Published in final edited form as:

Curr Opin Immunol. 2009 December ; 21(6): 679–686. doi:10.1016/j.coi.2009.09.007.

New Roles for Mast Cells in Modulating Allergic Reactions and Immunity Against Pathogens

Alison M. Hofmann^a and Soman N. Abraham^{b,c}

^a Division of Pediatric Allergy and Immunology, Duke University, DUMC 2898 Durham, NC 27710
alison.hofmann@duke.edu

^b Departments of Pathology, Immunology and Genetics & Molecular Microbiology, Duke University, DUMC 3020 Durham NC 27710 soman.abraham@duke.edu

^c Program in Emerging Infectious Diseases, Duke-National University of Singapore, Singapore

Abstract

Mast cells have primarily been associated with mediating the pathological secondary responses to allergens in sensitized hosts. In view of the recent evidence for a mast cell role in modulating primary immune responses to pathogens, the likelihood for a role of mast cells in influencing primary immune response to allergens has grown. New evidence suggests that mast cells drive the development of Th2 responses to allergens, particularly when allergen exposure occurs concomitantly with exposure to pathogen products present in the environment. These new roles for mast cells in allergy and infection suggest additional drug targets to prevent development of allergic disease and allergic exacerbations of established disease.

Introduction

Mast cells (MC) are highly specialized exocytic cells that line the surfaces of the body, including the skin, the respiratory tract and the gastrointestinal tract. [1] They interface with the external environment and are in prime position to react to foreign particles such as pollen and bacteria. Since their discovery in 1878, MCs have primarily been regarded as effector cells promoting harmful IgE mediated allergic reactions following secondary exposure to allergens. [2] However, in the last decade MCs are being increasingly recognized for their key role in pathogen recognition, and in initiating primary protective innate and adaptive immune responses. Given this unique role in immune surveillance, MCs could play a more central role in the development of allergic diseases. Indeed, there is evidence suggesting that MCs can participate in the initiation and polarization of the primary adaptive immune response to allergens, a process known as allergen sensitization. Here, we will briefly review the traditional role of MCs as effectors of secondary immune responses to allergens and their more recently described role in promoting protective innate and adaptive immune responses to pathogens. We will then discuss recent data pointing to the contribution of MCs in initiating and polarizing the primary immune responses to allergens and the importance of the co-stimulatory role of lipopolysaccharides (LPS) and peptidoglycan (PGN), common bacterial products in this response. We will describe how microbial infections may severely exacerbate allergy

Corresponding Author: Alison M. Hofmann, alison.hofmann@duke.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

symptoms in patients, through differential activation of MCs. In view of the additional roles of MCs in the development of immune responses to allergens and pathogens, strategies to modulate some of these MC activities that might be of therapeutic or prophylactic value will be reviewed.

Mast cells act as effector cells during allergic responses

MCs have long been recognized as the principle effector cell responsible for the IgE mediated allergic reactions following secondary contact with allergens. In atopic patients, allergens are recognized, processed and presented by antigen presenting cells such as dendritic cells, in association with MHC II and co-stimulatory molecules, to naïve T cells. Naïve T cells that recognize the allergen as foreign then differentiate into Th2 type T cells which produce cytokines that enhance allergen specific IgE synthesis by B cells. This primary immune response is termed allergen sensitization and has been previously thought to occur without involvement of mast cells. The IgE that is made to these allergens then binds to the high affinity IgE receptor (FcεRI) located on the MC surface. Upon secondary exposure, the allergen binds and crosslinks IgE on the MC surface resulting in a signaling cascade that is initiated from FcεRI that results in calcium mobilization and culminates in MC degranulation. MC granules contain proinflammatory preformed mediators such as histamine, proteases, TNFα and antimicrobial peptides that are released into the body upon degranulation.[3] Histamine, in particular, has been shown to be a potent mediator of immediate hypersensitivity reactions. [2] Upon IgE crosslinking, MCs also begin to synthesize lipid mediators such as prostaglandins and leukotrienes as well as cytokines and chemokines. [4] Prostaglandins and leukotrienes mediate some of the symptoms of allergic diseases such as bronchoconstriction. The cytokines and chemokines secreted by MCs recruit inflammatory cells such as eosinophils, basophils, and T cells to the site of allergen exposure. These cells then participate in the late phase response to allergen and promote many of the well known pathological sequelae associated with allergy. [4]

Mast cells initiate innate immune responses to pathogens

Although the role of MCs in mediating many of the pathological sequelae of allergy was well known, it was also recognized that an important physiologic role for these cells must exist. Within the last decade and a half, MCs have come to the forefront as triggers of innate immune defenses against pathogens. Because of their preferential location lining the various surfaces of the body, they are ideally positioned to be first responder cells during microbial attack.

