

Atopic NC/Nga Mice as a Model for Allergic Asthma: Cytokine Profiles and Eosinophil Productivity of Bone Marrow

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ABSTRACT. In previous study, NC/Nga mice with experimentally induced asthma showed severe eosinophilia. To explore the mechanism, profiles of representative cytokines interleukin (IL)-4, IL-5, and interferon (IFN)- γ were examined in bronchoalveolar lavage fluid. The level of only IFN- γ was lower in NC/Nga mice than control BALB/c mice. Furthermore, bone marrow cell culture system under the presence of eosinopoietic cytokines, which induce the differentiation of progenitor cells into mature eosinophils, showed that a larger number of eosinophils differentiated from NC/Nga mice derived bone marrow cells than from control BALB/c mice. These results may imply the possibility that severe eosinophilia in the NC/Nga mice are attributable to lower production of IFN- γ and higher eosinophil productivity of bone marrow cells.

KEY WORDS: asthma, eosinophil, NC/Nga mouse.

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Recently increasing evidence has suggested the potency of NC/Nga mice as models of human allergic diseases like atopic dermatitis, and bronchial asthma [14, 17, 18, 29]. In these models the NC/Nga mice showed severe eosinophilic inflammation and marked elevation of allergen specific IgE titer in plasma, which are known as common and major symptoms in allergic diseases. Although all these symptoms are intense in the NC/Nga mice than other mice strains in the asthmatic model, eosinophilic inflammation is remarkable because it is not only severe in scale, but also persistent [14].

Eosinophilic inflammation is considered to play a central role in allergic diseases because it correlates well with the severity of symptoms and its localization is accompanied by tissue destruction [27], and eosinophil granule proteins such as major basic protein and eosinophil cationic protein are known to damage the tissue structure and functions [7, 10, 20]. Such inflammatory reactions are mainly driven by Th2 cytokines, including interleukin (IL)-4, and IL-5 [15, 16, 23], while Th1 cytokines such as interferon (IFN)- γ compete to the function or secretion of Th2 cytokines [5, 6, 8], thus these cytokines constitute complex net works.

In an asthmatic model, we found that eosinophilic inflammation was accompanied by severe and persistent blood eosinophilia. In both the NC/Nga mice and control BALB/c mice the levels of blood eosinophils increased 6 hr after the challenge. However, they dropped to normal in control mice while high levels were maintained in the NC/Nga mice for more than 48 hr, suggesting the existence of an eosinophil progenitor supply in the bone marrow pool where differentiation of blood cells occurs.

To explore the cause for the severe inflammatory reaction in the NC/Nga mice, we examined the cytokine response in bronchoalveolar lavage fluid (BAL) after the antigen challenge. Furthermore, on the assumption that bone marrow from the NC/Nga mice has larger eosinophil productivity and that it may account for the severe and persistent eosinophilia in this mouse strain, we performed *in vitro* cultures of bone marrow cells to induce eosinophil differentiation.

Cytokine profiles in BAL fluid during the asthmatic response was determined as follows. Specific pathogen-free NC/NgaTnd mice, and control BALB/c mice were obtained from Charles River Japan Inc. (Kanagawa, Japan) and sensitized with peritoneal injection of saline containing 100 μ g of ovalbumin (OVA ;Sigma Chemical Co., St. Louis, MO) adsorbed in 1.6 mg of alum, 1 and 2 weeks before challenge. OVA-immunized and nonimmunized control mice were challenged by an intranasal administration of 50 μ l of saline containing 10 μ g of OVA under pentobarbital sodium anesthesia. At various time points following the antigen challenge a cannula was inserted into the trachea and BAL fluid was collected by gently washing with 500 μ l of PBS for four times. Levels of IL-4, IL-5, and IFN- γ in the supernatant of BAL fluid were determined by a quantitative sandwich ELISA kit (Amersham Backinghamshire, England).

