

Natural Killer Cells in the Development of Asthma

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Abstract Asthma is an immune-mediated disease of the airways characterized by reversible airway obstruction, bronchial eosinophilic inflammation, and airway hyperresponsiveness (AHR). The immune dysregulation in asthma has been attributed to the involvement of diverse immune cells that contribute to the immunopathology of the disease. Natural killer (NK) cells play critical roles in host defense against viruses and various cancers. Accumulating evidence demonstrates additional important roles for these cells in T cell priming, dendritic cell maturation, and the development of inflammation, all of which have the potential to enhance or dampen allergic responses. The ability of NK cells to produce Th2-type cytokines and their pivotal role in combating respiratory infections which cause airway dysfunction in asthmatics further suggest that they may directly contribute to the immunopathogenesis of allergic airway disease. In this review, we examine emerging evidence and discuss the putative roles of NK cells in the sensitization, progression, and resolution of asthma.

Keywords Natural killer cells · Asthma · Allergic inflammation · NKG2D · NK2

Introduction

Asthma has emerged as a major public health problem affecting over 300 million individuals worldwide. The increasing incidence of the disease and the urgent need to develop

efficacious therapeutic agents have been a major impetus for asthma research, with the aim of better understanding the mechanisms of disease pathogenesis and progression. The observation that T cells of the Th2 phenotype are a central mediator of asthma pathogenesis was a major breakthrough in the immunological understanding of the disease [1–3]. Subsequent findings elucidating the roles of various immune cells, cytokines, and chemokines have demonstrated that asthma is not merely an IgE-mediated disease as previous paradigms had suggested but requires the contribution of several immune components to achieve the full spectrum of the disease [4–17, 18•, 19, 20]. Furthermore, a number of studies have demonstrated that key cells of innate immunity including natural killer (NK) cells [21, 22•, 23•, 24•], natural killer T (NKT) cells [25–28], $\gamma\delta$ T cells [29], dendritic cells (DCs) [8], and innate lymphoid cells [30–33] play a pivotal role in shaping and influencing the outcome of asthma in both murine experimental models and in human patients. This review will focus on the emerging importance of the role of NK cells in the pathogenesis of asthma and allergic inflammation.

NK Cell Biology and Phenotype

NK cells play a critical role in the natural host defense to infectious pathogens [34, 35, 36•]. They are a distinct class of lymphocytes, which express several markers present on T and B cells, but do not rearrange germ line genes for the T and B cell receptors. As such, they are present in SCID and RAG^{-/-} mice, which cannot undergo VDJ recombination. In appearance, NK cells exhibit a large granulocyte morphology similar to activated cytotoxic T lymphocytes (CTL) and display killing functions dependent on perforin, granzymes, Fas-FasL, and TNF-related apoptosis-inducing ligand (TRAIL) [35]. A distinctive characteristic of NK cells is the presence of diverse surface receptors, which bind polymorphic major

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histocompatibility complex (MHC) class I molecules as well as non-MHC ligands and induce inhibition or stimulation of NK cell activity [37].

Phenotypically, NK cells share several markers with T cells, including CD28, CD11a, and CD69 [38]. Different subsets of NK cells also express B220, Thy1, Mac-1 (CD11b), and CD11c. However, conventional NK cells do not express CD3 and are generally identified as CD3[−] to distinguish them from NKT cells, which express NK and T cell markers. In C57BL/6 (B6) strains of mice, NK cells may be specifically identified based on the expression of NK1.1 (the NKR-P1C molecule) and the absence of CD3. In other strains of mice, they may be detected using a combination of surface antigens including DX5, 2B4, and CD94. In humans, NK cells express CD56, a marker present on all NK cells and some T cells, and can be divided into CD56^{bright} and dim subsets, which correlate with differing aspects of NK cell function [39]. The bright subset expresses low levels of CD16, has low cytotoxic activity, and has high cytokine secretion capacity, whereas the dim subset expresses high levels of CD16, has low cytokine secretion capacity, and has high cytotoxic activity. While the majority of human NK cells are CD56^{dim}, approximately 10 % are CD56^{bright} and can produce cytokines such as IFN- γ , TNF- β , GM-CSF, IL-10, and IL-13. More recently, NK cell subsets correlated to corresponding T cell subsets, such as NK1, NK2, and NKreg, have also been described [40, 41]. Finally, most NK cells express CD16, which induces antibody-mediated cellular cytotoxicity (ADCC).

