Immunity
Review

Innate Cells and T Helper 2 Cell Immunity in Airway Inflammation

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Activated mast cells, eosinophils, and basophils infiltrate the airways of asthmatics as a result of an overexuberant T helper 2 (Th2) cell immune response that drives the production of IgE, primes mast cells and basophils, and promotes tissue eosinophilia and mast cell hyperplasia. Recent evidence demonstrates that these innate effectors can be activated outside of this classical Th2 cell paradigm and that they have additional roles in promoting the development of innate and adaptive pulmonary inflammation. There is also an appreciation for the role of airway epithelial cells in orchestrating allergic pulmonary inflammation. Emerging data from basic research highlight the involvement of many unique pathways in the inflammation triggered by complex native allergens and microbes at the airway mucosal surface. Here, we review the role of effector cells and airway epithelial cells in augmenting and, at times, bypassing traditional Th2 cell-mediated allergic inflammation.

Introduction

Airway inflammation, reversible airflow obstruction, and an increased sensitivity to nonspecific irritants or bronchoconstricting agents, termed airway hyperresponsiveness (AHR), are the cardinal features of asthma. Although inflammation had been noted in asthmatic airway biopsies since the 1960s, it was not until the advent of the flexible bronchoscope that inflammation was recognized as a consistent feature of the asthmatic airway, seen even in mild, newly diagnosed adult and pediatric patients. Analysis of the cellular infiltrate in bronchial biopsies and bronchoalveolar lavage (BAL) of asthmatic patients demonstrated the presence of eosinophils, degranulated mast cells (MCs), and T helper 2 (Th2) lymphocytes generating interleukin-3 (IL-3), IL-4, IL-5, IL-9, IL-13, and granulocyte macrophage colony-stimulating factor (GM-CSF) (Bousquet et al., 2000; Robinson et al., 1992), which differentiated asthma from chronic obstructive pulmonary disease and focused research in airway inflammation for the ensuing decade.

The Th2 Cell Paradigm

The specific contribution of Th2 lymphocytes to asthma was noted in studies comparing Th2 lymphocyte infiltration to indices of airflow obstruction or clinical course. Classical allergen challenge studies, in which aerosolized allergen is given to sensitized asthmatics, demonstrate airflow obstruction, as measured by a fall in forced expiratory volume in 1 min (FEV1) that begins within several minutes, peaks by 30 min, and improves over several hours. This "early phase" response is followed by a second fall in FEV1 hours later, the "late phase." Both phases are blocked by pretreatment with inhibitors of MC degranulation or MC-derived bronchoconstricting mediators; however, the late phase is uniquely associated with an influx of eosinophils and T lymphocytes and an increase in Th2 cell cytokines in the BAL and bronchial mucosa. Furthermore, asthma severity correlates with the extent of T cell infiltration in the BAL, and persistent lymphocyte activation is seen in some patients with refractory steroid-resistant asthma (reviewed in Kay, 1997).

Mouse models of antigen-induced pulmonary inflammation have also supported a central role for Th2 cells and their cytokines. Adoptive transfer of TCR-transgenic Th2 cells, but not Th1 cells, from DO11.10 mice and subsequent ovalbumin (OVA) challenge of recipients induces eosinophilic airway inflammation and AHR (Cohn et al., 1997; Cohn et al., 1998), and depletion of $CD4^+$ T cells prior to challenge prevents both of these responses in OVA sensitized and challenged wild-type (WT) mice (Gavett et al., 1994). Furthermore, sensitization and airway challenge studies of mice with transgenic overexpression of cytokines, targeted deletion of cytokines, or blocking cytokine antibodies have demonstrated a central role for IL-4 in the generation of Th2 cells and IgE; for IL-5 in the promotion of airway eosinophilia; for IL9 in the recruitment, proliferation, and differentiation of MCs; and for IL-13 in induction of AHR, goblet cell metaplasia, and mucin production (reviewed in Holgate and Polosa, 2008). These studies have led to the dominant thesis that activated Th2 cells orchestrate the pulmonary immune response, stimulate B cell production of allergen-specific IgE, and elicit the structural changes in the lung that are seen in asthmatic patients.

Despite the strength of the Th2 cell paradigm, some features of human asthma are not been well explained by it alone. First, neutrophilic inflammation on bronchial biopsy is common in asthmatics, the degree of neutrophilia is roughly correlated with asthma severity, and neutrophilic inflammation without eosinophils is seen in a substantial subgroup of severe corticosteroid-dependent asthmatics, underscoring the heterogeneity of disease (Wenzel et al., 1999). Second, the histopathologic features of airway inflammation—including eosinophil influx and degranulated MCs—are similar in "intrinsic" asthma, in which there is no allergic trigger of symptoms, no family or personal history of atopy, and no evidence of allergen-specific

IgE or elevation in total IgE (Humbert et al., 1999), suggesting that additional pathways may be triggering the inflammation. Third, even in well-phenotyped allergic asthmatics, the administration of antibodies that prevent the binding of IgE to FcεRI or that neutralize IL-5 has had limited therapeutic efficacy and the success of interventions directed against IL-4 and IL-13 is, as yet, unproven (Holgate and Polosa, 2008). Fourth, viral infections are a common trigger for exacerbation in both atopic and nonatopic asthma. Finally, the antigen-induced AHR in the late phase of allergen challenge in human asthma or in sensitized and challenged mice is susceptible to inhibitors that are not capable of ameliorating the ''intrinsic'' or inflammation-independent AHR of the human disease. These observations suggest that additional mechanisms, beyond the classical activation of Th2 lymphocytes or crosslinking of IgE, may exist to drive neutrophilic and eosinophilic airway inflammation.

Beyond Th2 Cells

Although traditional mouse models of antigen-induced pulmonary inflammation that use intraperitoneal (i.p.) OVA sensitization (with or without aluminum hydroxide) and intranasal (i.n.), intratracheal (i.t.), or aerosolized OVA challenge have been instructive, they do not include the participation of a full mucosal immune response comparable to complex allergens such as dust mites or molds that contain protease activity and/or innate pattern-recognition receptor ligands for the Toll-like receptor (TLR), C-type lectin receptor (CLR), and/or nucleotide-binding domain, leucine-rich repeat-containing protein (NLR) families that facilitate their immunogenicity. Recent studies in mice with airway delivery of native allergens or respiratory viruses have underscored the critical role of innate signaling in the generation of allergic pulmonary inflammation and the complex immunologic response that includes the participation of Th1, Th2, Th17, NKT, and airway epithelial cells (Akbari et al., 2003; Bartlett et al., 2008; Grayson et al., 2007; Hammad et al., 2009; Kim et al., 2008; Phipps et al., 2009). In this review, we will discuss the role of the epithelial cell-derived cytokines IL-25, TSLP, and IL-33 as orchestrators of eosinophilic airway inflammation and the contribution of NKT cells to AHR. We will then review newly described functions for the classic effector cells, MCs, eosinophils, and basophils. In each section, we will highlight emerging data on the role of these innate cytokines and cells in augmenting and, at times, bypassing traditional Th2 cell-mediated allergic inflammation.

Epithelial Cell-Derived Cytokines: IL-25

IL-25 is a member of the IL-17 cytokine family but has limited homology to IL-17A (17%), uses IL17RB rather than the IL17RA and IL17RC heterodimer, and has distinct biologic functions. Although IL-25 was initially reported as a product of differentiated murine Th2 cells (Fort et al., 2001), IL-25 is made by activated eosinophils, by bone marrow-derived mast cells (BMMCs) and basophils after FcεRI-mediated activation, and by epithelial cells in response to allergens such as *Aspergillus oryzae*, *Aspergillus fumigatus*, ragweed, and the house dust mite, *Dermatophagoides pteronyssinus* (*Dp*) (Hammad et al., 2009 and reviewed in Saenz et al., 2008). Thus, IL-25 generation is induced in both innate and adaptive immune responses to allergens.

