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Basophil depletion downregulates *Schistosoma mansoni* egg-induced granuloma formation

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ABSTRACT

Granuloma formation around parasite eggs during schistosomal infection is considered to be controlled by Th2 cytokines. However, it is still controversial which cell populations are responsible for the host Th2 cytokine-dependent granuloma formation. Basophils have recently attracted attention because of their ability to produce large amounts of IL-4. Therefore, we investigated whether basophils play an essential role in the induction of granuloma formation induced by *Schistosoma mansoni* eggs. Together with our previous observation that basophil numbers increased markedly in the spleen at 7 weeks postinfection, immunohistochemical staining using anti-mMCP8 monoclonal antibody (mAb) showed basophil infiltration in the granulomatous lesions formed around parasite eggs. To examine the roles of basophils more directly, we treated mice with anti-CD200R3 mAb to deplete basophils. Depletion of basophils resulted in a reduction of basophil number with concomitant downregulation of egg granuloma formation at 7 weeks postinfection. Moreover, we observed a significant reduction in the size of egg granulomas formed in basophil-depleted mice in the pulmonary granuloma model. Taken together, these findings indicated that basophils are essential for *S. mansoni* egg-induced granuloma formation, and this may serve as a novel therapeutic target in ameliorating the pathology of schistosomiasis.

1. Introduction

Schistosoma mansoni infection induces an immunopathology that manifests as hepatosplenomegaly, which is the cumulative result of granuloma formation around parasite eggs trapped in the intrahepatic capillary vessels. Granulomatous inflammation is induced by immune responses to the parasite eggs, which are thought to be under the control of $CD4^+$ T cells [1–3]. Elevation of eosinophil number, a common feature of egg-induced granuloma during schistosomiasis, is a sequela of the Th2 cell response [4]. However, the details of the biological mechanisms underlying egg granuloma formation are still unclear. Analyses in murine models indicated that orchestration of Th2 cytokines, such as IL-4 and IL-13, seems to be important [4,5], but the principal conductor of this orchestration is still unknown.

Basophils are the least prevalent of all granulocytes in bone marrow, blood, or spleen under normal physiological conditions. However, basophils have recently attracted attention due to their vital role in the immune response repertoire based on their ability to produce IL-4 and IL-13 [6,7]. Even under certain pathological conditions, such as helminth infections where there is an increase in number of basophils [8,9], they still represent a small subpopulation among the total cellular components [10–13]. Furthermore, basophils have been viewed as redundant and surrogate cells of mast cells with which they share many characteristics, as they are generated from common $CD34^+$ progenitors in the bone marrow [14,15]. However, recent studies have shown that basophils and mast cells are not completely identical [16–18]. Differentiation of these cells takes different routes that result in distinct life cycles and localizations of these cells [12,19–21].

Indeed the advent of new tools and reagents over the past decade has heightened interest in basophil research, thereby providing new insight into basophil functions. These developments have underscored the crucial roles of basophils in allergic inflammation, antigen capture, and immunoregulation [13,22–25]. Moreover, basophils have been

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shown to secrete large quantities of IL-4 on activation [26,27]. In addition to the role of IL-4 in immunoregulation, it has been suggested to mediate inflammatory cell recruitment to sites in affected organs. Thus, basophils may be involved in immunopathology [13,23] of inflammatory lesions, although the details remain to be confirmed.

With regard to schistosomal infection, a few experimental studies have shown that basophils are activated in the presence of schistosome eggs [28,29]. However, the effector function of basophils in schistosome infection has not been well characterized. Progress in basophil studies has been achieved by the establishment of monoclonal antibodies (mAbs) reactive to basophils, such as anti-FcεR1 (MAR-1) [30,31] and anti-CD200R3 (Ba103) [23,24]. These two mAbs can effectively deplete basophils, and are therefore useful in functional studies of these cells.

In this study, we confirmed that mice infected with *S. mansoni* showed marked basophilia in the spleen, as well as infiltration of basophils into granulomatous lesions in the liver. Using Ba103, we confirmed a significant decrease in basophil number in the affected hepatic tissues and spleen during *S. mansoni* infection as well as lung tissues of mice intravenously injected with parasite eggs. The basophil depletion was accompanied by a decrease in size of granulomatous lesions in the liver (hepatic granuloma) and the lung (pulmonary granuloma). Here, we discuss possible roles of basophils in the clinical course of schistosomiasis mansoni and possible prospects to develop new strategies of prophylaxis and/or therapeutics for clinical manifestations of schistosomiasis.

2. Materials and methods

2.1. Mice and parasites

Female BALB/c and male C57BL/6 mice were purchased from CLEA Japan (Tokyo, Japan). The Puerto Rican strain of *S. mansoni* was maintained at Tokyo Medical and Dental University.

