellular signaling mechanism rather than a neutralizing or steric hindrance effect.

The inhibitory effects of IgG on IgE-mediated activation of BMDCs can also be modeled using monoclonal antibodies specific for a single epitope, such as the hapten trinitrophenyl (TNP). In this situation, IgG1 antibodies can be observed to have suppressive effects on IgE-mediated activation whether or not Fc γ RIIb is present. Critically, however, the presence of Fc γ RIIb dramatically enhances suppression (Burton, Logsdon, et al., 2014; Strait et al., 2006). At low concentrations of IgG, wild-type cells exhibit receptor-mediated inhibition, whereas at much higher concentrations, activation of both wild-type and Fc γ RIIb-deficient cells can be suppressed, presumably through epitope neutralization. In less reductionist systems, the contribution of Fc γ RIIb appears to dominate.

Anaphylactic food allergy in mice can be reversed by an OIT regimen facilitated by inhibition of mast cell activation with a small-molecule Syk inhibitor (Burton, Noval Rivas, et al., 2014). These desensitized mice exhibit multiple log increases in allergen-specific IgG of all isotypes, while specific IgE remains elevated (Fig. 4A). Using an *in vitro* adaptation of the Prausnitz–Küstner test, serum from food-allergic mice effectively sensitized mast cells for degranulation when challenged with allergen, whereas serum from post-OIT mice was far less effective (Fig. 5A). Moreover, the post-OIT serum contained a dominant suppressive factor capable of diminishing reactions induced by either pretreatment sera or exogenous monoclonal IgE (Fig. 5B and data not shown). When depleted of IgG using protein G depletion, the post-OIT serum failed to suppress and regained its ability to sensitize mast cells (Fig. 5B and data not shown). Mast cells deficient in FcγRIIb were not subject to suppression by post-OIT mouse serum, instead exhibited heightened responses to pretreatment serum, indicating the relevance of even normal low levels of IgG to silencing mast cell degranulation (Fig. 5C). Using a passive transfer model of anaphylaxis, serum from allergic mice effectively sensitized both wild-type and FcγRIIb-knockout mice, with the FcγRIIb-deficient animals displaying more severe anaphylaxis upon challenge (Fig. 5D). The addition of serum taken post-OIT largely abrogated anaphylaxis, provided that FcγRIIb was present (Fig. 5D) (Burton, Logsdon, et al., 2014).

We have recently reported that inhibitory IgG antibodies are elicited in subjects undergoing OIT for peanut allergy (Burton, Logsdon, et al., 2014). As in mice, human subjects successfully completing OIT and able to tolerate peanut ingestion challenges actually retain IgE levels that would normally predict significant allergic reactions upon challenge. In order to evaluate the effects of IgG antibodies induced during peanut OIT in humans, we developed a novel indirect basophil activation assay to interrogate sera for the inhibitory function. In this assay, basophils from nonallergic donors were sensitized with sera from peanut-allergic patients taken either before or after OIT. The basophils were then stimulated with peanut extract for 10 min, and basophil activation was assessed by flow cytometric detection of the activation marker CD63. Sera taken from high-risk peanut-allergic patients prior to OIT, which contained high levels of peanut-specific IgE, effectively sensitized basophils from nonallergic donors to react to peanut protein. Sera from the same patients taken 52 weeks later upon completion of OIT, however, not only showed a reduced ability to sensitize basophils but actually exerted a dominant suppressive effect when mixed with pretreatment sera,