NK Cell Development and Function

The development and maturation of NK cells is critically dependent on the bone marrow microenvironment and the production of cytokines such as stem cell factor (c-kit ligand), IL-7, flt-3 ligand, and IL-15 by bone marrow stromal cells [40]. IL-15, in particular, is critical for NK development, as seen by the complete absence of NK cells in mice lacking IL-15 or its receptor IL-15R α [42]. Mature NK cells migrate to the blood or become resident within tissues. In humans and mice, they constitute a minority of lymphoid populations, present as 5–10 % of peripheral blood lymphocytes in humans and about 3 % of splenocytes in mice. Higher numbers of NK cells have been found in the lung and liver, and they have also been observed in the draining lymph nodes, where they are able to interact with DCs and activated lymphocytes. Peripheral mature NK cells are quiescent and undergo low levels of proliferation [43]. They have a half-life of about 7–10 days based on results from adoptive transfer experiments [44–46]. The survival of mature NK cells in the periphery is dependent on IL-15 [44, 45, 47].

During infection or injury, NK cells are rapidly mobilized and carry out various effector functions including cellular cytotoxicity and the release of cytokines and chemokines, which affect neighboring cells [34, 48]. Individual NK cells express a wide array of inhibitory and activating receptors, which bind MHC class I and non-MHC ligands on target cells, and regulate their function [49, 50]. Activating receptors include the natural cytotoxicity receptors such as NKp46 [51], the Fc receptor CD16, and NKG2D. NKG2D, particularly, is a unique receptor that binds stress-induced ligands and is also expressed by other cell types, including CD8 T cells, $\gamma\delta$ T cells, NKT cells, and activated macrophages [52, 53]. The inhibitory receptors bind several MHC class I molecules and include C-type lectin-like homodimeric type II transmembrane proteins, such as the Ly49 molecules in mice, and the killer immunoglobulin-like receptors of the immunoglobulin (Ig) superfamily present in humans. The prototypic Ly49 receptor, Ly49A, is an inhibitory receptor that is expressed by specific subsets of NK cells and binds the ligand H-2Dd in BALB/c mice. The expression of inhibitory receptors on various NK cell subsets is stochastic, and they are specific for at least one of the six or fewer MHC alleles possessed by an individual, resulting in the protection of MHC class I-expressing endogenous cells, from NK cells having optimum inhibitory receptor expression. When MHC I is downregulated during inflammation, activating receptors are engaged due to the upregulation of various ligands, resulting in NK cell activation [54, 55].

Activation of NK Cells

NK cells are among the first cells to be activated early in the immune response [56, 40]. Triggers for NK cell activation include IFN- α/β produced by virally infected cells, double-stranded RNA, IL-12 and IL-18 [40], as well as contact with mature DCs that have been exposed to antigen and appropriate stimuli [57–61]. Activation results in the production of effector cytokines such as IFN- γ and TNF- α and the initiation of cellular cytotoxicity. Furthermore, activated NK cells can also reciprocally induce DC maturation and lyse immature DCs present in the immediate microenvironment, suggesting that NK cell cytotoxicity may play an important role during T cell priming and the development of adaptive immunity.

NK Cells and Asthma

The ability of NK cells to respond early to infectious stimuli or exogenous antigens and interact with diverse cell types including macrophages and DCs makes these cells ideal candidates for influencing T cell responses. As such, they have been shown to regulate the outcome of T cell responses in many