IL-25 acts on both recruited hematopoietic cells and native resident cells in the lung. IL-25 promotes the differentiation of murine Th2 cells in an IL-4- and STAT6-dependent manner in vitro (Angkasekwinai et al., 2007). IL-17RB is expressed on murine $CD4^+$ invariant NKT (iNKT) cells in which it identifies a potent IL-4- and IL-13-producing subset that, on adoptive transfer, can reconstitute AHR in sensitized and challenged iNKT cell-deficient mice (Terashima et al., 2008) (see NKT Cells). OVA-alum sensitization and OVA aerosol challenge of mice with the transgenic overexpression of IL-25 elicits elevated BAL eosinophils, IL-4, IL-5, and IL-13, which is abrogated with anti-CD4 prior to challenge or with crossing to *Stat6*$^{-/-}$ mice (Tamachi et al., 2006), suggesting that $CD4^+$ Th2 cells can mediate IL-25 effects in vivo. In an early report of IL-25 function in vivo (Hurst et al., 2002), WT and *Rag1*$^{-/-}$ mice treated with recombinant IL-25 (rIL-25) or an adenoviral construct expressing IL-25 generated high levels of IL-5 and IL-13 transcripts in the spleen, suggesting that a non-B/non-T (NBNT) cell could also mediate production of Th2 cytokines in response to IL-25. The i.n. administration of murine rIL-25 into C57BL/6 WT mice generates BAL eosinophilia and whole lung IL-5, IL-13, and eotaxin transcripts 24 hr later, each of which is significantly reduced in mice with an epithelial-specific knockout of Act1, an adaptor protein required for IL-17RB signaling, identifying a pathway for an epithelial cell response (Swaidani et al., 2009). Thus, IL-25 acts on an unusual range of hematopoietic and stromal cells to influence both innate and adaptive pulmonary inflammation.

Studies that use overexpression of IL-25 or administration of rIL-25 have consistently demonstrated a capacity for induction of Th2 cytokines and eosinophilia. Systemic administration of rIL-25 for 10 days in C57BL/6 mice induced peripheral blood eosinophilia, splenomegaly, eosinophilic infiltration of the spleen and lung, pulmonary goblet cell metaplasia, medial hypertrophy of the small and medium-sized pulmonary arteries, and elevated serum IgG1, IgA, and IgE, among other abnormalities (Fort et al., 2001). Adenoviral vector-mediated overexpression of IL-25 led to similar immunoglobulin abnormalities and histologic changes in the lungs with the addition of perivascular fibrosis. *Il4rα*$^{-/-}$ and *Il13*$^{-/-}$ mice infected with the same construct were protected from mucus production and goblet cell metaplasia, *Il4rα*$^{-/-}$ and *Il4*$^{-/-}$ mice were protected from the increased immunoglobulin levels and pulmonary vascular changes, and WT mice treated with anti-IL-5 were protected from peripheral blood eosinophilia, demonstrating that IL-25-dependent pathology is mediated entirely through downstream Th2 cytokines. Studies using the transgenic overexpression of murine IL-25 or IL-25 adenoviral vector infection reported similar abnormalities as well as elevations in circulating or BAL neutrophils (reviewed in Wang and Liu, 2009). Lastly, a recent study has shown that matrix matalloproteinase 7, an enzyme induced in allergen-activated airway epithelial cells, can both augment levels of IL-25 as well as cleave it to a more active form to promote Th2 cytokine production (Goswami et al., 2009).

Several studies have addressed the role of IL-25 in murine models of antigen-induced pulmonary inflammation and AHR. Balb/c mice sensitized with i.p. OVA-alum and challenged with aerosolized OVA have an upregulation of IL-25 transcript in the lung and eosinophils, $CD4^+$ T cells, and IL-5 and IL-13 protein in the BAL, each of which is reduced in mice treated with

a soluble IL-25 receptor fusion protein prior to each OVA challenge (Tamachi et al., 2006). The administration of anti-IL-25 to WT Balb/c mice prior to OVA-alum sensitization and prior to OVA aerosol challenge decreased AHR, pulmonary cellular infiltrate, BAL eosinophilia, OVA-specific serum IgE, and IL-5 and IL-13 generated by restimulation of parabronchial lymph node cells with OVA ex vivo (Ballantyne et al., 2007). Interestingly, when mice were treated with anti-IL-25 only during the OVA aerosol challenge phase, AHR was abolished and IL-5 and IL-13 levels in the BAL were diminished, but pulmonary cellular infiltrate and goblet cell metaplasia, OVA-specific serum IgE, and Th2 cytokines from restimulation of parabronchial LN cells with OVA ex vivo were intact. Direct intranasal injection of IL-25 elicited AHR in Balb/c WT, *Il13*$^{-/-}$, and *Il4*$^{-/-}$*Il5*$^{-/-}$*Il9*$^{-/-}$*Il13*$^{-/-}$ strains, suggesting an independent effect of IL-25. Taken together, murine in vitro and in vivo studies suggest that IL-25 can potentiate the induction of Th2 cell airway inflammation in the sensitization phase, can amplify Th2 inflammation in the challenge phase, and can induce AHR in the absence of Th2 cytokines.

There is limited data on the role of IL-25 in human asthma. IL-25 potentiates the proliferation of CD4^{+}CD45RO^{+}CRTH2^{+} human Th2 memory cells and augments their production of IL-4, IL-5, and IL-13 (Wang et al., 2007). IL-17RB protein is expressed on cultured primary human airway smooth muscle cells (Lajoie-Kadoch et al., 2006), on human fibroblast cell lines (Létuvé et al., 2006), and on human peripheral blood monocyte-derived macrophages cultured in the presence of IL-4 or IL-4 and TGF-β (Gratchev et al., 2004). Cultured human peripheral blood eosinophils also express IL-17RB that mediates production of IL-6, IL-8, MIP-1α, and MCP-1, prevents eosinophil apoptosis, and alters eosinophil adhesion proteins with an upregulation of ICAM-1 and a downregulation of ICAM-3 and L-selectin (Cheung et al., 2006; Wong et al., 2005). Bronchial biopsies of patients with asthma show elevations of IL-25 and IL-17RB transcripts as compared to normal controls. Immunohistochemical studies have also shown IL-25 protein in submucosal inflammatory cells of asthmatic bronchial biopsies (Létuvé et al., 2006), supporting a role for this mediator in human disease.

Epithelial Cell-Derived Cytokines: IL-33

IL-33 is a member of the IL-1 cytokine family. RT-PCR from human and mouse cDNA libraries have shown IL-33 transcript in murine and human epithelial cells, DCs, and activated macrophages and in human bronchial smooth muscle (Schmitz et al., 2005). In a murine model of antigen-induced pulmonary inflammation, intracellular cytokine staining revealed IL-33 in alveolar CCR3^{-}F4/80^{+} myeloid cells (Kurowska-Stolarska et al., 2008), whereas in a model with direct i.t. administration of house dust mite, *Dp*, IL-33 protein in the BAL was recovered from WT mice, but not *Tlr4*$^{-/-}$ mice or bone marrow chimeras with WT reconstitution of *Tlr4*$^{-/-}$ mice (Hammad et al., 2009), suggesting there are multiple cellular sources during both the innate and adaptive immune components of allergic inflammation.

IL-33 acts at the Toll/interleukin-1 (TIR) domain-containing receptor ST2. Soluble and transmembrane forms of the receptor, produced by differential mRNA splicing, act as a soluble decoy or transmembrane activator, respectively (see below). IL-33 can activate murine BMMCs to release TNF-α, IL-1β, IL-6, IL-13, Rantes, GM-CSF, MCP-1, MIP1-α, and MIP1-β (Ali et al., 2007; Ho et al., 2007; Kondo et al., 2008) and can activate murine bone marrow-derived basophils to produce IL-6, IL-4, IL-9, IL-13, Rantes, and MCP-1 (Kondo et al., 2008; Kroeger et al., 2009). Stimulation of committed murine Th2 cells with IL-33 elicits production of IL-4, IL-5, and IL-13 (Coyle et al., 1999; Kondo et al., 2008; Lohning et al., 1998), whereas stimulation of naive purified CD4^{+}CD62L^{+} DO11.10 T cells with IL-33 induces the generation of Th2 cells in a manner dependent on ST2 and MyD88 signaling, but independent of IL-4, IL-4Rα, and STAT6 (Kurowska-Stolarska et al., 2008). These in vitro studies emphasize that IL-33 can promote Th2 cell immunity through both the direct activation of innate effector cells and the generation and activation of IL-4-independent, MyD88-dependent Th2 cells.