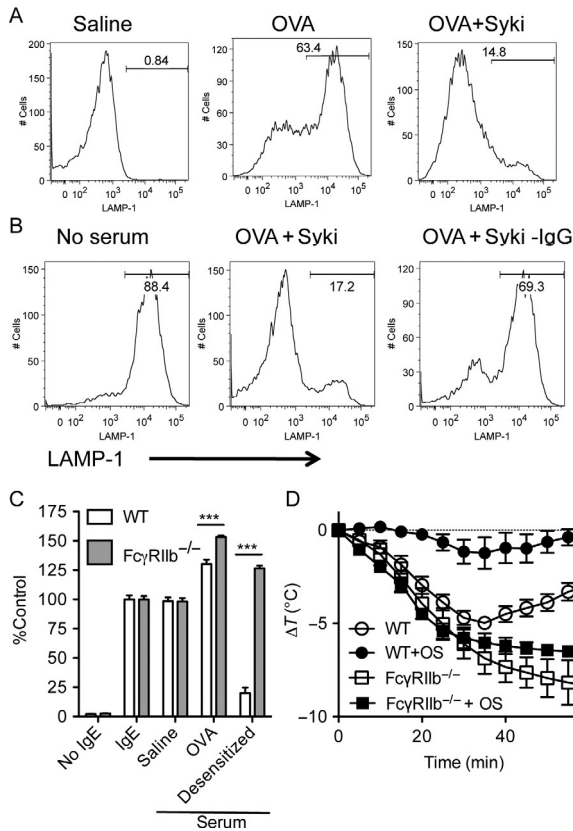


Figure 5 IgG induced during OIT inhibits $Fc\epsilon RI$ -driven anaphylaxis via $Fc\gamma RIIb$. (A) Upregulation of LAMP-1 (CD107a) on BMMCs following exposure to OVA (500 ng/ml). BMMCs were sensitized overnight with 2% serum from F709 mice treated as in Fig. 4. (B) Upregulation of LAMP-1 in IgE anti-OVA-sensitized (100 ng/ml) BMMCs challenged with OVA. Serum from desensitized mice was added before or after protein G-mediated depletion of IgG. (C) Activation of IgE anti-OVA-sensitized BMMCs from wild-type or $Fc\gamma RIIb^{-/-}$ mice with OVA. Serum was added from F709 mice fed saline, sensitized to OVA, or sensitized to OVA and then subjected to OIT with OVA plus Syk inhibitor. *** $P < 0.001$ by Bonferroni posttest on ANOVA. (D) Passive anaphylaxis (core body temperature) in wild-type and $Fc\gamma RIIb^{-/-}$ mice sensitized with serum from OVA-allergic F709 mice and challenged by i.p. injection with OVA (100 μ g). All groups received allergic serum. OS groups additionally received serum from post-OIT mice.

which was found to reside in the IgG fraction and was prevented by antibodies against $Fc\gamma RII$ (Burton, Logsdon, et al., 2014). This study provided the first direct indication that IgG antibodies, signaling via $Fc\gamma RIIb$, can exert a suppressive effect in $Fc\epsilon RI^{+}$ cells in human food allergy.

A previous report evaluating mechanisms of immunotherapy for hay fever failed to demonstrate an Fc γ RIIb-mediated blocking of IgG antibodies on basophils (Ejrnaes, Svenson, Lund, Larsen, & Jacobi, 2006). That study used a single donor and there is some interindividual variability in basophil activation so it is possible that this technical issue may have limited the ability to detect an IgG effect. Additional inhibitory mechanisms are activated during both allergen immunotherapy, which is administered chronically over months to years, and allergen desensitization, in which subjects receive a rapid escalation of allergen doses over hours, typically in the setting of drug allergy. These therapies have direct cell autonomous effects on basophils that affect IgE-mediated signaling pathways (Macglashan, Moore, & Muchhal, 2014; Thyagarajan et al., 2012; Wanich, Nowak-Węgrzyn, Sampson, & Shreffler, 2009). Fc γ RIIb is polymorphic in mice and humans, and mutations that reduce the inhibitory activity of the receptor are associated with atopy and IgE production as well as autoimmunity (Smith & Clatworthy, 2010; Wu et al., 2014). These associations implicate inhibitory IgG signaling as a physiologically relevant regulatory pathway.