inflammatory diseases including asthma [36•, 48, 55, 62–65, 66••]. Early indications that NK cells may affect asthma came from studies that correlated the activities of NK cells with the allergic phenotype in healthy and asthmatic subjects of various backgrounds [67–69]. These studies showed that patients with asthma had significantly higher numbers of NK cells in the peripheral blood and higher MHC-unrestricted cytotoxicity as compared with healthy controls. While some studies also showed that NK cell cytotoxicity decreased after allergen challenge, other studies indicated that bronchial challenge specifically increased it [70]. Further studies demonstrated that NK cells were selectively increased in the peripheral blood of asthmatic children, with certain subsets of NK cells that lack ICAM-1 being specifically elevated [71]. Similarly, elevated numbers of abnormally activated NK cells were also observed in a patient who presented with a hypereosinophilic syndrome [72]. The NK cells of children with asthma did not express early activation antigens such as CD69; however, its expression was upregulated on *in vitro* stimulation [73]. Lastly, other studies that show a potential role for NK cells in asthma include observations that NK cell levels and cytotoxicity may be correlated with serum IgE in healthy patients [74] and that administration of specific immunotherapy to patients with asthma also decreased NK cell activity [75]. Collectively, these data argue that NK cell activation, trafficking, and cytotoxicity may all contribute to the development of the allergic phenotype [76, 77].

The first evidence for a role for NK cells in an animal model of asthma was demonstrated by Korsgren et al. [21]. In this study, investigators specifically depleted NK and NKT cells using anti-NK1.1 (clone PK136) antibodies, prior to immunization with soluble ovalbumin (OVA) and alum. Induction of the challenge phase with aerosolized OVA in depleted animals resulted in an inhibition of lung tissue eosinophilia as measured by histochemistry; decrease of the Th1 and Th2 cytokines IL-12, IL-4, and IL-5; decrease of the systemic antibodies OVA-specific IgE and IgG2a; and systemic decrease of splenic IL-4. Interestingly, inhibition of asthma in this model was observed only when NK cells were depleted prior to immunization and not before challenge with OVA, suggesting that NK cells were critical during the initial priming phase and the activation of T cells rather than the subsequent acute phase of the disease. In contrast, Ple et al. demonstrated using anti-asialo GM1 antibodies (which depletes NK cells and subsets of T cells) that NK cells were recruited to the draining lymph nodes of allergic mice, where they expressed CD86 and were required during the acute inflammatory phase [23••]. While these studies demonstrate an important proinflammatory role for NK cells in asthma, the specific contributions of NK cells cannot be accurately delineated due to off-target effects (on NKT cells and subsets of T cells) of the depleting antibodies used. Furthermore, recent evidence demonstrating the critical role of NKT cells in

allergic inflammation [25, 78] highlights the necessity for more specific murine models of NK cell deficiency to address NK cell involvement. Neither NK cell depletion nor models of NKT cell deficiencies are sufficient in themselves.

To circumvent this limitation, Mathias et al. set out to demonstrate the role of NK cells using NK-deficient (NKD) mice, which are deficient in NK cells in peripheral organs such as the lung but have normal numbers of NKT cells [22••]. Induction of allergic disease in these animals resulted in a significant attenuation of lung inflammation and eosinophilia, as well as the decreased production of the Th2 cytokines IL-4, IL-5, and IL-13. Furthermore, in corroboration of the results observed by Ple et al., higher numbers of NK cells were observed in the lung tissue of wild-type mice after allergen challenge, suggesting that NK cells are recruited to the inflammatory site during the acute phase [22••]. Finally, using adoptive transfer of allergen-sensitized T cells into RAG^{-/-} recipients, which lack of T and B cells but not NK cells, they confirmed that NK cells are critical during the priming stage of the allergic response, as was initially observed in the Korsgren study [22••]. One limitation of this study is that the NK cell deficiency was achieved as a result of transgenic expression of Ly49A, an inhibitory receptor also present on T cell subsets [79]. However, B6 mice do not express the cognate ligand H-2Dd for this receptor, which is present in BALB/c mice. The importance of NK cells in this model (and also to rule out interference due to Ly49A expression) was further confirmed by supporting observations which demonstrated that depletion of Ly49A-, D-, and G-expressing cells in this model also resulted in attenuation of the allergic phenotype similar to that observed in NKD mice, suggesting that depletion of only a few subsets of NK cells can regulate the lung inflammatory response in allergic mice [22••].