Several studies have demonstrated a role for ST2 (encoded by *Il1r1*) in Th2 cell pulmonary inflammation. In models using direct OVA-alum sensitization and OVA aerosol or intranasal challenge, *Il1rl1*$^{-/-}$ mice on a Balb/c background had reduced eosinophilia and IL-5 in the BAL and reduced cellular influx into the lung (Kurowska-Stolarska et al., 2008). Similarly, administration of the ST2-specific mAb prior to the sensitization and challenge of WT Balb/c mice attenuated eosinophilia and IL-5 levels in the BAL, as well as serum OVA-specific IgE (Coyle et al., 1999). Two murine studies using adoptive transfer of Th2 cells from DO11.10 mice into WT recipients with subsequent OVA aerosol challenge have shown reductions in induced AHR, BAL eosinophilia, and BAL concentrations of IL-4, IL-5, IL-6, and IL-13 with the administration of either an ST2-specific mAb or an ST2-IgG soluble fusion protein prior to each OVA challenge (Coyle et al., 1999; Lohning et al., 1998), emphasizing a role for this pathway in the challenge phase. When WT or *Il4*$^{-/-}$ mice were subjected to an OVA-alum sensitization and OVA challenge protocol with only 10 ug of OVA for sensitization, the addition of exogenous IL-33 during sensitization triggered similar BAL eosinophilia, pulmonary inflammation, goblet cell metaplasia, and IL-5-producing CD4^{+}ST2^{+} T cells upon challenge (Kurowska-Stolarska et al., 2008), thereby demonstrating that IL-33 can specifically promote IL-4-independent Th2 cells that participate in antigen-induced immune responses. In a pharmacologic study, repeated i.p. injection of rIL-33 induced goblet cell metaplasia and AHR to methacholine in Balb/c WT and *Rag2*$^{-/-}$ mice similarly, demonstrating that the direct actions of IL-33 on innate effector cells can bypass the requirement for Th2 cells (Kondo et al., 2008). Lastly, studies using OVA-alum sensitization and OVA challenge with higher doses of antigen (100 μg) or longer protocols (28 days) have not shown a dependence on ST2 signaling (Hoshino et al., 1999; Kurowska-Stolarska et al., 2008), indicating that the IL33-ST2 pathway can be bypassed with continued immune stimulation. In sum, murine in vitro and in vivo studies demonstrate that IL-33 acts in the effector phase to augment Th2 cytokine production and inflammation via direct effects on both Th2 cells and innate effectors and can generate AHR and goblet cell metaplasia in the absence of adaptive immunity.

In studies of human cells, IL-33 stimulation potentiates the production of IL-4, IL-5, and IL-13 from CD3^{+}CRTH2^{+} Th2 cells in the peripheral blood and from cord blood CD4^{+} cells activated by anti-CD3 and anti-CD28 (Kurowska-Stolarska et al., 2008;

Pecaric-Petkovic et al., 2009). IL-33 can activate human MCs cultured from peripheral blood or cord blood-derived $CD34^+$ MC progenitors to generate IL-5, IL-13, GM-CSF, TNF-α, IL-10, IL-6, CXCL8, and CCL1, without degranulation or the production of prostaglandin D_2 or leukotriene C_4 (LTC_4) (Allakhverdi et al., 2007b). IL-33 can increase CD11b expression and enhance the survival of freshly isolated human peripheral blood eosinophils in an ST2-dependent fashion without degranulation or LTC_4 synthesis. IL-33 can also augment production of IL-8 by human eosinophils in response to IL-3, IL-5, and GM-CSF (Pecaric-Petkovic et al., 2009). ST2 is expressed by purified human basophils primed with IL-3 (Suzukawa et al., 2008), and IL-33 stimulation can increase basophil production of IL-4, expression of ICAM-1, VCAM-1, and CD11b, and release of histamine in response to FcεRI crosslinking (Suzukawa et al., 2008). Asthmatics have a baseline increase in serum ST2 levels as compared to normal controls, and there is a marked increase in these levels during asthma exacerbations. ST2 levels correlate inversely with peak expiratory flow, suggesting elaboration of a soluble trap for IL-33 with exacerbations in these atopic patients (Oshikawa et al., 2001).

Epithelial Cell-Derived Cytokines: TSLP

Thymic stromal lymphopoietin (TSLP) is an IL-7-like cytokine initially cloned from a murine thymic epithelial cell line and identified as a factor that supported the in vitro development of T and B cells (Friend et al., 1994; Sims et al., 2000). TSLP protein is detected in human bronchial epithelial cell cultures after stimulation with dsDNA, rhinovirus, peptidoglycan, or the combination of IL1β and TNF-α (Allakhverdi et al., 2007a; Kato et al., 2007) and in the BAL of WT mice after the i.t. administration of the house dust mite, *Dp* (Hammad et al., 2009). TSLP transcript is generated after mAb-mediated FcεRI crosslinking on human $CD34^+$ cell-derived MCs (Soumelis et al., 2002). TSLP protein is generated in murine basophil cultures after IgE crosslinking or in an innate response to proteolytically active papain (Sokol et al., 2008). Thus, TSLP can be produced in response to allergens, viruses, cytokines, and PAMPs in the setting of innate or adaptive immune responses.

TSLP stimulates human peripheral blood $CD11c^+$ DCs to upregulate the expression of HLA-DR and costimulatory molecules (CD40, CD80, CD86, and CD83) and to generate TARC and MDC, but not IL-12 p70, type I interferons, or the proinflammatory cytokines IL-1β, IL-6, and TNF-α (Soumelis et al., 2002). TSLP-treated DCs prime human naive $CD4^+$ T cells to produce IL-4, IL-5, IL-13, and TNF-α (Liu, 2006; Soumelis et al., 2002). The upregulation of OX40L on TSLP-treated DCs is central to their role in Th2 differentiation, given that this function is abrogated in the presence of OX40L neutralizing antibodies (Ito et al., 2005; Seshasayee et al., 2007). TSLP can also act directly on T cells to enhance Th2 cytokine production, as illustrated in a model of atopic dermatitis with i.p. OVA sensitization and epicutaneous OVA challenge in which the administration of TSLP antibodies prior to OVA challenge had no effect on $CD4^+$T cell recruitment to the tissue but significantly impaired cutaneous Th2 cytokine production and eosinophil recruitment (He et al., 2008). TSLP can also drive the proliferation of TCR-activated human $CD4^+$ T cells (Omori and Ziegler, 2007; Rochman et al., 2007). In conjunction with IL-1β and TNF-α, TSLP is a potent stimulus for human cord blood- or peripheral blood-derived MCs to generate IL-5, IL-13, IL-6, GM-CSF, IL-10, CXCL8, and CCL1 (Allakhverdi et al., 2007a). Thus, the diverse cell sources and functions recognized in vitro for TSLP suggest that it can potentiate allergic inflammation via several cellular targets.

Murine studies have highlighted a role for TSLP in Th2 immune responses in the lung. Mice with transgenic overexpression of TSLP in airway epithelial cells display spontaneous pulmonary inflammation with remarkable BAL cellularity and eosinophilia, pulmonary inflammation with goblet cell metaplasia and perivascular fibrosis, and AHR (Zhou et al., 2005). Conversely, TSLPR receptor- (TSLPR)-deficient ($Crlf2^{-/-}$) mice are protected from antigen-induced pulmonary inflammation in a model with OVA-alum i.p. sensitization and OVA aerosol challenge (Zhou et al., 2005). A second $Crlf2^{-/-}$ strain also showed attenuation of inflammation with OVA sensitization and challenge that could be reversed by adoptive transfer of WT but not $Crlf2^{-/-}$ $CD4^+$ T cells from OVA sensitized donors to the deficient mice prior to antigen challenge (Al-Shami et al., 2005). TSLP conditions DCs to express OX40L and treatment of mice with a neutralizing antibody to OX40L attenuated the serum IgE, pulmonary inflammation, and BAL IL-4, IL-5, and IL13 protein elicited by either direct intranasal injection of TSLP or by OVA-alum sensitization and OVA intranasal challenge (Seshasayee et al., 2007). The OX40L neutralizing antibody was efficacious even when administered in the challenge phase. Thus, TSLP can regulate both primary and secondary Th2 cell immune responses.