In many studies, the involvement and importance of Th2 cytokines, especially IL-4 and IL-5, in pathogenesis of asthma was discussed [3, 9, 22]. IL-4 is a primary determinant of Th2 cell differentiation and contributes to tissue recruitment of eosinophils [26]. IL-5 is well characterized as a differentiation factor of eosinophils [4, 25, 32], and tracheal application of this cytokine to guinea pig was shown to induce eosinophilic inflammation in lung [17]. In spite of the marked difference in the airway inflammation, which was shown in our previous study [14], we found no significant difference in their levels in BAL fluid between both

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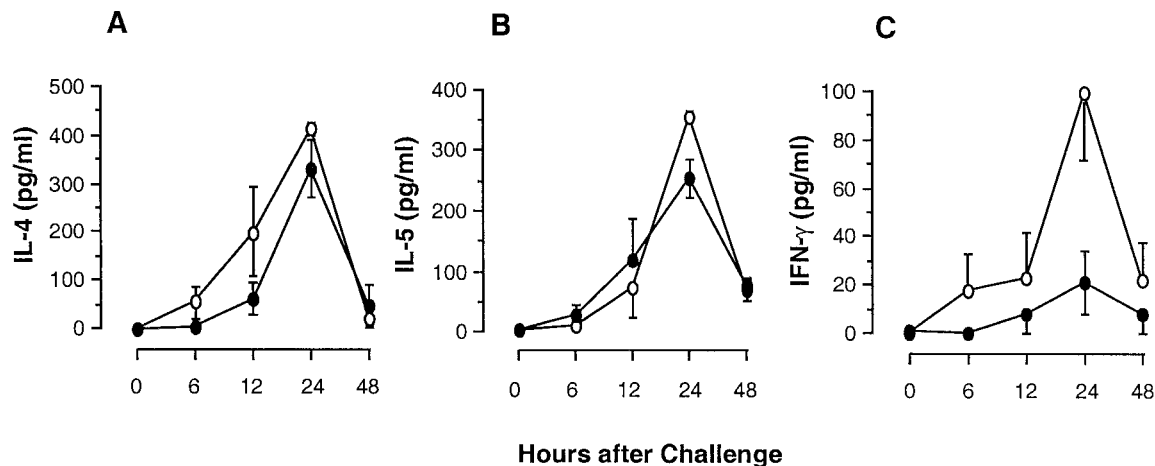


Fig. 1. Protein levels of IL-4 (A), IL-5 (B), and IFN- γ (C) in BAL fluid. Supernatants of BAL fluid were collected from BALB/c mice (open) and NC/Nga mice (closed) at various hours after the challenge, and contents of cytokines were measured by an ELISA. Each point represents the mean $\pm$ SE of three to five separate experiments.

NC/Nga mice and BALB/c mice (Fig. 1); both were rapidly increased after the challenge and peaked at 24 hr in both mice strains in comparable fashion. In contrast, BAL fluids from BALB/c mice contained significantly high levels of IFN- γ than NC/Nga mice, 24 hr after challenge (Fig. 1). Taking into account the reports that defective production in IFN- γ leads to Th2 inflammatory responses in the airways of mice and patients with asthma [6, 24], IFN- γ inhibits differentiation of eosinophils *in vitro* culture system [21], and that B cells in NC/Nga mice have high sensitivity to IL-4 [19], we speculate that low production of IFN- γ and high responsiveness to IL-4 and IL-5 may cause the severe eosinophilic inflammation in NC/Nga mice following the OVA challenge.

To elucidate further the mechanism of severe blood eosinophilia in the NC/Nga mice, we assumed the high eosinophil productivity of bone marrow, and performed the bone marrow cell culture systems, which induce differentiation of eosinophils [28]. Bone marrow cells were aspirated from femur and mononuclear cells were separated by density gradient centrifugation over 65% Percoll for 30 min at 5 g. The cells at the interface were collected and washed with RPMI1640 medium, then incubated for 3 hr in plastic flask at 37°C and 5% CO₂ to discard adherent cells. Mononuclear non-adherent cells were cultured in microassay 24-well plates, at the indicated density for 7 days. The culture medium was made up of 30% heat-inactivated fetal bovine serum, and eosinopoietic cytokines. The supplemented cytokines consisted of recombinant murine IL-5 (SIGMA, St. Louis, MO) only, which support the differentiation and maturation of the eosinophil precursor cells [4, 25, 32], to estimate the ability of eosinophil productivity of bone marrow from non-sensitized mice, or cocktail of recombinant murine IL-3, and GM-CSF (Genzyme, Boston, MA) in addition to IL-5, which stimulate the differentiation of progenitor cells into mature eosinophils. Seven days after the

initiation of incubation, the cells were counted with a hemocytometer and were differentially counted from cytopin preparations stained with Mayer's hematoxylin and Congo-red that stains eosinophilic granules.

