Eosinophils have long been recognized as terminal effector cells. However, recent reports have suggested that eosinophils might participate in initial stages of allergy development by modulating T-cell responses.

Moreover, pulmonary eosinophils are required for the localized recruitment of T cells. High-mobility group box 1 protein (HMGB1) is a non-histone DNA-binding nuclear protein that acts as an endogenous ‘Danger signal’ and triggers innate immune reactions. Previously, we reported that HMGB1 played a key role in the pathogenesis of asthma characterized by eosinophilic airway inflammation. This study was conducted to test our hypothesis that eosinophils could modulate T cell responses via HMGB1 in the pathogenesis of asthma characterized by eosinophilic airway inflammation. We performed in vitro experiments using eosinophils, dendritic cells (DCs), and CD4+ T cells obtained from a murine model of asthma. The supernatant of the eosinophil culture was found to significantly increase the levels of interleukin (IL)-4 and IL-5 in the supernatant of CD4+ T cells co-cultured with DCs. HMGB1 levels increased in the supernatant of the eosinophil culture stimulated with IL-5. Anti-HMGB1 antibodies significantly attenuated increases of IL-4 and IL-5 levels in the supernatant of CD4+ T cells co-cultured with DCs that were induced by the supernatant of the eosinophil culture. In addition, anti-HMGB1 antibodies significantly attenuated the expressions of activation markers (CD44 and CD69) on CD4+ T cells. Our data suggest that eosinophils modulate CD4+ T cell responses via HMGB1 in the pathogenesis of asthma.

Key Words: Asthma; eosinophil; high-mobility group box 1 protein (HMGB1); T cell
Evaluating the effect of eosinophils on CD4+ T cell functions

First, the levels of IL-4 and IL-5 in the supernatant of CD4+ T cells co-cultured with DCs were measured after their incubation with the supernatant (50 μL) of the eosinophil culture which was obtained after incubation with IL-5 (an eosinophil activator, 10 ng/mL) for 12 hours. Undetectable IL-5 in the supernatant of the eosinophil culture was confirmed before the supernatant was added to the co-culture of CD4+ T cells and DCs. Therefore, it is unlikely that IL-5 in the supernatant of the eosinophil culture affected IL-5 measurements in the supernatant of CD4+ T cells co-cultured with DCs. We evaluated whether the levels of HMGB1 increased in the supernatant of the eosinophil culture after stimulation with IL-5. We then measured the levels of IL-4 and IL-5 in the supernatant of CD4+ T cells co-cultured with DCs. Finally, we evaluated the expression of CD44 and CD69 on CD4+ T cells after incubation with the supernatant (50 μL) of the eosinophil culture alone or plus anti-HMGB1 antibodies (2 μg/mL). CD44 and CD69 were reported to be T cell activation markers. Levels of cytokine and HMGB1 in the culture supernatants were determined by ELISA. Expressions of marker were detected by flow cytometry using Cell Quest software (BD, Mountain View, CA, USA).

Statistical analysis

Results were assessed using paired two-tailed tests and expressed as means ± SEMs. A P values of <0.05 was considered as statistically significant.

RESULTS

The levels of IL-4 and IL-5 in the supernatant of CD4+ T cells co-cultured with DCs significantly increased after incubation with the supernatant of the eosinophil culture (Fig. 1). We then observed that HMGB1 levels were significantly elevated in the supernatant of the eosinophil culture stimulated with IL-5 (Fig. 2). Increases in IL-4 and IL-5 levels in the supernatant of CD4+ T cells co-cultured with DCs and CD44 and CD69 expressions on CD4+ T cells after incubation with the supernatant of the eosinophil culture were significantly attenuated when anti-HMGB1 antibodies were added (Fig. 3 and 4).