Biopsies from the mucosa of asthmatic patients show an elevated number of cells containing TSLP transcript as compared to normal controls (Ying et al., 2005). TSLP transcript is expressed in neutrophils, macrophages, MCs, epithelial cells, and endothelial cells, and the number of $TSLP^+$ cells is correlated with the severity of airflow obstruction, as assessed by FEV1. Although a supporting role for TSLP in generating, maintaining, or amplifying human asthma is suggested by the data in mice, direct translation to human asthma, as for IL-25 and IL-33, awaits support from further clinical studies and targeted interventions.

NKT Cells

NKT cells recognize both foreign and endogenous glycolipids presented by the MHC class I (MHC I)-like molecule CD1d on APCs. Type I iNKT cells express a restricted repertoire of αβ TCRs, with Vα24-Jα18 in human and Vα14-Jα18 in mice along with a limited set of Vβ chain gene segments, whereas type II or variant NKT cells utilize more diverse TCRs. iNKT cells recognize α-galactosylceramide (α-GalCer), which is loaded onto a tetramer to identify iNKT cells by flow cytometric analysis and is used as a ligand for their activation. In addition, iNKT cells can be activated by IL-12, IL-18, IL-33, and type 1 interferons generated from TLR signaling in DCs that amplify weak signals from endogenous glycolipid-loaded CD1d or bypass the CD1d requirement entirely (reviewed in Tupin et al., 2007).

Murine studies have demonstrated a role for iNKT cells in induction of IL-13-dependent AHR. Both $Cd1d1^{-/-}$ mice (lacking iNKT cells and variant NKT cells) on a Balb/c background or Jα18-deficient mice (lacking iNKT cells) on a Balb/c or a C57BL/6 background sensitized with i.p. OVA-alum and challenged with i.n. OVA had diminished AHR, as compared to their WT controls, that was restored with the transfer of tetramer-purified iNKT

cells (Akbari et al., 2003; Lisbonne et al., 2003). AHR was dependent on iNKT cell cytokine production because it was reconstituted by transfer of tetramer-purified iNKT cells from WT but not *Il4*$^{-/-}$*Il13*$^{-/-}$ mice into sensitized recipient Jα18-deficient mice prior to i.n. challenge with OVA. In a protocol of suboptimal i.p. sensitization with OVA-alum and i.n. challenge with OVA that used 2 ug of IL-25 for priming prior to OVA challenge, Jα18-deficient mice had attenuated AHR that was uniquely restored with transfer of a splenic CD4$^+$ iNKT cell subpopulation expressing IL-17RB (Terashima et al., 2008). Additionally, a recent murine study of Sendai virus-induced chronic pulmonary inflammation demonstrated that IL-13-producing CD4$^-$ iNKT cells identified in the lung 21 and 49 days after infection promoted the recruitment and activation of CD68$^+$IL13$^+$ alternatively activated macrophages (Kim et al., 2008). Macrophage activation was critical for subsequent airway goblet cell hyperplasia and AHR in WT mice and was abrogated in both *Cd1d1*$^{-/-}$ and Jα18-deficient strains. Thus, iNKT cells, activated in the setting of antigen, proinflammatory cytokines, or viral infection can promote AHR and structural airway changes, termed airway remodeling.

Notably, studies with *B2m*$^{-/-}$ strains, which also lack iNKT cells, have not demonstrated an impairment in AHR in immunization protocols that are more severe (Brown et al., 1996; Koh et al., 2008), demonstrating the ability to bypass the iNKT cell contribution to AHR. Although one study has reported a significant decrement in BAL eosinophilia and antigen-specific IgE after sensitization and challenge of C57 Jα18-deficient mice (Lisbonne et al., 2003), studies using stronger immunization protocols in *B2m*$^{-/-}$ mice (Brown et al., 1996; Das et al., 2006; Koh et al., 2008; Zhang et al., 1996) and in *Cd1d1*$^{-/-}$ mice (Akbari et al., 2003; Das et al., 2006) have not demonstrated any impairment. Thus, the aggregate studies in *B2m*$^{-/-}$, *Cd1d1*$^{-/-}$, and Jα18-deficient strains in models of antigen-induced pulmonary inflammation indicate a circumscribed role for NKT cells in IL-13-dependent AHR (but no clear effect on the cellular infiltrate) that can be bypassed with stronger protocols.

iNKT cells are <1% of peripheral blood CD4$^+$ cells and <1% of BAL CD4$^+$ cells in nonasthmatic subjects. An initial study reporting a high percentage of CD4$^+$$\alpha$-GalCer-loaded CD1d tetramer$^+$ cells in the BAL and bronchial biopsies of patients with moderate to severe asthma has not been duplicated (Akbari et al., 2006; Thomas et al., 2006; Vijayanand et al., 2007). However, some increase in CD4$^+$ α-GalCer-loaded CD1d tetramer$^+$ cells was seen in the BAL of asthmatic children as compared to normal controls (Pham-Thi et al., 2006), and an increased percentage of Vα24$^+$6B11$^+$ iNKT cells was seen in the BAL of adult asthmatics, as compared to peripheral blood (Thomas et al., 2007). Finally, another recent study demonstrated that CCR9$^+$Vα24$^+$ INF-γ-producing iNKT cells isolated from the peripheral blood of asthmatics, but not normal controls, can direct Th2 cytokine generation from cocultured conventional CD3$^+$ T cells in a manner dependent on CD226, a member of the Ig superfamily that mediates cell-cell adhesion (Sen et al., 2005). This same CCR9$^+$Vα24$^+$ IFN-γ-producing iNKT cell population was found in the bronchial biopsies of asthmatics, but not normal controls, and was associated with upregulation of mucosal Th2 cytokine transcripts as well as the CCR9 ligand CCL25, suggesting a role for IFN-γ-producing iNKT cell in bronchial asthma.

Mast Cells

MCs are well-established effectors in allergic airway inflammation. Crosslinking of FcεRI by IgE-bound inhaled antigen can trigger degranulation, defined by the release of the preformed secretory granule complex and subsequent extracellular dissociation of preformed mediators (e.g., histamine and certain proteases). Activation is accompanied by the rapid synthesis of lipid mediators (e.g., cys-LTs, dihydroxy leukotrienes, and prostaglandin D2), and the induction of cytokines and chemokines (e.g., IL-4, IL-13, IL-12, IL-1, IL-18, TNF-α, etc.). Through the sequential and/or synergistic actions on the same target tissue, this array of inflammatory mediators can elicit and sustain a vascular leak, constrict smooth muscle, and initiate inflammatory cell infiltration. MC mediator release can also be triggered by innate signals, and although innate MC activation can be part of a protective immunity to pathogens (reviewed in Marshall, 2004), it also expands the variety of signals that can elicit pathologic airway inflammation in asthma. Here, we review recent information on MC activators beyond the antigen-induced crosslinking of FcεRI, as well as MC participation in initiating and amplifying immunologic responses.