Attenuation of eosinophilia and Th2 responses suggest an important proinflammatory role for NK cells; however, their function and effector mechanisms need to be clearly identified. The above data argue that NK cells may be important during both the sensitization and allergen challenge phases of the asthmatic response. NK cells may influence the initial activation of T cells by a variety of means. Bogen et al. showed that the earliest detectable response to subcutaneous administration of OVA with alum was the presence of IFN- γ -producing NK cells at the site of immunization [80]. Thus, NK cells may be mobilized immediately after immunization and induced to produce different cytokines that can affect the immune environment. An analysis by Mathias et al. of phenotypic changes in NK cell markers after sensitization with OVA and alum did not yield any significant differences [22••]. Interestingly, however, they observed that immunization with OVA and alum resulted in the immediate activation of NKT cells in both wild-type and NKD mice, as was observed by the expression of CD69 and CD25, suggesting that these cells may also influence the early stages of immune sensitization.

Activated NKT cells may further regulate the activation of NK cells as has been previously shown [81]. Mathias et al. further demonstrated that while the numbers of splenic and lung DCs in naïve NKD mice is normal, they are significantly attenuated during acute challenge with allergen [22•]. This suggests that NK cells can regulate the numbers of DCs available for antigen presentation during allergen sensitization and/or challenge, thus impacting the T cell response. Interestingly, while the numbers of splenic and lung DCs were reduced, their functionality was not [22•]. The DCs in NKD mice expressed equivalent levels of costimulatory molecules and normally induced the activation of allogeneic T cells, suggesting that in the absence of NK cells, the magnitude of antigen presentation is affected, but not the quality. Hence, NK cell activation during sensitization, either by cytokines produced by macrophages and DCs or directly by NKT cells that have been activated by DCs, may result in further activation or lysis of DC populations, ensuring that sufficient numbers of DCs are present and mature for antigen presentation to occur. Lastly, NK cells can also be induced to produce chemokines such as MIP-1 α or cytokines like IL-10 that can regulate the immune environment during allergic sensitization [82].

During the challenge phase, NK cells may potentiate the allergic response by further inducing maturation of DCs in the lung or producing Th2 cytokines such as IL-5 and IL-13. NK cells have been shown to produce both IL-5 and IL-13 [83–85], and the ability of NK cells to regulate eosinophilia *in vivo* by IL-5 production has been shown in a mouse model of allergic peritonitis [86]. NK cells also have the capacity to differentiate into NK1 and NK2 clones, which have the ability to produce cytokines corresponding to Th1 (IFN- γ) and Th2 (IL-4, IL-5, and IL-13) subsets, thus having the potential to regulate the outcome of the T cell response [87, 88, 41]. As such, Wei et al. demonstrated the presence of IL-4-producing NK2 cell subsets in asthmatics [89], and Wingett et al. showed that the enhancement of CD40L expression on allergic T cells is associated with an NK2 cell subset that expresses CD86 [90]. Similarly, Kaiko et al. showed that NK cell deficiency during respiratory syncytial virus (RSV) infection resulted in subsequent Th2-mediated allergic responses due to the lack of the regulatory effect of IFN- γ produced by NK cells [91]. Also, Scordamaglia et al. demonstrated that patients with allergic rhinitis and intermittent asthma had deficiencies in a NK cell subset that could produce IFN- γ and induce the maturation of DCs [92]. Interestingly, Wei et al. also demonstrated that clinical therapy reversed the NK2 phenotype in asthmatics to a protective IFN- γ -producing NK1 subset [89]. Finally, NK cells may also be triggered during allergen challenge by IgE antibodies and interaction with mast cells. Arase et al. demonstrated that IgE is able to bind to CD16 on NK cells and induce their activation [93], while Vosskuhl

et al. demonstrated a similar role for LPS-activated mast cells [94].