To function as immune surveillance cells, MCs must be able to interact with incoming pathogens. MC activation occurs through at least 3 mechanisms of pathogen recognition: (i) direct binding of pathogens or their components by pathogen associated molecular pattern (PAMP) receptors located on the MC surface; (ii) binding of opsonized bacteria or their products by complement receptors or immunoglobulin receptors, or (iii) recognition of endogenous peptides produced by infected or injured host cells. [2] One class of PAMP receptors are the Toll like receptors (TLRs). Each TLR binds a specific component of different pathogens. Human MCs have been shown to express TLR-1,-2,-3,-4,-5,-6,-7 and -9 under certain conditions.[5,6] When ligands bind to TLRs, MCs release specific cytokines such as IL6 and TNFα. [7] Activation of each individual TLR results in MC secretion of a unique profile of cytokines and chemokines. The two most studied TLRs on MCs are TLR 2 and TLR 4. Human MCs seem to express TLR4 only in the presence of IL4 while TLR 2 is ubiquitously expressed. [8,9] TLR2 mediates responses to peptidoglycan (PGN) from gram positive bacteria, gram negative bacteria, and mycobacteria, and zymosan from yeast. [10] TLR 4 binds to lipopolysaccharide (LPS) from gram negative bacteria, lipid A, fibrinogen and *Mycobacterium tuberculosis*. [11] When TLR2 or TLR 4 is activated, human MCs release

TNF α , IL-5, IL-10, and IL-13 which facilitate the recruitment of immune cells to sites of infection. [8]

As stated earlier, MCs have granules that contain prestored cytokines such as TNF α which are critical for neutrophil recruitment to sites of infection. [12,13] Other cytokines such as IL4, IL5 and IL6 are rapidly synthesized and released by mast cells in response to infection. [14] These cytokines and chemokines serve to attract phagocytic as well as other immune cells such as neutrophils and eosinophils to infection sites and influx of these cells is crucial to control and eliminate invading pathogens. Studies have shown that MC deficient mice are less able than wild type mice to clear *E.coli* in an acute peritonitis model, and *Klebsiella pneumoniae* and *Francisella tularensis* in a lung infection model. In all of these models, MC deficient mice have decreased survival due to impaired innate host responses thereby indicating that MCs are critical to the innate immune response against pathogens. [13-16]

In addition to recruiting inflammatory cells, MCs can also directly kill opsonized bacteria. Bacteria coated with complement components such as C3b or IgG antibody can bind to specific complement receptors or IgG Fc γ receptors on MCs. These bacteria are then endocytosed via an endosome lysosome pathway. Bacteria that enter into MCs via endocytosis are then killed via both oxidative and nonoxidative pathways. ([17,18] MCs also produce antimicrobial peptides such as LL37 which are released upon MC degranulation and have potent killing activity. [19] Recent studies have shown that MCs also exhibit extracellular killing activities that rely on trapping of the bacteria in unique extracellular structures composed of DNA, histones, tryptase and LL-37. [17,18] In summary, MCs function first to alert and activate the innate immune system to invasion by foreign pathogens and secondly to eliminate pathogens through mechanisms such as opsonization of bacteria.

Mast cells coordinate adaptive immune responses to pathogens

In addition to activating the innate immune system during infection, MCs also have profound effects on two of the central cells of adaptive immunity: dendritic cells and T cells. Peripheral immature dendritic cells can take up antigens but are unable to act as antigen presenting cells due to low expression of MHC II and co-stimulatory molecules, such as CD80 and CD86. [20] They are also unable to migrate to peripheral lymph nodes where critical interactions with T cells occur. [20] TNF α , a mast cell granule component, has been shown to increase dendritic cell expression of CCR7, a chemokine important for dendritic cell homing to lymph nodes. [21,22] Studies have shown that MCs degranulate at the site of infection thereby releasing pre stored TNF α , which functions in migration of dendritic cells to lymph nodes through upregulation of CCR7. [23-25] CCR7 expression allows dendritic cells to respond to the homing chemokines, CCL19 and CCL21 which are specifically produced by the draining lymph nodes [26, Shelburne et al submitted]. TNF α also induces maturation of dendritic cells so that dendritic cells express antigen collected from the periphery in association with MHC II and co-stimulatory molecules thereby becoming an antigen presenting cell for T cells.[22] In addition to its local action, MC derived TNF α also drains to proximal lymph nodes and retains lymphocytes circulating from the blood in the nodes. [27, Kunder et al submitted] These lymphocytes are then in prime position to engage the dendritic cells entering lymph nodes from the infection sites. Although dendritic cells exposed to pathogens can become mature and migrate to lymph nodes independently of mast cell activation, mast cell activation of local dendritic cells through TNF alpha seems to result in faster migration of dendritic cells to lymph nodes and therefore a more rapid adaptive immune response.[24]

MCs may also profoundly influence the type of adaptive immune response made to a pathogen through their modulation of T cell responses. Upon activation, MCs release Th2 polarizing cytokines such as IL4, IL10 and IL13 which can induce a stimulated naïve CD4⁺ T cell to

become a Th2 cell when activated in the lymph node. [10] These Th2 cells induce humoral immune responses to pathogens by producing cytokines that stimulate B cell production of antibody. In support of this concept, one study has shown that MC activators, when paired with an antigen, can induce strong humoral immune responses that are protective against antigen challenge. [28] MCs can also enhance proliferation of suboptimally activated T cells both through an FcεRI dependent pathway and through a mechanism independent of FcεRI signaling. [29] This type of MC dependent activation of T cells may occur in situations in which T cells are already exposed to antigen but at suboptimal concentrations. [29] These observations further expand the role of MCs in direct modulation of effector lymphocyte subsets and the adaptive immune response.

