Tropical Medicine and Health Vol. 38 No. 1, 2010, pp. 13-22

Original article

Schistosome eggs have a direct role in the induction of basophils capable of a high level of IL-4 production: Comparative study of single- and bisexual infection of *Schistosoma mansoni in vivo*

William K. Anyan[1,2], Takashi Kumagai[1], Rieko F. Shimogawara[1], Takenori Seki[1], Nobuaki Akao[1], Kazushige Obata[3], Bethel Kwansa-Bentum[1,2], Kwabena Mante Bosompem[2], Daniel A Boakye[2], Michael D. Wilson[2], Hajime Karasuyama[3], Nobuo Ohta[1*].

Received 4 December, 2009 Accepted 18 December, 2009 Published online 25 February, 2010

Abstract: Immunobiological roles of schistosome eggs during murine experimental infection were investigated with special reference to the induction of basophilic leukocytes. After single- or bisexual infection with *Schistosoma mansoni* in BALB/c mice, splenomegaly and liver granulomas were observed only in bisexual infection in parallel with deposition of mature parasite eggs. Comparison of the kinetics of basophil response revealed a marked increase in number in the bone marrow of mice with bisexual infection at the 7th week post infection as opposed to a marginal increase in single- sex infections. In the spleen, bimodal response was observed in the basophil responses; a small but repeatable peak at the 4th week after infection, increasing again at the 8th week, which corresponded to the initiation and maturation of parasite eggs in the affected organs of infected mice. The same time course was observed for IL-4 production by the splenocytes from mice of bisexual infection. To obtain more concrete evidence of the role of eggs in the induction of basophils, we tested using the intravenous egg injection model. Injection of eggs induced basophilia, and it was accompanied by the up-regulation of IL-4 production in splenocytes from the 8th day. Basophils induced in this model showed a high level of IL-4 production confirmed by flow cytometry, while faint levels of IL-4 production were observed for $CD4^+$ T cells at this time point. In addition, we demonstrate that egg deposition is the trigger of basophil induction and activation in the murine experimental model of *S. mansoni* infection, which might play an essential role in the initiation of Th1/2 conversion during the course of *S. mansoni* infection *in vivo*.

Key words: *Schsitosoma mansoni*, Immunoregulation, Basophils, Granuloma, Eggs antigens

INTRODUCTION

Schistosoma mansoni, a blood dwelling dioecious helminth, is one of the three major schistosome species widely known to affect man in developing nations in Africa, South America and the Carribeans [1, 2]. Based on available information, it is estimated to be responsible for more than half of the over 200 million schistosome-affected people globally [1, 3]. Several reports indicate that the pathology of schistosome infection is $CD4^+$ T cell dependent [4, 5]. Two patterns of cytokine profile have been reported from $CD4^+$ T cells in host animals. Host T cells in early infection stages secrete Type 1 cytokines such as IFNγ and IL-2. Later, hosts with egg-producing adult worms skew from Type 1 to Type 2 polarization with IL-4, IL-5, IL-6, IL-10 and IL13 production, among which IL-4 plays a central role in Th2 response [6-8]. Consistently elevated serum IgE and tissue eosinophilia which are under the control of Th2 cells, are acknowledged as dominant characteristics of the pathology and immune response [9].

Ironically, the shift from Th0 stage to Th2 cell polarization requires an initial IL-4, and there is controversy about its origin. For instance, $CD4^+$T cells are reported to generate the initial IL-4 [10], which is at variance to other research showing non-T, non-B cells as being the prime source of IL-4 [11, 12]. These differing opinions make the

[1] Department of Environmental Parasitology
[2] Department of Parasitology, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon.
[3] Department of Immune Regulation Tokyo Medical and Dental University, Tokyo113-8519, Japan.
[*] Corresponding author:
Department of Environmental Parasitology Tokyo Medical and Dental University Graduate School of Medical and Dental Sciences,1-5-45 Yushima, Bunkyoku, Tokyo113-8519, Japan
Tel/Fax. +81-3-5803-5191
E-mail: matata.vip@tmd.ac.jp

assertion of the origin of Th1/Th2 balance in helminth infections quite controversial. Nevertheless, accumulating evidence shows that basophil, which accounts for only about 0.5% of all leukocytes, is the early and major IL-4 producing potential [13-16] and appears to be responsible for inducing Th2-dominant response [17-21]. Basophils, as well as mast cells and eosinophils, are considered to originate from a common $CD34^+$ progenitor cell [22] and therefore to share many characteristics with mast cells [23]. In some cases, basophils were considered as "redundant mast cells" or at least a poor sister to mast cells and so were obscured [24]. Previously held notions, however, are rapidly being demystified by the advent of sophisticated tools for research on basophils [25-27].