In asthmatic patients and experimental animal models it has been reported that bone marrow cells from antigen challenged patients or experimental animals produced increased eosinophil colonies in cultures with IL-5 [11, 13, 31]. Others have shown the existence of IL-3, GM-CSF and IL-5 in the blood of asthmatic patients [30], and the increase in T cell numbers, which produce GM-CSF and IL-5, after the antigen challenge [2]. These reports imply that the bone marrow is stimulated to produce eosinophil progenitors by eosinopoietic cytokines during the asthmatic response. In the bone marrow cell culture, which were supplemented only with recombinant murine IL-5 and known to support the differentiation and maturation of the eosinophil precursor cells, we found no significant difference in number of eosinophils between the two mice strains (Fig. 2A). This suggests that under non-sensitized condition, eosinophil precursor cells in bone marrow of both NC/Nga mice and BALB/c mice have comparable abilities to produce the same number of mature eosinophils. In contrast, when we cultured the bone marrow cells in cocktail with IL-3 and GM-CSF, in addition to IL-5, which induce progenitor cells differentiation into mature eosinophils, significant differences in eosinophil numbers between two mice strains were observed as the IL-5 concentration increased (Fig. 2B). Although we did not determine the levels of IL-3 and GM-CSF in BAL fluid of both mice, based on the current findings, there is a possibility that NC/Nga mice either have high levels of the two cytokines or that the bone marrow cells from this mouse strain have a high sensitivity to stimulation by IL-3 and GM-CSF. A report that patients undergoing bone marrow transplants also contracted asthmatic disease [1] raised the possibility that part of the predisposi-

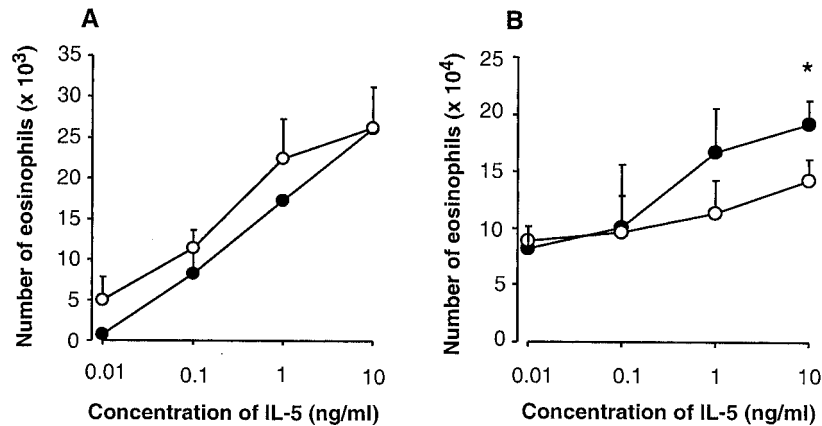


Fig. 2. Number of eosinophils in bone marrow cells cultured in the presence of IL-5 (A), or the cocktail of IL-3, GM-CSF and IL-5 (B). Femur bone marrow cells from BALB/c (open) and NC/Nga (closed) mice were cultured in the presence of indicated concentrations of IL-5 with or without IL-3 (1 ng/ml), and GM-CSF (1 ng/ml) for 7 days at the density of 5×10^4 /ml (A) or 5×10^5 /ml (B). Each point represents the mean $\pm$ SE of four separate experiments. * $p < 0.05$, when compared with BALB/c mice; statistical significance was determined by Student's *t* test.

tion to allergic inflammation may be due to a character of bone marrow cells. This character may explain, at least in part, the severe and persistent eosinophilia in the NC/Nga mice in experimentally induced allergy.

In conclusion, we found lower expression levels of IFN- γ in the NC/Nga mice than control BALB/c mice in the course of asthmatic inflammation, which may contribute to Th2 response regularly observed in asthmatic patients [12]. We further have shown that the bone marrow cells from the NC/Nga mice produced larger amount of eosinophils under the stimulation of IL-3, GM-CSF, and IL-5 *in vitro*, but not under the stimulation of IL-5 only. This result imply that bone marrow cells in NC/Nga mice may be stimulated by IL-3, GM-CSF and IL-5 and produce larger number of eosinophils in the course of airway inflammation after the antigen challenge than BALB/c mice. The characteristic features of experimentally induced allergy in NC/Nga mice observed in this study were consistent with some clinical features of asthma, therefore putting all the facts together, we suggest that NC/Nga mice could be a suitable model to study the pathogenesis of asthma.

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