In addition to influencing the polarity of T cell responses, MCs can directly activate T cells by functioning as antigen presenting cells under certain conditions. MCs have been shown to express MHC I and MHC II in limited circumstances. [30,31,] Studies by Malaviya and colleagues showed that MCs have the ability to endocytose bacteria bound by complement or antibody and to express these bacterial antigens in association with MHC I on the MC surface. Expression of MHC I and bacterial antigens by MCs is likely the cellular basis for induction of CD8 T cell responses to pathogens that enter MC vacuole compartments. [30] Controversy exists in the literature regarding the ability of MCs to express MHC II. However, two recent studies suggest that certain conditions can induce MC expression of MHC II. Kambayashi et al. demonstrated that murine bone marrow derived MCs (BMMCs) as well as peritoneal murine MCs express MHC II when treated with LPS and Interferon γ (IFN γ). [31] Furthermore they showed that in vivo inflammatory stimuli such as LPS or bacteria increase the number of MHC II expressing MCs in the lymph nodes. In these experiments with LPS, MHC II expressing MCs seemed to have traveled from the periphery to draining lymph nodes much like dendritic cells do after antigen encounter. These MCs also expressed co-stimulatory molecules such as CD80 and CD86, which are necessary for T cell activation. Although the MHC II expressing MCs were poor stimulators of naïve CD4 T cells, they were able to re-stimulate previously activated CD4 T cells. [31] Other studies by Nakano and colleagues demonstrated that Notch signaling induced expression of MHC II and OX40L, a key co-stimulatory molecule, on the surface of murine BMMCs. These MHC II, OX40 L BMMCs were able to promote proliferation of naïve CD T cells into Th2 cells in vitro.[32] Further work is needed to clarify if human mast cells are able to express MHC II and how MHC II bearing MCs influence T cell activation in vivo. Cumulatively these studies suggest that MCs can directly modulate the initiating as well as subsequent steps leading to the development of an effective adaptive immune response to pathogens. In summary, MCs initiate and coordinate the adaptive immune response through recruitment and maturation of dendritic cells, through secretion of T cell polarizing cytokines, and possibly through direct antigen presentation to T cells.

Mast Cells contribute to the initiation of the primary immune responses to allergens

Over the past decade, ample evidence has accumulated showing the critical role that MCs play in immune responses to pathogens, or dangerous foreign substances. Less is known about the role of MCs in initiating innate and adaptive immune responses to innocuous substances such as pollen or food. However, new evidence is emerging to suggest that MCs may coordinate and drive a Th2 immune response to innocuous agents particularly when these innocuous agents are encountered in the setting of endotoxin exposure. [33-35] Endotoxins and PGN are common contaminants in the environment and can act on MCs via TLRs as described previously. [36] When an individual encounters allergen and low doses of endotoxin or PGN in the environment together, MC activation through TLR signaling may lead to MC secretion of cytokines such as TNF α into the local environment. Dendritic cells that have taken up the innocuous allergen may then become activated by TNF α to express co-stimulatory molecules

and to migrate to the lymph node leading to an inappropriate Th2 immune response to the innocent allergen in a “bystander phenomenon” resulting in allergen sensitization. [33-35] Once allergen sensitization has occurred, MCs expressing allergen specific IgE may amplify the allergic response by acting as antigen presenting cells to further drive allergen specific Th2 proliferation. In addition, MCs bearing allergen specific IgE may increase cytokine production markedly in the setting of allergen exposure and infection through simultaneous activation of both FcERI by allergens and PAMP receptors by pathogens thereby exacerbating allergic disease. Thus in this scenario, the critical role that MCs play in defense against pathogens acts detrimentally to induce allergic sensitization and to amplify allergic responses.

Recent studies have given weight to the potential role for MCs in allergen sensitization. In a mouse model of asthma, intranasal administration of low dose LPS and antigen resulted in markedly enhanced eosinophil accumulation in the lung upon antigen rechallenge as compared to mice that were sensitized with antigen only or sensitized with high dose LPS and antigen. [33-35] In addition, serum antibodies in mice treated with low dose LPS and antigen showed a Th2 pattern (high IgE and IgG1) as opposed to those from mice treated with high dose LPS and antigen (high IgG2a-Th1 pattern). [33] When this experiment was performed in MC deficient mice, eosinophilic infiltration was markedly low and was restored when MC deficient mice underwent MC repletion prior to exposure to LPS and allergen thereby indicating that MCs were important in the process of allergen sensitization. [34,35] Interestingly, when MC deficient mice were reconstituted with MCs made from TLR4 knockout (KO) mice, and were then sensitized with LPS and antigen, eosinophilic infiltration failed to occur upon antigen challenge suggesting that MC activation through LPS interaction with TLR4 on the MC surface was a key event during allergen sensitization. Furthermore, MC deficient mice that were reconstituted with wildtype BMMCs pretreated with LPS demonstrated increases in eosinophilic lung infiltration upon allergen challenge which were absent in MC deficient mice repleted with LPS treated TLR4 KO MCs. [34-35] Other studies showed that the Th2 immune response to low dose LPS and antigen in TLR4 knockout mice could be restored when these mice were treated with antigen and TNF α , a cytokine stored preformed in MC granules. [33] These experiments suggest that in the setting of TLR4 activation by low doses of endotoxin, MCs initiate and prime Th2 responses to allergens resulting in allergen sensitization.