In spite of recent works showing the presence of egg components inducing activation of basophils, details on the initiator and activator of basophils *in vivo* remain less characterized [28]. In the present study, we investigate the dynamics of basophil responses in infected mice with *S. mansoni* and attempt to determine whether it is under the control of schistosome eggs *in vivo*. Considering the probable role of matured eggs in basophil activation, we compared the phenomena of presence and absence of mature eggs in mice. For that purpose, we prepared bisexual (mixed-sex) and single-sex infection, the latter revealing no egg production in the host mice. In this study, we demonstrate the evidence of schistosome eggs responsible for the induction and activation of basophils *in vivo*, which might be related to immnoregulation during murine experimental *S. mansoni* infection.

MATERIALS AND METHODS

Animals and parasite infection:
Female BALB/c mice of 4 weeks old were purchased from CLEA Japan (Tokyo, Japan). A Puerto Rican strain of *S. mansoni* cercariae maintained in our laboratory was infected to 30 mice at a dose of 100 cercariae/mouse by percutaneous exposure for an hour. In the single-sex male and female infection groups, 30 mice each were exposured to cercariae obtained from snails to which a single miracidium had been exposed. This indicates that cercariae shedding from snails are male or female only. Non-infected mice were maintained as controls.

To evaluate the direct role of schistosome eggs in the basophil response *in vivo*, 5,000 viable eggs were injected into the tail vein by the method described previously [29]. As controls, we injected 5,000 beads of the same size as the schistosome eggs (Dynabeads M-450 Dynal Biotech, Norway). In this mouse model, the eggs or beads were trapped in the lung capillary vein, and the immune response to egg antigen was induced in the absence of adult worms.

All experiments in the present study were conducted in conformity with the regulatory guidelines and approved by the committee of animal ethics of Tokyo Medical and Dental University.

Eggs, worms and sera collection from mice:
On a weekly basis starting from week 1, mice were euthanized and bled by cardiac puncture. From the 4^{th} week, worms were perfused from the hepatic portal system of each mouse, and the worm burden was enumerated [30]. This was repeated every week up to the 12^{th} week and the recovered worms were enumerated. The liver from each mouse was collected, weighed, and a third cut and placed in 10% formalin for histology to test for granuloma formation. The remaining liver samples from mice with bisexual infection were minced. Parasite eggs were obtained by filtering through a 38 μm mesh and purified by centrifugation and later quantified using a light microscope. Worms and eggs were washed and centrifuged 3 times with PBS and kept frozen at -80 ℃ until use.

Cells preparation:
Bone marrow (BM) and spleen cells were prepared under aseptic conditions from the hind leg bone and spleen respectively. Cells were resuspended in RPMI-1640 medium (Wako, Tokyo, Japan), and the viable cells were enumerated by the trypan blue dye exclusion method. Following subsequent washing, cells were resuspended and, for RNA extraction, a portion of the spleen cells was placed in TRIzol (Invitrogen, San Diego, USA) and stored at -80 ℃ until use. The remaining cells were used for FACS analysis (BD, San Jose, CA). In the case of mice injected with viable eggs intravenously, spleen, BM and lung cells were prepared on day 1, 4, 8, 12 and 16 post injection.

Flow cytometry analysis:
The antibodies used for FACS analyses were as follows: FITC-conjugated monoclonal antibodies (mAb) specific for CCR3 and CD4, Phycoerythrin (PE)-conjugated mAb specific for c-kit and IL-4, biotinylated Gr-1 (BD PharMingen, Franklin Lakes, USA). Allophycocyanin (APC)-conjugated strepavidin was used for detection of biotinylated reagents (BD PharMingen) FITC-conjugated mAb specific for CD49 b (DX5) and Gr-1, PE-conjugated mAb specific for Gr-1 and FcεRIα; and APC-conjugated mAb specific for IFN-γ and rat IgG1 was purchased from eBioscience (San Diego, USA). FITC-conjugated mAb CCR3 was purchased from R&D systems (Minneapolis, USA). Unlabelled anti-CD16/32 mAb (2.4G2) was purchased from BD PharMingen.