Mature MCs develop in peripheral tissues after transendothelial migration of circulating MC progenitors (MCps). In mice, the recruitment of MCps to the lung is dependent on the binding of integrins $\alpha_4\beta_1$ and $\alpha_4\beta_7$ on MCp to endothelial vascular cell adhesion molecule 1 (VCAM-1), the expression of which is regulated by CXCR2 (Hallgren et al., 2007). The development of MCps and their tissue maturation is dependent on the stimulation of Kit by stem cell factor, which is a lineage-specific requirement. There are two major MC subtypes, the connective tissue MC (CTMC) sustained by SCF alone, and the intraepithelial mucosal MC (MMC) that is dependent on costimulation by Th2 cytokines. In addition to their different tissue distributions, these two MC populations differ in the composition of their secretory granule proteoglycans to which cationic proteases and amines are bound. In rodents, the MMC secretory granule proteoglycan is primarily composed of chondroitin sulfates that bear, in mice, two β-chymases, mouse MC protease 1 (mMCP-1), and mMCP-2, whereas the CTMC proteoglycan is composed predominantly of heparin glycosaminoglycans, which carry diverse proteases that include chymases (mMCP-4 and mMCP-5), tryptases (mMCP-6 and mMCP-7), and carboxypeptidase A. In human MCs, the secretory granule proteoglycan has both chrondroitin sulfate and heparin chains, and the MMC carry tryptase, whereas the CTMC carry tryptase, chymase, and carboxypeptidase A (reviewed in Stevens and Adachi, 2007).

In vitro studies of rodent and of human MCs, generally obtained by culture of progenitors, have demonstrated that innate stimuli induce cytokines and LTC_4 generation from MCs. TNF-α, for example, can be generated by MCs in response to signaling via TLR2 and TLR1, TLR2 and TLR6, TLR3, TLR4, TLR7, and TLR9 (reviewed in Marshall, 2004). MC exocytosis and LTC_4 generation require the mobilization of calcium, which is triggered by TLR2 and Dectin-1 signaling (Marshall, 2004; Olynych et al., 2006). Whereas these studies highlight the wide variety of innate signals that can activate MCs, they also underscore the selectivity of mediator release in response to these signals and the propensity for calcium mobilization and eicosanoid production to be restricted to TLR2 and CLR signaling, as

has been seen in macrophages and DCs (Barrett et al., 2009; Buczynski et al., 2007; Suram et al., 2006). Although there is now evidence that major crude allergens carry innate signals for TLRs and CLRs, as well as epitopes for an IgE response (Barrett et al., 2009; Hammad et al., 2009; Phipps et al., 2009), the integrated role of allergen-derived innate signals in MC activation is not known.

MCs can modulate DC antigen presentation and T cell activation. Murine BMMCs treated with IL-4 and the P815 and MC-9 MC lines spontaneously release exosomes, small endocytic vesicles that contain soluble antigen as well as membrane-bound MHCII and the costimulatory molecules CD80, CD86, and CD40 (Skokos et al., 2003). Antigen-loaded exosomes are potent activators of DCs and stimulate robust production of serum IgG1 and IgG2a when injected s.c. into mice, which is dependent on the MC exosomal proteins hsp60 and hsc70 and the DC endocytic receptor for many heat shock proteins, CD91. Another study has shown antigen transfer from MCs to DCs by a distinct mechanism (Kambayashi et al., 2008). BMMCs or freshly isolated peritoneal MCs "sensitized" with OVA-specific IgE demonstrate endocytosis of IgE-bound OVA and activation of $CD4^+$ OT-II splenocytes that is dependent on apoptosis of the BMMCs and their phagocytosis by BMDCs. MCs can provide costimulation to activated T cells, given that BMMCs sensitized with IgE and activated with antigen can augment the cell proliferation and cytokine production by cocultured CD3 mAb-activated splenic T cells in a manner that is dependent on cell contact and that is neutralized with antibody to OX40L, a molecule that is expressed on activated BMMCs (Nakae et al., 2006).

The role of MCs in antigen-induced pulmonary inflammation has been approached by use of the MC-deficient mouse strains $Kit^{W/W\text{-}v}$ or $Kit^{W\text{-}sh/W\text{-}sh}$ with disruptions in the stem cell factor receptor c-kit, which is required for normal MC growth and survival. Because these strains have other hematologic abnormalities, ($Kit^{W/W\text{-}v}$ are leukopenic and anemic [Galli et al., 2005]; $Kit^{W\text{-}sh/W\text{-}sh}$ have splenomegaly, neutrophilia, and thrombocytosis [Nigrovic et al., 2008]), MC engraftment achieved 8–12 weeks after adoptive transfer of BMMCs is used for defining the MC contribution to the disease phenotype. This approach does not restore the number and location of mature MCs to the WT phenotype. Nonetheless, a MC contribution was demonstrated in a study using OVA i.p. sensitization without alum and in repeated aerosol challenges over 9 weeks in both $Kit^{W/W\text{-}v}$ and $Kit^{W\text{-}sh/W\text{-}sh}$. The MC-deficient mice had substantially reduced BAL eosinophils, neutrophils, and lymphocytes, pulmonary eosinophilia, goblet cell metaplasia, and induced AHR that was reversed with engraftment of WT BMMCs before initiating the protocol (Yu et al., 2006). Engraftment of MC-deficient mice with FcεR1 γ chain-deficient BMMCs, which cannot be activated through either FcεRI or FcγRIII, could not fully restore any aspect of the phenotype, indicating a role for MCs in amplifying tissue inflammation. In a model using intranasal OVA and LPS for sensitization and OVA alone for challenge in $Kit^{W/W\text{-}v}$ mice or the WBB6F1 controls, the MC-deficient strain had reduced eosinophilic inflammation in the BAL and lung histopathology. This was reversed on adoptive transfer of BMMCs from C57BL/6 WT but not from $Tlr4^{-/-}$ mice, suggesting that MC activation by TLR4 can provide critical signals to promote eosinophilic inflammation (Nigo et al., 2006). Although the MC mediator was not identified, TNF-α is a prominent product of LPS-activated murine BMMCs (McCurdy et al., 2001), and TNF-α can augment lung DC migration to draining lymph nodes after intranasal injection of FITC-OVA, given that the number of FITC-OVA^+ DCs in the draining lymph nodes of $Tnf^{-/-}$, $Tnfrsf1a^{-/-}$, MC-deficient $Kit^{W/W\text{-}v}$ or $Kit^{W\text{-}sh/W\text{-}sh}$, or $Kit^{W\text{-}sh/W\text{-}sh}$ mice reconstituted with BMMCs from $Tnf^{-/-}$ mice was decreased as compared to WT or $Kit^{W\text{-}sh/W\text{-}sh}$ mice reconstituted with BMMCs from WT mice (Suto et al., 2006). Thus, FcεRI-activated MCs augment tissue inflammation in the challenge phase and TLR4-activated MCs can generate TNF-α for DC mobilization in the sensitization phase. Nonetheless, the contribution of B cells, IgE, and MCs to antigen-induced inflammation and AHR is strictly context dependent such that MC-deficient mice display no defects in AHR or pulmonary eosinophilia in antigen sensitization and challenge protocols that use strong systemic adjuvant, high doses of antigen, or prolonged challenges (Hamelmann et al., 1999; Takeda et al., 1997; Williams and Galli, 2000).

A role for MCs in human asthma is evident in studies using allergen challenge in sensitized asthmatics. Although the early phase is associated with MC degranulation and an increase in plasma histamine and urinary LTE_4, both phases are attenuated by pretreatment with sodium chromoglycate (an inhibitor of MC degranulation), a 5-lipoxygenase inhibitor (preventing cys-LT synthesis), a CysLT1 receptor antagonist, or antihistamines (reviewed in Bradding, 2003). The MC contribution to asthma is underscored by two recent findings. Omalizumab, an anti-IgE mAb that blocks IgE binding to FcεRI, decreases asthma exacerbations and allows reductions in corticosteroid use (Busse et al., 2001; Milgrom et al., 1999), supporting a role for MCs in chronic allergic inflammation. In an anatomic study of patients with allergic asthma and with eosinophilic bronchitis, a disease characterized by steroid-responsive cough and sputum eosinophilia without airway hyperresponsiveness, bronchial biopsies demonstrated equal numbers of submucosal MCs and eosinophils in both groups. In contrast, there was a selective increase in tryptase and chymase positive MCs in the smooth muscle layer of subjects with either atopic or nonatopic asthma. MC infiltration correlated with AHR, suggesting that localization of MCs to the bronchial smooth muscle is a unique feature of airway hyperresponsiveness (Brightling et al., 2002).