Activation of TLR2 on MCs may also function in the allergen sensitization phase of allergic diseases. In a murine model of food allergy, mice sensitized orally to low doses of ova or peanut in the presence of *Staphylococcus Aureus* enterotoxin B (SEB), a TLR2 agonist, developed Th2 immune responses to peanut including elevations of peanut specific IgE and a Th2 cytokine profile. Furthermore, when these sensitized mice were challenged with ova or peanut, they developed signs and symptoms of anaphylaxis. [37] As in the case of LPS, SEB likely binds TLR2 on MCs resulting in release of mediators such as TNF α which coordinates an adaptive immune response to the allergen, and IL-4 which influence Th2 development, thereby resulting in a Th2 immune response to a co-exposed innocuous allergen.

Interestingly, the results of these murine experiments add insight into the hygiene hypothesis which states that infections and unhygienic contact may confer protection from the development of allergic illnesses. [38] Epidemiologic evidence has shown that children growing up on farms where there is exposure to large amounts of microbial products are protected from the development of asthma, hay fever and allergic sensitization. In this epidemiologic study, children from a neighborhood in the same village but much less exposed to farm life, and therefore to microbial products, served as controls and had higher rates of allergic disease.[38] The murine asthma experimental data described previously concurs with the hygiene hypothesis and human epidemiologic data under conditions of high endotoxin exposure. High dose endotoxin exposure resulted in Th1 responses to the allergen and protection from allergy in the mice similar to the observation that farm life and exposure to

microbial products protects humans from allergic disease. However, low dose endotoxin exposure resulted in the development of Th2 responses to the allergen and in asthma in the mice. Furthermore these Th2 allergic responses seemed to depend on MCs since MC deficient mice failed to develop allergic inflammation after allergen and low dose endotoxin exposure. Perhaps the control children who did not live on farms experienced concomitant allergen and low dose endotoxin exposure given its ubiquitous presence in the environment thereby leading to higher rates of allergy. These results suggests that there is a dose threshold to the hygiene hypothesis in which high doses of endotoxin leads to Th1 responses and protection and low doses of endotoxin lead to Th2 responses and allergy. This dose response may be explained by differential TLR4 signaling of MCs via either the MyD88 pathway in low dose LPS exposure or via the TRIF signaling pathway in high dose LPS exposure which could differentially skew the T cell response to either a Th2 or Th1 phenotype through secretion of different cytokine patterns. [33]

Mast Cells amplify exacerbations of allergic diseases

While the role of TLR ligands in the development of the primary immune response to allergens is still being investigated, there are ample examples of exacerbations of allergic disease, including asthma in the setting of microbial infections which likely involve TLR signaling and MC activation [39] Allergic asthma exacerbations, in the setting of infection, may be explained by dual activation of TLR4 and TLR2 on the MC surface by LPS and PGN, in association with FcεRI stimulation by allergen. Studies have shown that this dual activation of TLR4 or TLR2 by bacterial products and FcεRI by allergen acts synergistically to increase cytokine production by MCs. [7] In the setting of dual TLR-FcεRI signaling, MCs produced augmented amounts of IL-6, IL-13, and TNFα. [7] IL-6 and TNFα have been shown to recruit neutrophils into the site of MC activation while IL-13 promotes a Th2 response in T cells and initiates IgE class switching and IgE production in B cells, all of which serve to exacerbate allergic asthma. [11] In addition, TNFα is also thought to modulate the contractility of airway smooth muscle and has been associated with development of airway hyperresponsiveness. [40]. Overall, activation of TLR signaling in addition to FcεRI signaling in the setting of infection and allergen exposure causes enhanced MC activation and worsening of established allergic disease.

Although bacterial infections can worsen asthma symptoms, allergic asthma exacerbations are more commonly associated with viral infections. [39,41] The role of TLRs and MCs in viral infections is still under investigation. One group has shown that cultured human MCs express TLR3 which recognizes double stranded RNA from viruses and induces production of type I IFNs. [42] Any synergistic effect of TLR3 activation by viruses in the setting of FcεRI activation by allergens on MC cytokine production remains to be determined. Some viruses, such as Respiratory Syncytial Virus (RSV), a known asthma trigger, contain proteins such as HsP60 and F-protein which can activate TLR4. [11] In theory, dual activation of TLR 4 and FcεRI on MCs in the setting of simultaneous allergen exposure and RSV infection could lead to increased cytokine production and TNFα release by MCs thereby worsening asthma symptoms as detailed earlier. In addition, TLR4 polymorphisms affecting MC cytokine production could also influence exacerbations of asthma in the setting of either viral or bacterial infections. Clinical trials are underway to understand the effect of polymorphisms in TLR4 genes to induce varying levels of chemokine and cytokine production with the goal of understanding the role of innate immune responses to RSV in developing asthma. [43]