The single IgG subclass most closely associated with effective allergen immunotherapy responses is IgG4, as mentioned above. The requirement for Fc γ RIIb in the basophil-silencing effects of blocking antibodies calls into question the importance of IgG4, since this isotype has quite low affinity for the receptor (Paul, 2003). Allergen-specific IgG4 levels are normally extremely low or even undetectable prior to allergen immunotherapy, but increase massively during OIT even reaching parity or beyond with the other isotypes (Burton, Logsdon, et al., 2014; Vickery et al., 2014). While IgG4 provides a clean signal (and low baseline background), we observe that all isotypes of IgG increase during OIT (Fig. 2A) (Burton, Logsdon, et al., 2014), and the known stronger interactions of Fc γ RIIb with IgG1 and IgG3 suggest their involvement in the IgG inhibitory effects. IgG4 may still have important non-Fc γ RIIb-mediated functions in blocking binding of allergen to IgE⁺ B cells or inhibiting facilitated antigen presentation (Holm, Willumsen, Wurtzen, Christensen, & Lund, 2011; James et al., 2011). It is also possible that the mass amounts of IgG4 generated during OIT may provide sufficiently avid interaction with Fc γ RIIb, compensating for the low affinity.

7.3.2 Fc γ RIIb and Fc ϵ RI Signaling Interactions

Unlike Fc ϵ RI, which is fully occupied by IgE due to high-affinity binding, Fc γ RIIb, Fc γ RIII, and Fc γ RIIa (in humans) are low-affinity receptors,

requiring IgG to aggregate around antigen to increase binding (Nimmerjahn & Ravetch, 2008; Paul, 2003). Fc γ RIIb is unique among Fc receptors in that it carries an ITIM, which when activated recruits inhibitory phosphatases, including Src homology 2 (SH2) domain-containing phosphatase (Shp)-1, Shp-2, and SHIP (Kepley et al., 2000; Waterman & Cambier, 2010). These signals can essentially cancel out activation through immunoreceptor tyrosine-based activation motif (ITAM)-containing receptors, such as Fc ϵ RI, BCR, and activating Fc γ receptors. Although the naming of Shp and SHIP proteins reflects the shared SH2 motif, their actions are quite different, with Shp acting on other proteins and SHIP being restricted to IP₃, a phospholipid.

It is thought that Fc γ RIIb becomes phosphorylated primarily upon coligation with a second, activating receptor. In the case of mast cells, cross-linking with Fc ϵ RI drives phosphorylation of the ITIM on Fc γ RIIb through Lyn (Malbec et al., 1998). While Shp-1, Shp-2, and SHIP can all associate with phosphorylated Fc γ RIIb, most reports suggest that only SHIP does so in Fc ϵ RI:Fc γ RIIb-cross-linked mast cells (D'Ambrosio, Fong, & Cambier, 1996; D'Ambrosio et al., 1995; Fong et al., 1996; Malbec et al., 1998; Ono, Bolland, Tempst, & Ravetch, 1996). Shp-1 is generally considered to be dispensable for Fc γ RIIb-mediated effects in B cells and mast cells (Fong et al., 1996; Nadler, Chen, Anderson, Wortis, & Neel, 1997), and SHIP has been found to be the requisite phosphatase for the actions of Fc γ RIIb in silencing mast cell degranulation (Damen, Ware, Kalesnikoff, Hughes, & Krystal, 2001; Isnardi, Bruhns, Bismuth, Fridman, & Daeron, 2006). Since Fc ϵ RI-mediated activation of Lyn is required for Fc γ RIIb phosphorylation, it follows that Syk, which is a direct target of Lyn, remains phosphorylated in mast cells following Fc γ RIIb ligation (Malbec et al., 1998). SHIP acts to hydrolyze phosphatidylinositol (3,4,5)-trisphosphate, generating inositol triphosphate, which drives calcium flux and degranulation in mast cells (Damen et al., 2001). Although limiting this pathway would have some effect on the activation of PKC, Btk, PDK1, and Akt, it is unclear how this would account for the dramatic effects of Fc γ RIIb in abrogating mast cell cytokine production. Concerning this issue, it has been demonstrated that Dok activation downstream of SHIP provokes an interaction with Grb2 and RasGAP, leading to reductions in MAP kinase activation, including p38, ERK, and JNK (Kepley et al., 2004; Ott, Tamir, Niki, Pandolfi, & Cambier, 2002). Although a Dok:Fc ϵ RI interaction is sufficient to mimic the effects of Fc γ RIIb ligation, it is not required for the inhibition of mast cell activation, suggesting redundancies in signaling or the predominance of an alternative pathway (Ott et al., 2002).