Eosinophils

Eosinophils are circulating granulocytes that are expanded in bone marrow and that are recruited to peripheral tissues in the settings of parasitic infection and allergic disease. The terminal differentiation and mobilization of eosinophils from the bone marrow is under the direction of IL-5. Eosinophils can generate lipid mediators such as LTC_4; cytokines such as IFN-γ and IL-2 (Th1) and IL-4, IL-5, IL-10, IL-13, and TNF-α (Th2); and Th2 cell chemokines including CCL17 and CCL22 (reviewed in Akuthota et al., 2008). Here, we review recent human studies that highlight the importance of eosinophils in airway remodeling (Flood-Page et al., 2003) and in asthma exacerbations in a particular subgroup of patients (Haldar et al., 2009; Nair et al., 2009), and murine studies that extend its role beyond the classic effector functions.

Eosinophils can modulate adaptive immune processes and even act as antigen-presenting cells. Murine airway eosinophils

harvested via BAL from sensitized and challenged mice have elevated expression of MHCII, CD80, and CD86, can present OVA antigen to memory T cells in vitro, and can stimulate activation and proliferation of memory DO11.10 T cells when transferred to previously sensitized recipients (Shi et al., 2000). Ova-pulsed eosinophils from naive IL-5 transgenic mice can activate naive DO11.10 TCR transgenic T cells when transferred into WT recipients 24 hr after i.v. infusion of DO11.10 $CD4^+$ cells (Wang and Weller, 2008), demonstrating their ability to act as professional APCs. However, human eosinophils cultured with IL-3, IL-5, or GM-CSF can support the proliferation of T cell clones to antigenic peptides, but not intact antigen, from tetanus toxoid and from influenza hemaglutinin, demonstrating their limited antigen-presenting function (Celestin et al., 2001). Human eosinophil-derived neurotoxin (EDN) can activate human monocyte-derived DCs to upregulate costimulatory molecules and generate IL-6, IL-8, IL-12p70, and TNF-α in a MyD88-dependent fashion (Yang et al., 2008). EDN acts as a TLR2 ligand and immunization of C57BL/6 mice with OVA and EDN substantially augments anti-OVA IgG1 titers and splenic T cell proliferation and cytokine production upon antigen restimulation ex vivo, all of which is abrogated in *Tlr2*$^{-/-}$ mice. Eosinophils can augment B cell activation in other settings. In a study of WT, eosinophil-deficient dblGATA mice with deletion of the high-affinity GATA binding site in the GATA-1 promoter and dblGATA mice after i.v. infusion of eosinophils, splenic B cell activation and the production of antigen-specific antibodies was dependent on the presence of eosinophils (Wang and Weller, 2008). Thus, by acting as an APC, augmenting DC antigen presentation, and enhancing immunoglobulin production, eosinophils can have important effects on the generation of adaptive immunity.

Two eosinophil-deficient mouse strains have demonstrated a discrepancy in the requirement for eosinophils in antigen-induced pulmonary inflammation. The PHIL mouse (Lee et al., 2004), with the selective expression of diphtheria toxin A under the EPO promoter, is on a C57BL/6 background, whereas the dblGATA mouse (Humbles et al., 2004) is on the Balb/c background. Whereas PHIL mice had reduced antigen-induced AHR in a protocol of OVA-alum sensitization and OVA challenge, dblGATA mice did not. However, when dblGATA mice were crossed to a C57 background, they also had attenuation of antigen-induced AHR, which was reversed by eosinophil infusion (Walsh et al., 2008). Both the PHIL and dblGATA mice on a C57BL/6 background have a failure to recruit $CD4^+$ T cells to the lung with deficits in generation of pulmonary chemokines CCL7, CCL11, and CCL24 and chemokines CCL17 and CCL22, respectively (Jacobsen et al., 2008; Walsh et al., 2008). $CD4^+$ T cell recruitment to the lung is restored either by infusion of eosinophils (Jacobsen et al., 2008) or by i.n. delivery of eotaxin-1 during OVA challenge (Walsh et al., 2008), again demonstrating that eosinophils are not simply terminal effector cells but rather active participants during an adaptive Th2 cell response in mice. Lastly, the Balb/c dblGATA mice had a decrement in peribronchial collagen deposition and airway smooth muscle cell proliferation (Humbles et al., 2004), and both PHIL mice and C57BL/6 dblGATA mice had a decrement in PAS-positive goblet cell metaplasia (Lee et al., 2004; Walsh et al., 2008), suggesting a role for eosinophils in features of airway remodeling.

Many clinical studies have documented a correlation between pulmonary eosinophilia and asthma. Eosinophils are recruited to the lung after segmental allergen challenge of atopic asthmatics. Activated eosinophils are found in the induced sputum, BAL, and mucosal biopsies of patients with asthma, and the degree of peripheral blood and BAL eosinophilia correlates with disease severity. Furthermore, the degree of eosinophilia in mucosal biopsies correlates with inherent airway hyperresponsiveness (reviewed in Bousquet et al., 2000). Increases in sputum eosinophilia can predict the loss of asthma control, and several randomized controlled trials have shown that adjusting inhaled glucocorticoid doses to reduce sputum eosinophils can decrease exacerbations without an increase in total inhaled or oral corticosteroid use (reviewed in Petsky et al., 2007). Although these studies argue for the eosinophil as a marker of disease activity, data supporting a pathobiologic role for eosinophils in the clinical course of human bronchial asthma have been more elusive until recently. Two randomized controlled trials using a monoclonal antibody to IL-5 in patients with severe asthma and persistent eosinophilia despite high doses of corticosteroids have shown a reduction in clinical exacerbations associated with reductions in peripheral blood and sputum eosinophils (Haldar et al., 2009; Nair et al., 2009). Although this confirms a pathobiologic role for eosinophils in this highly selected clinical subgroup of asthma, two prior studies of unselected asthmatic patients demonstrated no benefit (Flood-Page et al., 2007; Leckie et al., 2000), suggesting that eosinophils may play a critical role in a small subset of patients. The possible benefit of anti-IL-5 in other subsets of asthmatics (aspirin-intolerant asthmatics) with eosinophilia remains to be determined. Notably, anti-IL-5 has been shown to diminish the supepithelial deposition of extracellular matrix proteins in mild asthmatics not on inhaled corticosteroids, suggesting that, in parallel with the mouse data, eosinophils play a role in airway remodeling (Flood-Page et al., 2003).

Basophils

Basophils are mature circulating granulocytes recruited to peripheral tissues in the setting of allergic inflammation or helminth infection. In the mouse, circulating basophils have been difficult to assess by routine hematology, and studies have relied on their characteristic ultrastucture with fewer, larger, and more-uniform granules than eosinophils and PMNs. Murine basophils have recently been identified by flow cytometry, on the basis of their expression of CD49b ($DX5^+$) and FcεRI and the absence of Kit, although the routine histology of this cell shows poor granulation (Lee and McGarry, 2007). Nonetheless, the ability to identify this cell has led to an appreciation of new immune functions as a facilitator of Th2 cell development and even as a primary inducer of a Th2 cell response.

Mediator release from basophils can be triggered by both FcεRI-dependent and -independent mechanisms (reviewed in Schroeder and Frederick, 2009). Whereas antigens can induce specific crosslinking of FcεRI to elicit histamine, lipid mediators, and cytokines from human basophils, the HIV-1 glycoprotein (gp) 120 and the *Schistosoma mansoni* egg glycoprotein IPSE-alpha-1 can activate basophils in an IgE-dependent but antigen-independent manner, via nonspecific interactions that crosslink FcεRI. Basophils can also be activated by C5a to degranulate and generate LTC_4 and IL-4, and by IL-33 and PGN to elicit

cytokines and augment IgE-dependent histamine, LTC_4, and cytokine release. Comparable studies of degranulation, eicosanoid formation, and cytokine production from antigen stimulation of IgE sensitized mouse basophils are lacking but cytokine production is apparent in the studies detailed below.