Another allergic disease in which bacteria may exacerbate MC mediated symptoms is atopic dermatitis or allergic skin inflammation characterized by pruritis and erythema. Approximately 90% of patients with atopic dermatitis have skin colonization with *Staphylococcus aureus*, a gram positive bacteria which contains PGN which can bind to TLR2. [44] As opposed to some

of the other TLRs, TLR2 has been shown to be consistently expressed by human mast cells. [6] PGN produced by *S. aureus* may bind to TLR 2 on MCs in the skin thereby enhancing release of proinflammatory cytokines such as TNF α , IL-4, IL-5, IL-6, and IL-13 which can initiate or exacerbate the skin inflammation characteristic of atopic dermatitis. [45]

Although MCs seem to have a prominent role in allergic exacerbations in the setting of infection and endotoxin exposure, MCs may also play a role in the amplification of the allergic response in the uninfected host by acting as antigen presenting cells in the setting of allergen re-exposure after sensitization. MCs have the ability to phagocytose antigens that bind to surface receptors and to process and display them on the MC surface in the setting of MHC I or even, perhaps, MHC II under certain conditions. Because MCs can phagocytose antigens using its receptors, it is a possibility that MCs may act similar to B cells and participate in facilitated antigen binding. In a facilitated antigen binding model, allergens that bind to IgE-Fc ϵ RI complexes on the MC surface theoretically may be endocytosed, processed, and expressed on the cell surface in the context of MHC I or MHC II. [46] MCs bearing MHC allergen complexes could then stimulate CD 8+ or CD 4+ effector T cells located in peripheral sites such as the skin or lungs where MCs are naturally found leading to expansion and proliferation of the particular allergen specific T cell line. Increased numbers of allergen specific CD8 T+ cells and CD 4 T+ cells may contribute to exacerbations of allergic diseases especially asthma.

Novel treatments for allergic diseases

Because of the many roles that MCs play in both allergic sensitization and allergic exacerbations, new treatments aimed specifically at MCs are needed. Two treatment strategies which may help to limit the IgE independent role of MCs in allergic diseases are inhibition of MC activation and inhibition of the mediators released by MCs.

MCs rely critically on TLR binding for activation in both allergic sensitization and exacerbations of allergic disease as well as in initiation of innate and adaptive immune responses. One possible future treatment for allergic diseases may be antibodies that act as decoys and bind LPS and PGN thereby preventing them from binding to TLR4 and TLR2 on the MC surface. Although this may effectively prevent the induction of Th2 responses to allergens encountered in the presence of LPS or PGN, this strategy is unlikely to affect innate and adaptive immune responses to pathogens because pathogens are recognized via multiple mechanisms and receptors on MCs and other cell types.

Another treatment strategy for allergic diseases is inhibition of the mediators released by MCs. One novel treatment incorporating this concept is the ongoing clinical investigations of TNF α blockade to treat severe refractory asthma. Several clinical trials have investigated the efficacy of different TNF α inhibitors in severe and moderate asthma with varying results. In an initial open label investigation of the TNF α blocker, etanercept, for treatment of severe asthma, improvement in methacholine airway hyperresponsiveness as well as improvement in lung functions and asthma symptoms was found. [47] A randomized, double blind placebo controlled trial of etanercept in patients with severe asthma showed a small but significant improvement in asthma control. [48] However another clinical trial of a human monoclonal antibody against TNF α , golimumab, showed no clinical benefit. [49] Furthermore golimumab seemed to be associated with increased risk of infection and malignancy. [49] Interestingly, this increased risk was found in patients taking inhaled or oral corticosteroids. It may be that TNF α inhibition in the context of corticosteroids results in such potent immunosuppression that there was failure of innate and adaptive immune responses to pathogens and malignant cells. The question remains whether TNF α inhibition could be a steroid sparing therapy for patients with severe asthma.

Other potential MC derived mediator targets for treatment and prevention of allergic diseases are IL-4 and IL-13. IL-13 has been shown to be a critical mediator of asthma pathology and is one of the Th2 cytokines released by MCs upon TLR2 and TLR4 stimulation. IL-4 is known to influence the development of Th2 cells and is also released by TLR stimulation of MCs. There are many clinical trials in progress assessing the safety and efficacy of different IL4 and IL13 inhibitors in the treatment of asthma and allergic rhinitis. These include studies of a monoclonal antibody against IL13, monoclonal antibody inhibitors of the IL4 and IL13 receptors, and a recombinant human IL4 variant which inhibits IL4 and IL13 receptors. [50-52] One preclinical study of an anti IL13 antibody in allergic macaques showed decreases in allergic inflammation upon allergen exposure but had no effect on lung function. [53] The human clinical trials of these agents for therapy of asthma and allergic rhinitis are ongoing.

Conclusion

All allergic diseases including asthma, allergic rhinitis and food allergy are increasing worldwide. For many years, MCs were considered only as the effector cells in allergic diseases. We now know that MCs are much more diverse players in both allergy and infection. We know that MCs can enhance initiation of both innate and adaptive immune responses to foreign pathogens as well as to allergens in the setting of co-exposure to bacteria or its products. For pathogens, these immune responses are protective. For allergens, these immune responses are pathologic. MCs also seem to play a critical role in exacerbations of allergic disease in the setting of infection. Therefore by increasing our understanding of the role of MCs in innate and adaptive immunity to infectious pathogens, we may be able to discover better treatments to prevent development of allergic disease and allergic exacerbations of established disease since the pathways and mechanisms involved in allergen sensitization, allergic exacerbations, and infection control are likely shared.