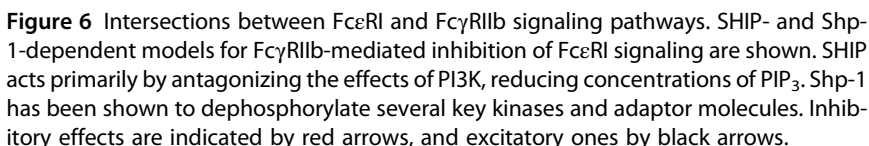
An alternative signaling pathway involving Shp proteins rather than SHIP has been invoked in some studies. A report by Mertsching et al. using an engineered protein comprised of the Fc portions of IgE and IgG demonstrated recruitment of Shp-1 and Shp-2 in addition to SHIP to Fc γ RIIb in murine mast cells. Consistent with this alternative theory, Shp-1 has been suggested to be the mediator of Fc γ RIIb's inhibitory effects in human basophils (Kepley et al., 2000; Saxon, Zhu, Zhang, Allen, & Kepley, 2004; Zhu, Kepley, Zhang, Zhang, & Saxon, 2002). Interestingly, this Fc ϵ -Fc γ construct diminished phosphorylation of Syk as well as Erk, which could readily explain reductions in cytokine synthesis (Mertsching et al., 2008). Shp-1 is thought to dephosphorylate several early molecules in Fc ϵ RI signal transduction pathway, including Syk, LAT, SLP76, and the β/γ chains of Fc ϵ RI itself (Nakata et al., 2008; Xie, Zhang, & Siraganian, 2000). SHIP and Shp-1 can also associate with Fc ϵ RI and regulate its function independently of Fc γ RIIb (Huber, Kalesnikoff, Reth, & Krystal, 2002; Nakata et al., 2008); it may be that coligation of Fc γ RIIb simply prolongs and amplifies these existing inhibitory signals (Kepley et al., 2004; Fig. 6).

7.3.3 Other Effects of Fc γ RIIb on Mast Cells

In addition to deactivating ITAM-containing receptors (Daeron et al., 1995), Fc γ RIIb ligation has been demonstrated to reduce signaling by transmembrane tyrosine kinase receptors, including c-Kit. Simultaneous ligation of c-Kit and Fc γ RIIb causes cell cycle arrest in mast cells, inhibiting their proliferation (Malbec, Attal, Fridman, & Daeron, 2002; Malbec, Fridman, & Daeron, 1999; Malbec et al., 2001). As with Fc ϵ RI, costimulation of c-Kit and Fc γ RIIb effectively negates the positive growth signal delivered by SCF, canceling MAP kinase activation and preventing the induction of cyclins (Malbec et al., 2002). IgG has also been reported to act through Fc γ RIIb to induce internalization of IgE bound to Fc ϵ RI on mast cells. Not only would this loss of surface IgE reduce the likelihood of reactions to allergen, but it would also eliminate the prosurvival effect of IgE on mast cells, potentially triggering their apoptosis (Asai et al., 2001; Kalesnikoff et al., 2001; Uermosi et al., 2014). While such effects remain to be conclusively demonstrated *in vivo*, these findings suggest that IgG may have nearly as potent an effect in regulating mast cell survival as IgE.