Recent murine studies have highlighted a role for basophils in the effector phase of chronic allergic inflammation in vivo. The subcutaneous administration of TNP-conjugated OVA into the ear of Balb/c or C57BL/6 mice passively sensitized with a prior i.v. injection of TNP-specific IgE leads to a triphasic ear swelling with a dramatic third cellular phase at 48 hr consisting of eosinophil and neutrophil infiltration in the tissue (Mukai et al., 2005). This third phase is abolished in FcRγ-deficient mice that lack FcεRI signaling, but re-established in BM chimeras with a $DX5^+$ basophil-enriched population for reconstitution of irradiated FcRγ-deficient recipients. In a confirmatory study, the same investigators showed that depletion of basophils with the basophil-specific antibody BA103 24 hr prior to passive sensitization and challenge abrogated the third phase, but left the immediate and late-phase responses intact (Obata et al., 2007).

Basophils can also participate in immunoglobulin production. In a study in which mice were sensitized and challenged with APC, PE, or pneumococcal surface protein A (PspA), depletion of basophils prior to the challenge with an antibody to the alpha chain of FcεRI (MAR-1) decreased antigen-specific antibody production in all Ig subgroups and significantly increased sepsis in mice that had been vaccinated to PspA and infected with *S. pneumoniae* (Denzel et al., 2008).

A role for the basophil in facilitating Th2 cell immune responses has recently been uncovered. Mice with a targeted deletion in the gene encoding the interferon regulatory factor 2 (IRF-2) transcription factor exhibit a Th2 cell-polarized phenotype with high serum IgE titers and robust ex vivo IL-4 production by splenic $CD4^+$ T cells. This phenotype is associated with spontaneous expansion of the splenic $Fc\varepsilon RI^+DX5^+Kit^-B220^-$ basophil compartment (Hida et al., 2005). In ex vivo coculture experiments, splenic cell preparations from IRF-2-deficient mice directed OT-II TCR transgenic T cells to produce IL-4. IL-4 production by OT-II cells was diminished with basophil depletion and was abrogated by anti-IL-4, revealing a role for basophils in Th2 cell development. In a unique approach to recognizing basophil function, the $Fc\varepsilon RI^+DX5^+Kit^-$ cell population in liver and bone marrow was expanded by administration of IL-3 to WT mice through a miniosmotic pump (Oh et al., 2007). These basophils could induce naive PCC peptide-stimulated 5C.C7 transgenic $CD4^+$ T cells to produce IL-4 in a dose-dependent fashion, and basophil-mediated skewing was abrogated when *Il4*$^{-/-}$ basophils were used. Further, naive transgenic T cells that were adoptively transferred into *Rag2*$^{-/-}$ mice primed with IL-3, but not untreated controls, responded to challenge with PCC peptide by developing $CD4^+$ IL-4^+ Th2 cells, demonstrating that in vivo Th2 cell development was augmented in an environment with IL-3-expanded basophils (Oh et al., 2007). Another study used the "4get" mice, which contain a bicistronic construct encoding enhanced green fluorescent protein in the *Il4* locus that can be used to track IL-4 competent cells in vivo during the development of Th2 cell immune responses (Sokol et al., 2008). Immunization of these mice with the model protease allergen papain generated $CD4^+DX5^-$IL-4-$eGFP^+$ Th2 cells in the draining lymph node on day 4 that were preceded by TSLP-producing $DX5^+$IL-4-$eGFP^+$ basophils. Th2 cell generation was abrogated with basophil depletion with the MAR-1 antibody or with a neutralizing antibody to TSLP, thus demonstrating that basophil-derived IL-4 and TSLP are critical checkpoints in the development of Th2 cell immunity.

Recent studies have expanded the function of basophils to include antigen presentation. In an in vitro system using TCR Tg DO11.10-"4get" T cells and OVA peptide, the addition of purified bone marrow-derived basophils (BMB) to the culture system in the absence of any other APC produced Th2 differentiated cells, as measured by IL-4-eGFP expression in T cells. This effect was abrogated by antibodies to MHC II and by using basophils from *Il4*$^{-/-}$ mice (Sokol et al., 2009). Th2 cell generation was augmented in the presence of papain, which increases basophil expression of MHC II, IL-4, and TSLP, indicating an innate response of the basophil that augments direct presentation of a soluble antigen. Basophils were able to generate Th2 cell immune responses in vivo in the absence of other contaminating APCs because OVA-pulsed basophils transferred into C57BL/6 WT mice or *Ciita*$^{-/-}$ mice (which do not express MHC II) induced comparable production of IL-4 by antigen-restimulated $CD4^+$ LN T cells ex vivo. In a complementary study, BMB and splenic NTNB $Fc\varepsilon RI^+Kit^-$ basophils primed by *Strongyloides venezuelensis* infection of WT mice were able to act as APCs and generate Th2 cells in the DO11.10-OVA system (Yoshimoto et al., 2009). In a nontransgenic system, WT Balb/c mice sensitized with DNP-OVA- and anti-DNP-IgE-treated basophils and challenged 4 days later with OVA protein had elevated serum OVA-specific IgE and IgG1 titers and increased production of IL-4 and IL-13 by OVA-restimulated splenic $CD4^+$ T cells ex vivo. Conversely, basophil depletion with the MAR-1 antibody prior to i.v. immunization with DNP-OVA/anti-DNP Ag-IgE in WT mice decreased OVA-specific IgG1, IL-4, and IL-13 production from OVA-restimulated splenic $CD4^+$ T cells. A different study using transgenic mice with MHC II expression restricted to $CD11c^+$ cells revealed impaired generation of LN IL-4, IL-5, and IL-13, persistent histologic inflammation, and a higher worm burden after infection with *Trichuris muris* (Perrigoue et al., 2009). Conversely, CD11c-DTR mice with DT-dependent $CD11c^+$ cell depletion had no impairment in Th2 cytokines or worm expulsion, revealing that DCs were neither sufficient nor required for productive helminth immunity. Basophils were identified in the spleen of infected WT mice, and depletion of basophils with the MAR-1 antibody resulted in decreased IL-4 transcript from colonic tissue, increased inflammation, and increased worm burden, suggesting that basophils are critical for Th2 cell-dependent immunity. Taken together, these recent studies in mice reveal that basophils can direct a T cell response to Th2 cell polarization in vitro and in vivo, but there are no data showing direct Ag presentation by these cells in allergic pulmonary inflammation.

Basophils are detected in the lung sections of patients with fatal asthma (Kepley et al., 2001) and in the bronchial biopsies of atopic asthmatics during the late phase after allergen challenge (Macfarlane et al., 2000). Basophils recovered from the BAL of mild asthmatics after segmental allergen challenge produce IL-4 protein assessed by flow cytometry on freshly isolated cells and by ELISA on supernatants from short-term

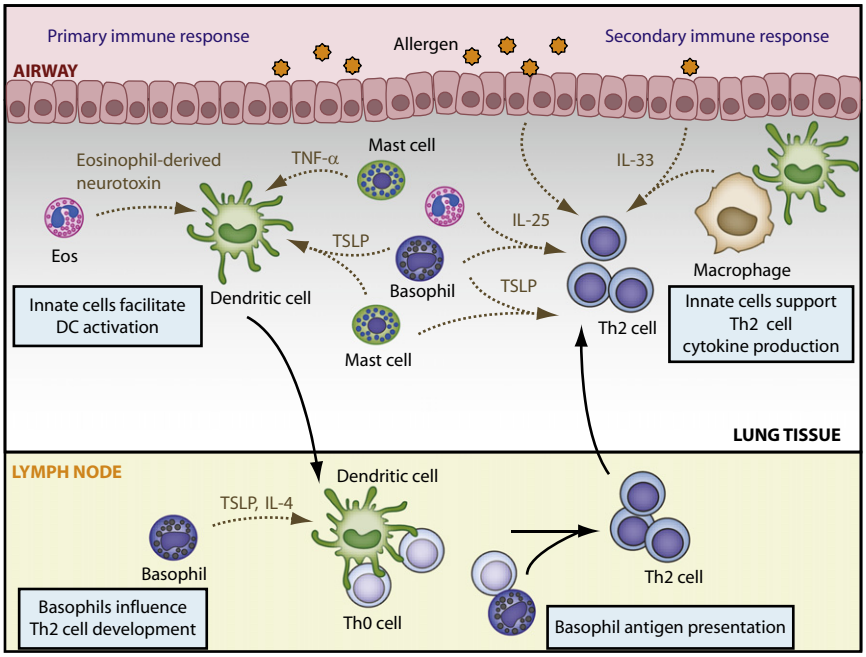

Figure 1. Innate Cells and Their Mediators Modulate the Development of Th2 Cell Immune Responses