Acknowledgments

We would like to thank Dr. David Zaas, Dr. John Sundry and Ashley St. John for their helpful comments in reviewing this manuscript.

AM Hofmann is funded by the Pediatric Scientist Development Program and SN Abraham is funded by the NIH.

References

1. Heib V, Becker M, Taube C, Stassen M. Advances in the understanding of mast cell function. *Br J Haematol* 2008;142(5):683–94. [PubMed: 18513284]
2. Rao KN, Brown MA. Mast Cells: multifaceted immune cells with diverse roles in health and disease. *Ann NY Acad Sci* 2008;1143:83–104. [PubMed: 19076346]
3. Brown JM, Wilson TM, Metcalfe DD. The mast cell and allergic diseases: role in pathogenesis and implications for therapy. *Clin Exp Allergy* 2008;38(1):4–18. [PubMed: 18031566]
4. Minai-Fleminger Y, Levi-Schaffer F. Mast cells and eosinophils: the two key effector cells in allergic inflammation. *Inflamm Res*. 2009 May 8;
5. Tkaczyk C, Jensen BM, Iwaki S, Gilfillan AM. Adaptive and innate immune reactions regulating mast cell activation: from receptor-mediated signaling to responses. *Immunol Allergy Clin North Am* 2006;26(3):427–50. [PubMed: 16931287]
6. Bischoff SC. Role of mast cells in allergic and non-allergic immune responses: comparison of human and murine data. *Nat Rev Immunol* 2007;7(2):93–104. [PubMed: 17259966]
- *7. Qiao H, Andrade MV, Lisboa FA, Morgan K, Beaven MA. FcepsilonR1 and toll-like receptors mediate synergistic signals to markedly augment production of inflammatory cytokines in murine mast cells. *Blood* 2006;107(2):610–8. [PubMed: 16174756] This study shows that TLR2 and TLR4 ligands enhance antigen induced production of cytokines by mast cells without affecting degranulation, arachidonic acid production, or calcium mobilization. TLR signaling seemed to

increase MAP kinase activity to promote increased production of cytokines. No in vivo data was presented and all work was done in mc primary cultures or mc cell lines.

8. Varadaradjalou S, Féger F, Thieblemont N, Hamouda NB, Pleau JM, Dy M, Arock M. Toll-like receptor 2 (TLR2) and TLR4 differentially activate human mast cells. *Eur J Immunol* 2003;33(4):899–906. [PubMed: 12672055]
9. McCurdy JD, Olynich TJ, Maher LH, Marshall JS. Cutting edge: distinct Toll-like receptor 2 activators selectively induce different classes of mediator production from human mast cells. *J Immunol* 2003;170(4):1625–9. [PubMed: 12574323]
10. Stelekati E, Orinska Z, Bulfone-Paus S. Mast cells in allergy: innate instructors of adaptive responses. *Immunobiology* 2007;212(6):505–19. [PubMed: 17544835]
11. Marshall JS, McCurdy JD, Olynich T. Toll-like receptor-mediated activation of mast cells: implications for allergic disease? *Int Arch Allergy Immunol* 2003;132(2):87–97. [PubMed: 14600420]
12. Malaviya R, Gao Z, Thankavel K, van der Merwe PA, Abraham SN. The mast cell tumor necrosis factor alpha response to FimH-expressing *Escherichia coli* is mediated by the glycosylphosphatidylinositol-anchored molecule CD48. *Proc Natl Acad Sci U S A* 1999;96(14):8110–5. [PubMed: 10393956]
13. Echtenacher B, Männel DN, Hültner L. Critical protective role of mast cells in a model of acute septic peritonitis. *Nature* 1996;381(6577):75–7. [PubMed: 8609992]
14. Féger F, Varadaradjalou S, Gao Z, Abraham SN, Arock M. The role of mast cells in host defense and their subversion by bacterial pathogens. *Trends Immunol* 2002;23(3):151–8. [PubMed: 11864844]
15. Ketavarapu JM, Rodriguez AR, Yu JJ, Cong Y, Murthy AK, et al. Mast cells inhibit intramacrophage *Francisella tularensis* replication via contact and secreted products including IL-4. *Proc Natl Acad Sci U S A* 2008;105(27):9313–8. [PubMed: 18591675]
16. Sutherland RE, Olsen JS, McKinsty A, Villalta SA, Wolters PJ. Mast cell IL-6 improves survival from *Klebsiella pneumoniae* and sepsis by enhancing neutrophil killing. *J Immunol* 2008;181(8):5598–605. [PubMed: 18832718]
17. von Köckritz-Blickwede M, Nizet V. Innate immunity turned inside-out: antimicrobial defense by phagocyte extracellular traps. *J Mol Med.* 2009 May 16;
18. von Köckritz-Blickwede M, Goldmann O, Thulin P, Heinemann K, Norrby-Teglund A, Rohde M, Medina E. Phagocytosis-independent antimicrobial activity of mast cells by means of extracellular trap formation. *Blood* 2008;111(6):3070–80. [PubMed: 18182576]
19. Metz M, Maurer M. Mast cells--key effector cells in immune responses. *Trends Immunol* 2007;28(5):234–41. [PubMed: 17400512]
20. Galli SJ, Nakae S, Tsai M. Mast cells in the development of adaptive immune responses. *Nat Immunol* 2005;6(2):135–42. [PubMed: 15662442]
21. Sozanni S, Allavena P, D'Amico G, Luini W, Bianchi G, Kataura M, Imai T, Yoshie O, Bonecchi R, Mantovani A. Differential regulation of chemokine receptors during dendritic cell maturation: a model for their trafficking properties. *J Immunol* 1998;161(3):1083–6. [PubMed: 9686565]
22. Ritter U, Meissner A, Ott J, Körner H. Analysis of the maturation process of dendritic cells deficient for TNF and lymphotoxin- α reveals an essential role for TNF. *J Leuk Bio* 2003;74:216–222.
23. Yamazaki S, Yokozei H, Satoh T, Katayama I, Nishioka K. TNF- α , RANTES, and MCP-1 are major chemoattractants of murine Langerhans cells to the regional lymph nodes. *Exp Dermatol* 1998;7(1):35–41. [PubMed: 9517920]
24. Suto H, Nakae S, Kakurai M, Sedgwick JD, Tsai M, Galli SJ. Mast cell-associated TNF promotes dendritic cell migration. *J Immunol* 2006;176(7):4102–12. [PubMed: 16547246]
25. McLachlan JB, Hart JP, Pizzo SV, Shelburne CP, Staats HF, Gunn MD, Abraham SN. Mast cell-derived tumor necrosis factor induces hypertrophy of draining lymph nodes during infection. *Nat Immunol* 2003;4(12):1199–205. [PubMed: 14595438]
26. Förster R, Davalos-Misslitz AC, Rot A. CCR7 and its ligands: balancing immunity and tolerance. *Nat Rev Immunol* 2008;8(5):362–71. [PubMed: 18379575]
27. Young AJ, Seabrook TJ, Marston WL, Dudley L, Hay JB. A role for lymphatic endothelium in the sequestration of recirculating gamma delta T cells in TNF- α -stimulated lymph nodes. *Eur J Immunol* 2000;30(1):327–34. [PubMed: 10602056]