DISCUSSION

The present study was conducted to test our hypothesis that eosinophils could modulate T cell responses via HMGB1 in the pathogenesis of asthma. We performed in vitro experiments using eosinophils, dendritic cells (DCs), and CD4+ T cells obtained from a murine model of asthma. Our results revealed that the supernatant of the eosinophil culture significantly increased the levels of IL-4 and IL-5 in the supernatant of CD4+ T cells co-cultured with DCs. In our study, HMGB1 levels increased in the supernatant of the eosinophil culture stimulated with IL-5, suggesting that HMGB1 might be secreted from the activated eosinophil. Our results also demonstrated that anti-HMGB1 antibodies significantly attenuated the increases of IL-4 and IL-5 levels in the supernatant of CD4+ T cells co-cultured with DCs that were induced by the supernatant of the eosinophil culture. HMGB1 antibodies also significantly reduced the expressions of activation markers (CD44 and CD69) on CD4+ T cell. It was reported that intratracheal transfer of eosinophil into IL-5 null mice exposed to antigen resulted in the restoration of asthma phenotypes, strongly suggesting CD4+ T cell...
mediated inflammatory signals as well as signals derived from eosinophils cooperatively contributed to the development of asthma. Taken together, it is possible that HMGB1 may be one of the important mediators released from eosinophils.

Previous studies reported that DC-conditioned medium containing HMGB1 polarized CD4+ T cells toward the Th1 phenotype, which is different from our findings. The discrepancy could be due to the fact that previous studies used naïve T cells whereas our study used CD4+ T cells obtained from a murine model of asthma. Those CD4+ T cells might have already been primed under the Th2-deviating microenvironment. The effects of HMGB1 on naïve CD4+ T cells might be different from those on Th2 primed CD4+ T cells. For example, the cooperative role of the CD4+ T cell-mediated inflammatory signals and signals derived from eosinophils were only seen in OVA-treated IL-5-/- mice, but not in naïve IL-5-/- mice. Detectable levels of baseline

Fig. 1. IL-4 and IL-5 levels in the supernatant of CD4+ T cells co-cultured with dendritic cells after incubation with the supernatant of the eosinophil culture.

Fig. 2. HMGB1 levels in the supernatant of the eosinophil culture before and after IL-5 stimulation.

Fig. 3. IL-4 and IL-5 levels in the supernatant of CD4+ T cells co-cultured with dendritic cells after incubation with the supernatant of the eosinophil culture alone or plus anti-HMGB1 antibodies.
IL-4 and IL-5 in the supernatant of CD4+ T cells co-cultured with DCs (as seen in the ‘Culture media only’ group in Fig. 1) and detectable levels of baseline HMGB1 in the supernatant of the eosinophil culture (as seen in the ‘Before IL-5 stimulation’ group in Fig. 2) could be explained by the fact that all cells were derived from an established murine model of asthma. Therefore, effects of HMGB1 on CD4+ T cells may differ depending on the functional states of CD4+ T cells.

It was reported that the cDNA sequence of a conserved lymphokine elements-0 (CLE0) binding protein (CLEBP-1) was almost identical to the cDNA sequences of HMGB1.13 CLEBP-1 binds to CLE0 in the promoter region of IL-5 which is essential for the expression of IL-5.14 A murine model for spontaneous osteoarthritis exhibited not only elevated levels of HMGB1 in hyperplastic synovium but also elevated levels of IL-5 in serum.15 In addition, the HMGB1 protein and messenger RNA levels of HMGB1 and IL-5 were significantly higher in eosinophilic chronic rhinosinusitis with nasal polyps than those from controls or non-eosinophilic chronic rhinosinusitis with nasal polyps.16 Taken together, these findings suggest that HMGB1 might be closely linked to IL-5 production.

In conclusion, our in vitro data revealed that eosinophils modulated CD4+ T cell responses via HMGB1 in the pathogenesis of asthma characterized by eosinophilic airway inflammation, although in vivo experiments are needed to confirm our findings. Along with our previous report, we conclude that HMGB1 might be a therapeutic target in the treatment of asthma.

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