The activation of NK cells during immune responses is stringently controlled by the balance of activation and inhibition signals received via various receptors. Activating and inhibitory receptors have been associated with a number of NK cell functions *in vitro* and *in vivo* [37, 52, 54], especially NK cell cytotoxicity. Additionally, they have been found to uniquely affect the maturation and enhancement of DC function in *in vitro* studies and models of viral infection [95]. Farhadi et al. recently demonstrated that the proinflammatory role of NK cells in house dust mite (HDM)-induced asthma is mediated by NKG2D [24•]. NKG2D-deficient mice were resistant to the induction of allergic inflammation and exhibited attenuated eosinophilia, fewer airway Th2 cells, and no rise in serum IgE, despite repeated allergen exposure. However, they showed no alterations in responses to RSV. These data therefore suggest that the function of NK cells during asthma is regulated by NKG2D and possibly other activation receptors, which may contribute to the cytotoxic functions and/or cytokine secretion capacity of NK cells, in the context of the allergic response. The allergic phenotype in NKG2D-deficient mice was restored when wild-type but not granzyme B-deficient NK cells were adoptively transferred, suggesting that the NKG2D-mediated effect is dependent on granzyme B-mediated cytotoxicity. In contrast, Mathias et al. demonstrated that perforin-mediated cytotoxicity was not essential for NK cell function in their model of asthma, as perforin-deficient mice developed eosinophilia and Th2 responses at comparable levels with wild-type animals [22•]. Taken together, these data suggest that activation of NK cells by NKG2D and subsequent NK cell-mediated cytotoxicity are critical for the observed effects of NK cells during asthma. However, since both perforin and granzyme B are required for the cytotoxic function of NK cells, it is possible that in the absence of perforin, granzyme B can enter target cells in a perforin-independent manner, using perhaps granulysin or another candidate molecule. Similarly, other mechanisms of NK cell cytotoxicity may include FasL and TRAIL-mediated killing. In summary, the findings demonstrated by Farhadi et al. confirm early observations reported in studies of human patients with asthma, which suggest a significant link between NK cell cytotoxicity and allergic inflammation [67, 70, 68, 74].

Lastly, Haworth et al. demonstrated that NK cells and the expression of NKG2D are also required for the resolution of allergic inflammation [96•]. Increased numbers of NK cells were present in the lungs and lymph nodes of wild-type mice after the cessation of allergic responses, and depletion of NK cells or blocking of NKG2D resulted in delayed clearance of airway eosinophils and antigen-specific CD4 T cells. Moreover, these NK cells expressed the receptor for resolvin E1, an endogenous proresolving

mediator for allergic airway responses. These data therefore suggest that NK cells are involved not only in the pathogenesis of asthma but also in the resolution of disease once effector responses subside.

Conclusion

In light of the data described above, the role for NK cells during allergic responses may be summarized as follows (Fig. 1): The first step in the sequence of events leading to asthma occurs at the site of sensitization. At this stage, NK cells may be activated either by cytokines produced by cells such as macrophages and DCs or directly by activated NKT cells. Activated NK cells now have the potential to directly interact with DCs and induce their maturation. NK-DC interaction at this stage may also affect the expansion of mature DCs and consequently the magnitude of antigen presentation. These interactions likely involve the engagement of activating receptors, cytotoxic mechanisms, as well as the production of cytokines such as IFN- γ and TNF- α . Furthermore, the

production of NK cell cytokines at this stage may also play a role in inducing the T helper cell phenotype and upregulating CD40L expression, although this needs to be investigated in further detail. During the acute allergic response, activated NK cells may continue to aid antigen presentation by inducing the maturation of DCs in the lung interstitium and regulating their proliferation and function. This also likely involves the engagement of activating receptors and cytotoxic mechanisms. Additionally, NK2 subsets may contribute to Th2 responses by producing cytokines such as IL-3, IL-5, or IL-13. Finally, NK cell migration to the airways and activity in response to resolvins E1 may aid in the resolution of allergic inflammation and the healing process, likely dependent on engagement of activation receptors and due to the cytotoxicity of inflammatory cells.