Surface staining was done by the method described

Table 1: Parasite infectivity and maturation in single- or mixed-sex infection, and egg production and splenomegaly in mixed-sex infection

Period (weeks)	Male only TWR (% mature)	Female only TWR (% mature)	Mixed-sex TWR (% mature)	Worm pairs	Parasite eggs ($\times 10^5$)
4	36.3 (52.3)	18.3 (63.6)	31.3 (54.8)	3.3	0.00230
5	51.3 (81.2)	27.3 (75.3)	40.3 (96.3)	8.7	0.129
6	20.0 (88.3)	51.7 (93.5)	52.0 (99.7)	14.0	3.05
7	40.0 (99.0)	33.0 (99.0)	31.7 (100)	13.3	9.57
8	37.3 (99.1)	47.0 (100)	31.7 (100)	10.3	14.2
10	16.7 (100)	65.0 (100)	28.5 (100)	12.0	20.7
12	9.7 (100)	45.7 (100)	23.0 (100)	10.1	27.9

TWR: Total worms recovered

elsewhere [25] with slight modification. In brief, 1×10^6 of BM or spleen cells were pre-incubated with 2.4G2 and normal rat serum in FACS buffer (PBS with 1mg/ml of BSA and 0.5mg/ml of NaN_3) on ice for 15 min to eliminate non-specific binding of irrelevant antibodies. The cells were subsequently incubated on ice with the conjugated mAbs for 30 min, and then second antibodies were added for 10 min and finally propidium iodide was added as substrate. Stained cells were analyzed using FACScalibur (BD Bioscience) equipped with CellQuest software.

Cytoplasmic staining was done by the standard protocol [27]. Freshly prepared spleen cells were suspended at 2×10^6 cells in 200 µl of complete medium (10% FCS added to RPMI-1640 with 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM L-glutamine and 50 µM 2-mercapto ethanol). Cells were treated with 10 ng/ml phorbol myristate acetate (PMA) plus 500 ng/ml Ionomycin (Iono) and 2 µM monensin in 96 well plates for 4 hr at 37℃ in 5% CO_2. Cells were taken through all the steps of surface staining as described above, except for the addition of propidium iodide. Cells were then treated with cytofix/cytoperm and perm/wash solution (BD Bioscience) according to the manufacturer's instructions, and then stained for intracellular cytokines detected by FACScalibur with CellQuest software.

Histopathology of the liver:
Liver specimens from each week (1-12weeks) were fixed in 10% formalin and kept at room temperature until tissue section preparation. Each liver specimen was decalcified and then embedded in paraffin. The tissue sections of 4 µm were stained with haematoxylin and eosin for microscopic observation.

Real-time reverse-transcriptase polymerase chain reaction:
Total RNA was extracted from the spleen and BM cells using TRIzol reagent (Invitrogen), and cDNA synthesis was performed as described elsewhere [31]. We measured mRNA transcripts for IL-4 and IFNγ using the following primers: for IL-4 (forward 5'-CATCGGCATTTTGAACGA-3' reverse 5'-GTGGTGTCTCTCACTCGAGA-3'); for IFNγ (forward 5'-GGAGGAACTGGCAAAAGGAT-3', reverse 5'-GGAGTCTGAGAAACTTCAGAACTT-3'), relative to that of β-actin (forward 5'-GCTCTAGACTTCGAGCAGGAGA-3', reverse 5'-TCTTCCCGATACTCGACGGA-3'). We performed product amplification with the SYBR Green I kits (Roche, Mannheim, Germany) as described by the manufacturer using the LightCycler^R (Roche) with the following profile: 95℃ for 5 min, 45 cycles at 95℃ for 10 sec, 60℃ for 10 sec, 72℃ for 4 sec and finally 50℃ for 30 sec. The relative expression levels were calculated based on ratio of target gene to house keeping gene.

Statistical analyses:
Statistical analyses were done using student *t*-test. *P* values of <0.05 were considered significant.

RESULTS

Parasite infectivity, pairing and eggs production in host:
Parasite ability to infect host ranged between 9.7% and 65.0% in the single (male or female) and mixed sex (bisexual) infection (Table 1). More than 50% adult worms appeared by the 4th week, and by the 7th week all worms were morphologically matured in bisexual infection when maturation was determined by the presence of distinct suckers and worm shape, the single-sexes showing close to 99% morphologically matured worms. In the bisexual infection, marked egg production was realized from the 6th and 7th week and progressively increased through to the 12th week (Table 1). The spleen from single-sex infection revealed no significant increase in size as compared with controls, while bisexual infection showed enlarged spleen size correspond-