- **28. McLachlan JB, Shelburne CP, Hart JP, Pizzo SV, Goyal R, Brooking-Dixon R, Staats HF, Abraham SN. Mast cell activators: a new class of highly effective vaccine adjuvants. *Nat Med* 2008;14(5): 536–41. [PubMed: 18425129] This study shows that intranasal instillation or subcutaneous administration of a mast cell activator plus an antigen generates mast cell dependent antigen specific antibody responses in the sera that correlate with dendritic and T cell movement into the lymph node.
29. Nakae S, Suto H, Kakurai M, Sedgwick JD, Tsai M, Galli SJ. Mast cells enhance T cell activation: Importance of mast cell-derived TNF. *Proc Natl Acad Sci U S A* 2005;102(18):6467–72. [PubMed: 15840716]
30. Malaviya R, Twosten NJ, Ross EA, Abraham SN, Pfeifer JD. Mast cells process bacterial Ags through a phagocytic route for class I MHC presentation to T cells. *J Immunol* 1996;156(4):1490–6. [PubMed: 8568252]
31. Kambayashi T, Allenspach EJ, Chang JT, Zou T, Shoag JE, Reiner SL, Caton AJ, Koretzky GA. Inducible MHC class II expression by mast cells supports effector and regulatory T cell activation. *J Immunol* 2009;182(8):4686–95. [PubMed: 19342644]
- **32. Nakano N, Nishiyama C, Yagita H, Koyanagi A, Akiba H, Chiba S, Ogawa H, Okumura K. Notch signaling confers antigen-presenting cell functions on mast cells. *J Allergy Clin Immunol* 2009;123(1):74–81. [PubMed: 19130928] Intriguing study showing that upon DeltaII/Notch signaling, bone marrow derived MCs and peritoneal MCs express MHCII and upregulate OX40L expression which allowed these MCs to function as antigen presenting cells. These MHCII OX40L BMMCS induced naïve T cells to proliferate and differentiate into Th2 cells. This study lacks in vivo corroborative evidence.
33. Eisenbarth SC, Piggot DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K. Lipopolysaccharide-enhanced, Toll-like Receptor 4-dependent T Helper Cell Type 2 Responses to Inhaled Antigen. *J Exp Med* 2002 Dec;196(12):1645–1651. [PubMed: 12486107]
34. Nigo YI, Yamashita M, Hirahara K, Shinnakasu R, Inami M, Kimura M, Hasegawa A, Kohno Y, Nakayama T. Regulation of allergic airway inflammation through Toll-like receptor 4-mediated modification of mast cell function. *Proc Natl Acad Sci U S A* 2006;103(7):2286–91. [PubMed: 16461458]
35. Yamashita M, Nakayama T. Progress in allergy signal research on mast cells: regulation of allergic airway inflammation through toll-like receptor 4-mediated modification of mast cell function. *J Pharmacol Sci* 2008 Mar;106(3):332–5. [PubMed: 18360088]
36. Singh J, Schwartz DA. Endotoxin and the lung: Insight into the host-environment interaction. *J Allergy Clin Immunol* 2005;115(2):330–3. [PubMed: 15696090]
37. Ganeshan K, Neilsen CV, Hadsaitong A, Schleimer RP, Luo X, Bryce PJ. Impairing oral tolerance promotes allergy and anaphylaxis: a new murine food allergy model. *J Allergy Clin Immunol* 2009;123(1):231–238. [PubMed: 19022495]
38. von Mutius E. Allergies, infections and the hygiene hypothesis --the epidemiological evidence. *Immunobiology* 2007;212(6):433–9. [PubMed: 17544828]
39. Newcomb DC, Peebles RS Jr. Bugs and asthma: a different disease? *Proc Am Thorac Soc* 2009;6(3): 266–71. [PubMed: 19387028]
40. Brightling C, Berry M, Amrani Y. Targeting TNF-alpha: a novel therapeutic approach for asthma. *J Allergy Clin Immunol* 2008;121(1):5–10. [PubMed: 18036647]
41. Sykes A, Johnston SL. Etiology of asthma exacerbations. *J Allergy Clin Immunol* 2008;122(4):685–8. [PubMed: 19014758]
42. Kulka M, Alexopoulou L, Flavell RA, Metcalfe DD. Activation of mast cells by double-stranded RNA: evidence for activation through Toll-like receptor 3. *Allergy Clin Immunol* 2004;4(1):174–82.
43. Innate Immunity and Respiratory Syncytial Virus (RSV) Infection in Children. <http://clinicaltrials.gov/ct2/show/NCT00593918?term=tlr4+and+rsv+and+asthma&rank=1>
44. Abeck D, Mempel M. Staphylococcus aureus colonization in atopic dermatitis and its therapeutic implications. *Br J Dermatol* 1998;139:13–6. [PubMed: 9990408]