To test the roles of basophils in *S. mansoni* infection, we employed two routes of infestation. First, BALB/c mice were exposed to cercariae of *S. mansoni* at 100 cercariae/mouse percutaneously via the tail (infection model). These mice were sacrificed at 7 weeks postinfection, and the liver and spleen were recovered. Second, C57BL/6 mice were given 5000 *S. mansoni* eggs/mouse intravenously via the tail vein (pulmonary granuloma model), and mice were euthanized on day 12 after egg injection. The lung and spleen were recovered, and used for the subsequent experiments.

All experiments in this study were conducted in accordance with the Guidelines for Animal Use and Experimentation of Tokyo Medical and Dental University (0130374A).

2.2. In vivo depletion of basophils by treatment with Ba103

One day prior to cercarial infection or egg injection, mice in each model (infection model and pulmonary granuloma model) were divided into two groups ($n = 3$–5 each). Each mouse from one group of the two models was pretreated in vivo with i.v. injection of anti-CD200R3mAb (Ba103) at 100 μg diluted in 200 μl of PBS [22]. Each mouse in the other group was administered rat IgG (rIgG) as a control antibody. Due to its relatively short time efficacy [22], Ba103 was injected repeatedly into mice in the infection model or pulmonary granuloma model every 7 days until sacrifice.

2.3. Cell preparation and flow cytometric analysis

Single-cell suspensions of spleen, lung or liver cells were prepared as described previously [10,32], and cell suspensions from each mouse were examined by flow cytometric analysis for surface staining (FACSCalibur; BD Bioscience, San Jose, CA). Staining of surface markers was performed with the indicated mAbs and analyzed as described previously [27]. The following antibodies were used for flow cytometric analyses: PE-conjugated mAbs specific for c-kit and biotinylated mAbs specific for IgE (BD Pharmingen, Franklin Lakes, NJ). FITC-conjugated anti-CD49b (DX5) and APC-conjugated avidin were purchased from eBioscience (San Diego, CA). Unlabeled anti-CD16/32 mAb (2.4G2) was purchased from BD Pharmingen. Control antibodies of the same isotypes were also prepared. Rat IgG2 and HRP-conjugated goat anti-rat IgG were used for immunohistochemical staining (BD Pharmingen).

2.4. Histology and granuloma measurement

After treatment of mice with Ba103, the liver (infection model) or lungs (pulmonary granuloma model) were removed and fixed in 4% formalin. The tissues were processed and stained with hematoxylin/eosin (HE) or anti-mMCP-8 mAb (TUG8) for histopathological observation. Immunohistochemical staining of basophils with TUG8 was performed as described previously [18] and then counterstained with HE for microscopic examination.

Granuloma areas formed around a single parasite egg were measured in a blinded manner in the histological sections using ImageJ software (NIH, Bethesda, MD). The mean areas were computed for each group, and mean sizes were compared between groups.

2.5. Statistical analysis

Statistical significance was analyzed by Student's *t* test or Welch's *t* test, and $P < 0.05$ was taken to indicate significance.

3. Results

3.1. Basophilia and basophilic infiltration during the time course of *S. mansoni* infection

Although there is as yet no standard protocol for detecting basophils in flow cytometry, probable criteria of basophils on flow cytometry are ckit$^-$FcεRI$^+$CD49b$^+$ or c-kit$^-$IgE$^+$CD49b$^+$ cells [10,23,27]. In the present study, we employed the definition of basophils as c-kit$^-$IgE$^+$CD49$^+$. As described in our previous report [27], the frequencies of basophils were markedly elevated in the bone marrow (BM) and spleen at 6–8 weeks postinfection (data not shown). The peak of basophilia in the spleen seemed to be concordant with maximal granuloma formation in the liver, which was observed at 7–8 weeks postinfection. To determine whether peripheral basophilia is related to egg granuloma formation, immunohistochemical staining using TUG8 was performed to detect basophils in the granulomatous lesions in the liver. Basophils were detected in granulomatous lesions in the liver (Fig. 1), suggesting that these cells were recruited into the pathological sites. We did not detect basophils in the livers of uninfected control mice (data not shown).

3.2. Reduced number of basophils by treatment with Ba103 at sites of pathological lesions

To investigate the effects of Ba103 treatment on depleting basophils from organs affected in the course of *S. mansoni* infection, we counted basophil number by flow cytometry. Treatment with Ba103, but not rIgG, resulted in significant reduction of splenic basophils in the infection model (Fig. 2a). In mice in the infection model, we observed a reduction in frequency and number of basophils in the liver and spleen at 7 weeks postinfection in Ba103-treated mice compared to rIgG treatment (Fig. 2b–e).

Furthermore, immunohistochemical staining of granulomas in the livers of Ba103-treated mice in the infection model showed few or no cells stained with TUG8 at 7 weeks postinfection, whereas many cells stained with TUG8 were observed in the livers of infected mice treated with rIgG (Fig. 2f).