In summary, the studies described above suggest that NK cells can play critical proinflammatory and immunomodulatory roles during the development of allergic inflammation. The specific roles of NK cells at these various stages, and especially in the context of virus-induced airway dysfunction, need to be further investigated. Furthermore, the interactions

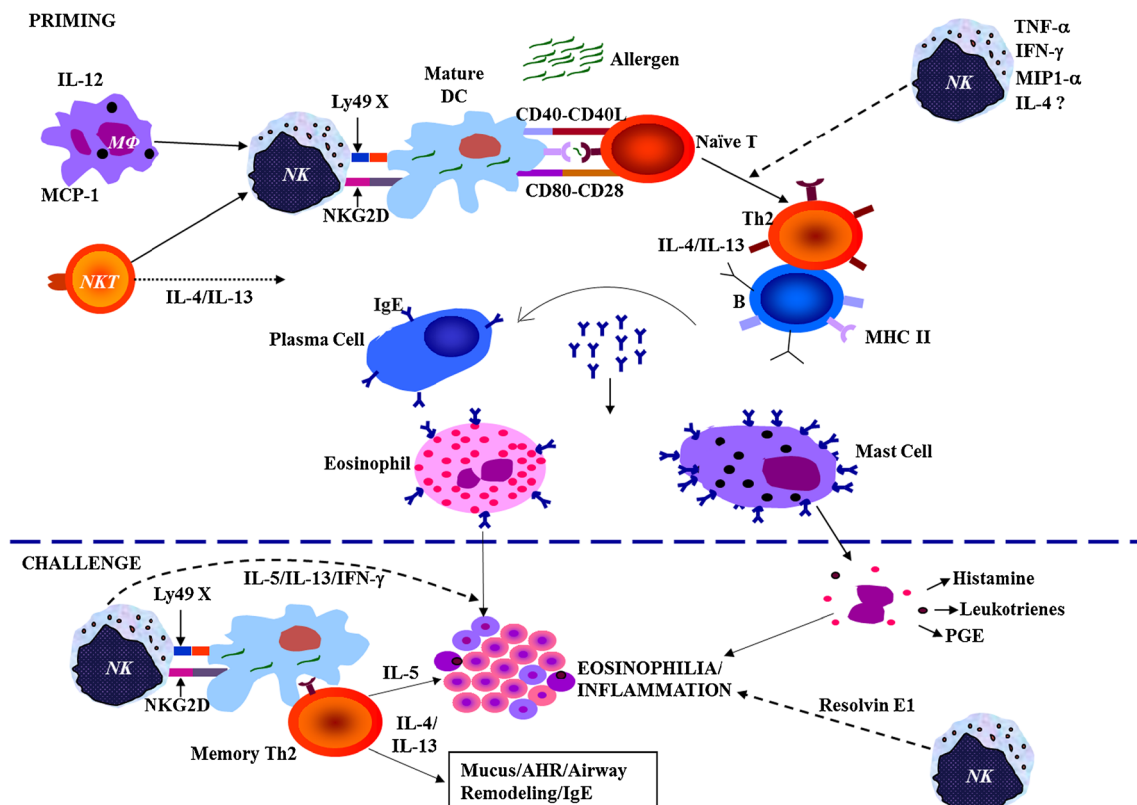


Fig. 1 Proposed roles for NK cells during allergic responses. During allergic sensitization, activated NK cells may regulate the extent of DC maturation and thus the magnitude of antigen presentation to naïve CD4 T cells. The production of inflammatory cytokines by NK cells can subsequently affect the development of allergen-specific Th2 cells by modulating the immune microenvironment. While cytokines such as IFN- γ produced by NK1 cells may potentially inhibit the development

of Th2 cells, whether NK2 cells can produce cytokines such as IL-4, which may enhance Th2 development, remains to be shown. During allergen challenge, activated NK cells may further potentiate the response by producing cytokines such as IL-5, IL-13, and IFN- γ , which can enhance or dampen airway inflammation. Finally, NK cell cytotoxic activity can aid in the resolution of allergic inflammation by clearance of eosinophils and allergen-specific Th2 cells

of NK cells with other immune cells as well as the mucosal epithelia during the course of the allergic response need to be elucidated. These studies will hopefully not only shed further insight into the mechanisms by which NK cells regulate the airway inflammatory response during asthma but also enhance our overall understanding of allergic inflammation and provide novel approaches to developing therapeutic strategies.

Compliance with Ethics Guidelines

Conflict of Interest Clinton B. Mathias declares no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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