45. Supajatura V, Ushio H, Nakao A, Akira S, Okumura K, Ra C, Ogawa H. Differential responses of mast cell Toll-like receptors 2 and 4 in allergy and innate immunity. *J Clin Invest* 2002;109(10):1351–9. [PubMed: 12021251]
46. Wilcock LK, Francis JN, Durham SR. IgE-facilitated antigen presentation: role in allergy and the influence of allergen immunotherapy. *Immunol Allergy Clin North Am* 2006;26(2):333–47. viii–ix. [PubMed: 16701148]
47. Howarth PH, Babu KS, Arshad HS, Lau L, Buckley M, McConnell W, Beckett P, Al Ali M, Chauhan A, Wilson SJ, Reynolds A, Davies DE, Holgate ST. Tumour necrosis factor (TNFalpha) as a novel therapeutic target in symptomatic corticosteroid dependent asthma. *Thorax* 2005;60(12):1012–8. [PubMed: 16166100]
48. Morjaria JB, Chauhan AJ, Babu KS, Polosa R, Davies DE, Holgate ST. The role of a soluble TNFalpha receptor fusion protein (etanercept) in corticosteroid refractory asthma: a double blind, randomised, placebo controlled trial. *Thorax* 2008;63(7):584–91. [PubMed: 18245148]
49. Wenzel SE, Barnes PJ, Bleecker ER, Bousquet J, Busse W, Dahlén SE, Holgate ST, Meyers DA, Rabe KF, Antczak A, Baker J, Horvath I, Mark Z, Bernstein D, Kerwin E, Schlenker-Herceg R, Lo KH, Watt R, Barnathan ES, Chanez P, T03 Asthma Investigators. A randomized, double-blind, placebo-controlled study of tumor necrosis factor-alpha blockade in severe persistent asthma. *Am J Respir Crit Care Med* 2009;179(7):549–58. [PubMed: 19136369]
50. Phase 1 Study of AIR645 in Healthy Subjects and Subjects With Controlled Asthma. <http://clinicaltrials.gov/ct2/results?term=il13+and+asthma>
51. A. Phase IIa Study of Subcutaneous AER 001 on Antigen Challenge In Atopic Asthmatic Subjects (28 Day Study). <http://clinicaltrials.gov/ct2/show/NCT00535028?term=il13+and+asthma&rank=7>
52. Phase II Study to Evaluate the Efficacy of AMG 317. <http://clinicaltrials.gov/ct2/show/NCT00436670?term=il13+and+asthma&rank=10>
53. Martin PL, Fisher D, Glass W, O'Neil K, Das A, Martin EC, Li L. Preclinical safety and pharmacology of an anti-human interleukin-13 monoclonal antibody in normal macaques and in macaques with allergic asthma. *Int J Toxicol* 2008;27(5):351–8. [PubMed: 19037805]

Figure 1.
Mast Cell Roles in Allergic Disease

1. MCs play a key role in allergen sensitization when allergen exposure occurs in the presence of bacterial byproducts. 2. Allergen triggers release of MC granules leading to allergic disease. 3. Allergen and bacterial products act synergistically to increase MC granule release. 4. MCs may act as antigen presenting cells and stimulate allergen specific Th2 cell proliferation.