Activated eosinophils (Eos) release eosinophil-derived neurotoxin, which acts at DC TLR2 to mature DCs, to generate IL-6, IL-8, IL-12p70, and TNF-α, and to enhance the production of antigen-specific Th2 cell cytokines and serum IgG1. MC-derived TNF-α facilitates DC migration to the lymph node. MCs facilitate DC antigen presentation by taking up antigen and either releasing it in exosomes or apoptosing for subsequent DC phagocytosis and activation. Activated MCs and basophils produce TSLP, which upregulates OX40L on DCs for the subsequent generation of Th2 cell immunity. In the lymph node, basophils direct Th2 cell development with the generation of IL-4 and TSLP. Basophils can present soluble antigen to direct Th2 cell development, which is augmented by the presence of antigen-specific IgE and critical to the generation of Th2 cell immunity during helminth infection. IL-33, IL-25, and TSLP potentiate the proliferation of committed Th2 cells as well as their cytokine production.

culture in medium alone (Schroeder et al., 2001), and IL-4 mRNA and protein colocalizes with basophils in the bronchial mucosa of atopic asthmatics after segmental allergen challenge. Basophil activation, as assessed by CD69 expression, is upregulated in basophils from the peripheral blood of asthmatics as compared to normal controls (Yoshimura et al., 2002). The use of anti-IgE in patients with allergies and atopic asthma results in a rapid decrement in serum IgE and the subsequent gradual reduction of FcεRI on MCs and basophils. In a study of patients with perennial allergic rhinitis, treatment with anti-IgE decreased FcεRI surface expression on human basophils from pretreatment levels of 220,000 receptors per cell to 8,000 receptors per cell and correlated with a 90% reduction in basophil degranulation in response to house dust mite antigen in vitro (MacGlashan et al., 1997). In patients with seasonal allergic rhinitis, free IgE titers were reduced by 96% after 3 days of omalizumab, but a reduction in nasal PD30 response to ragweed challenge was only noted after 14 days of treatment and this was associated with a 70% reduction of FcεRI expression on circulating basophils (Lin et al., 2004). Thus, whereas studies using omalizumab suggest a role for basophils and mast cells in FcεRI-mediated allergic responses, their contributions based on the mouse studies could be cooperative or distinct, with the basophil implicated in facilitating Th2 cell adaptive immune responses by supplying IL-4 and/or TSLP or by acting as an APC for select allergens.

Concluding Remarks

Asthma is a heterogeneous disease with a variable clinical presentation, association with atopy, profile of recruited inflammatory cells, and response to targeted therapies, suggesting involvement of immune pathways beyond the canonical Th2 cell paradigm. An emerging body of evidence from human in vitro studies and mouse models demonstrates that innate stromal and hematopoietic effectors cells can promote $CD4^+$ Th2 cell immune responses by activating DCs, supplying IL-4 and TSLP, acting as APCs, and directly activating committed memory Th2 cells (Figure 1). However, these cells can themselves generate the full repertoire of Th2 cytokines, lipid mediators, eosinophilic, and neutrophilic inflammation that is the hallmark of asthma (Figure 2). Thus, whereas the generation of inflammatory mediators from epithelial cells, iNKT cells, MCs, eosinophils, and basophils has previously been viewed as a mechanism to amplify established inflammation, complex allergens that provide immunogenic epitopes and PAMPs for TLR, CLR, and/or NLR signaling can activate these innate cells to initiate the development of innate and adaptive host immune responses. Our current understanding of these innate responses, even in mice, has substantial gaps. They include the distinction between studies with pure proteins (with or without adjuvants) and those conducted with clinically relevant allergens or viral models of airway inflammation; the contribution of neutrophils, if any, to these innate responses; the role of myeloid DCs and macrophages, which can produce leukotrienes and inflammatory mediators in response to PAMPS, allergens, and chitin (Barrett et al., 2009; Reese et al., 2007; Suram et al., 2006) as effector cell participants in allergic inflammation (Figure 2); and the delineation of which innate pathways are relevant in the absence of T and B cell immunity. It seems reasonable to consider the roles and magnitude of these innate signals in humans with nonatopic asthma, in viral exacerbations of asthma, and in neutrophil-predominant asthma. What is clear is that exposure of the lung to diverse environmental elements can activate many pathways to airway inflammation and that many new potential targets for therapy are emerging from basic research.

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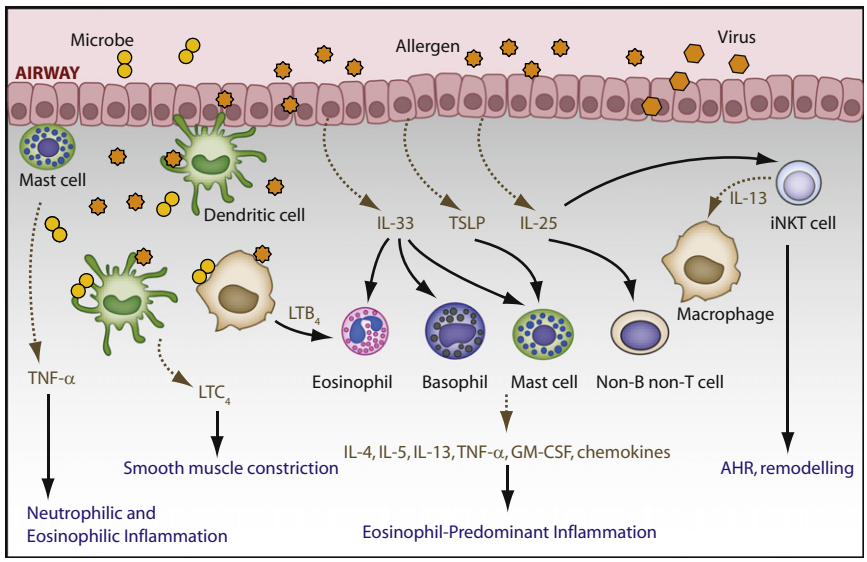

Figure 2. Airway Inflammation, Smooth Muscle Constriction, and Airway Remodeling Generated by Innate Effectors

PAMPs associated with microbes or allergens such as peptidoglycan, zymosan, chitin, and mannose-rich ligands from dust mites and fungi directly elicit arachidonic acid metabolism and inflammatory cytokines from tissue-resident effector cells such as DCs, MCs, and macrophages. Allergens stimulate epithelial cells to secrete IL-33, TSLP, and IL-25. Although TSLP induces MC generation of Th2 cell cytokines, IL-33 acts more broadly on MCs, eosinophils, and basophils to elicit Th2 cell cytokines. IL-25 induces IL-4, IL-5, and IL-13 production from a non-B non-T cell population, and stimulates iNKT cells to generate IL-13 and promote airway hyperreactivity (AHR) and airway remodeling. IL-25 can induce AHR in $Il4^{-/-}Il5^{-/-}Il9^{-/-}Il13^{-/-}$ mice. dsDNA or viral infection induces epithelial cells to produce TSLP, which activates MCs to generate Th2 cell cytokines. iNKT cells, activated by viral infection, generate IL-13 and activate IL-13-producing macrophages, in the absence of $CD4^{+}$ cells, to promote AHR and remodeling.

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