7.3.4 Engineering Fc γ RIIb Binding and Other Therapeutic Approaches

Activation of Fc γ RIIb provides a potential means of silencing mast cell and basophil activation in allergic disease. Several approaches have been



developed in efforts to take advantage of this negative regulatory pathway. As discussed above, constructs that artificially cross-link FcεRI and FcγRIIb using the Fc portions of IgE and IgG effectively suppress mast cell and basophil activation (Zhu et al., 2002). Similar constructs developed using specific allergens linked to Fcγ fragments appear to have equal or greater potency, but would require individual constructs for each allergen to be useful in clinical application (Liu et al., 2013; Zhang, Zhu, Kepley, Terada, & Saxon, 2007). Naturally occurring allergen-specific IgGs are present in commercial immunoglobulin preparations and should be fairly easy to obtain. These could conceivably be used to treat allergic individuals either directly to prevent allergic reactions or as adjuncts to allergen immunotherapy including OIT. In an alternative approach, monoclonal allergen-specific IgG antibodies could also be engineered to increase their specificity for binding to FcγRIIb over the other activating Fcγ receptors (Li & Ravetch, 2011; Mimoto et al., 2013; Nimmerjahn & Ravetch, 2008), which would likely enhance their therapeutic potential.



8. SUMMARY AND CONCLUSIONS

These are exciting times in the study of mast cell biology. New models of allergic diseases along with powerful genetic tools have provided opportunities to carefully test a range of hypotheses regarding the induction and regulation of allergic responses. These same approaches allow previously held dogma regarding the functions of mast cells to be revisited. Recent studies have made it clear that IgE antibodies, acting via FcεRI on mast cells, not only trigger immediate hypersensitivity reactions but also exert previously unappreciated immunomodulatory effects, supporting Th2 responses while suppressing Treg induction. These mast cell effects are now known to be modulated by inhibitory effects of IgG antibodies, transmitted via FcγRIIb. The field is now fertile both for the further analysis of the

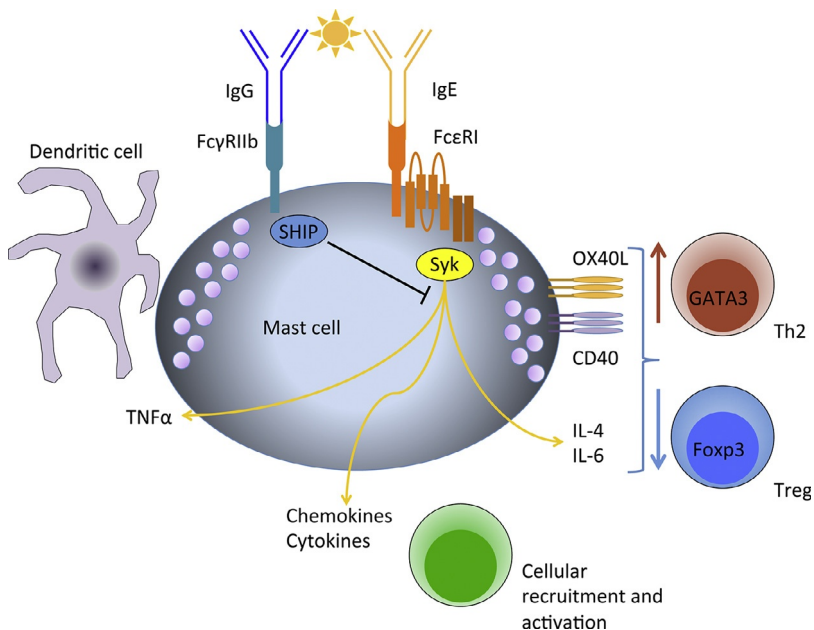


Figure 7 Pathways of immune modulation by mast cells and IgE. Cross-linking of FcεRI-bound IgE by allergen activates the kinase Syk, leading to production of cytokines and chemokines by the mast cell. The ensuing inflammatory milieu recruits and activates innate and adaptive immune cell types, altering the presentation of the allergen by dendritic cells, disrupting regulatory responses to the allergen, favoring Th2 induction, and encouraging the growth of effector cells such as neutrophils and eosinophils that will establish a chronic allergic inflammatory state.

relevance of these mechanisms to human disease and for their exploitation, using blockers of IgE or its signaling pathways or enhancers of IgG-mediated inhibition, in the development of novel strategies to treat allergic disorders (Fig. 7).

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