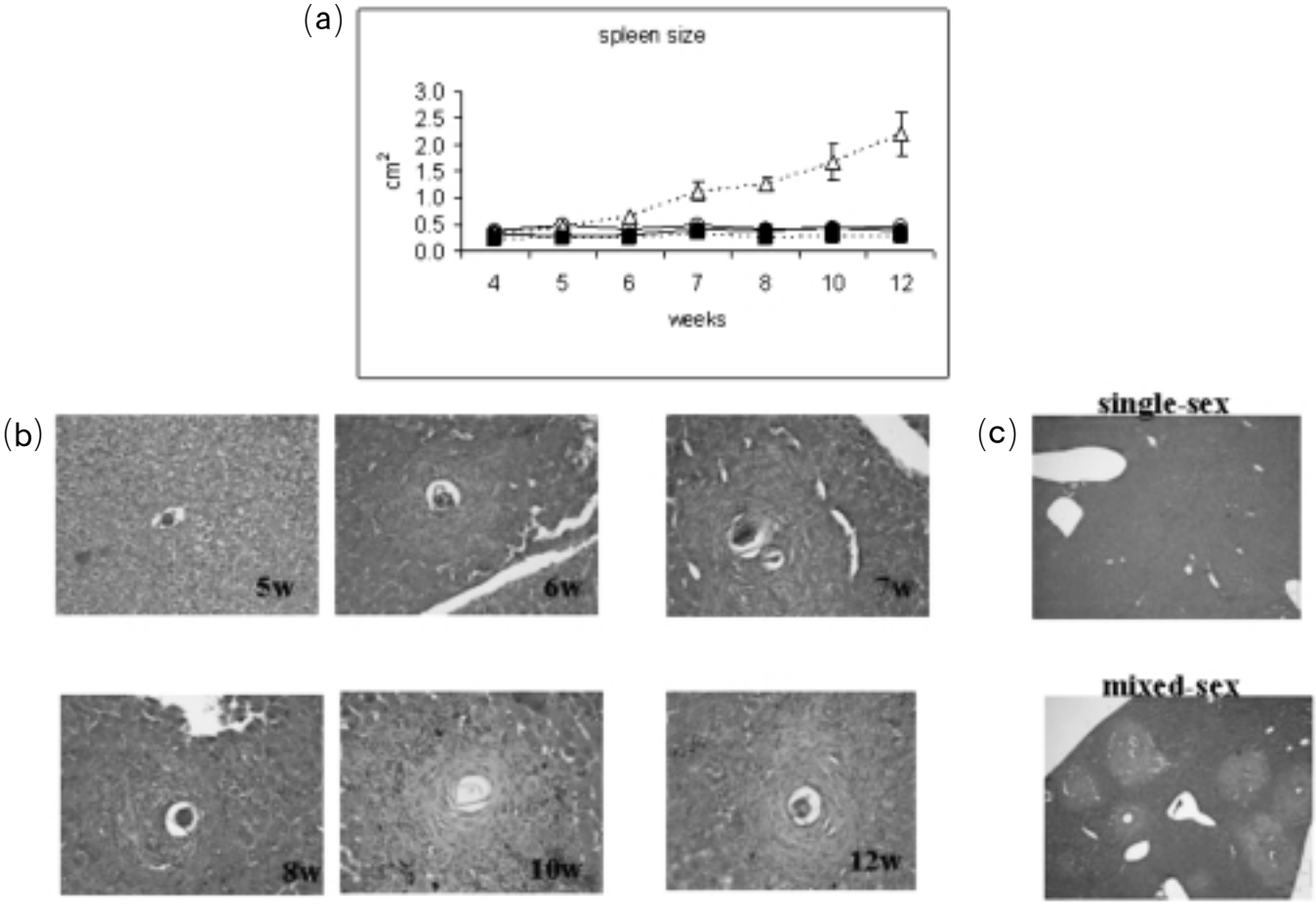

Fig. 1
Pathological parameters in bisexual and single-sex infection of *S. mansoni*. (a) Splenomegary of single-sex or bisexual infection is shown. ■: infection-free control,□: single sex male alone, ●: single sex female alone, and △: bisexual infection. (b) Schistosome eggs deposited in the liver and granuloma formation during bisexual infection are shown. All lesions are shown at x200 magnification. (c) Comparison of liver lesions between single-sex and bisexual infection is shown (x40).

ing closely with egg production seen from the 6th and 7th weeks (Fig. 1a). In bisexual infections, eggs were found in the liver in the 4th and 5th weeks, but no granuloma formation was observed at those time points (Fig. 1b). Moreover, none of the single sex infected mice liver showed any granuloma formation (Fig. 1c).

Dynamics of granulocyte response in BM and spleen during the course of S. mansoni *infection:*
Basophils were identified in FACS by the use of antibodies to CD49b and FcεRⅠ. It was possible to detect basophils as a double-positive stained population (Fig. 2). FACS patterns for eosinophils and neutrophils are also shown. Basophils in BM from mixed sex infection began to show apparent but modest induction from the 3rd week with steady increase thereafter, all peaking by the 7th week (Fig. 3a). Contrary to the situation in the BM, the level of splenic basophils from mixed- and single-sex (female) infection reveal an equally modest increase at the 4th week (Fig. 3b). However, the splenic basophilia became significantly elevated after the 6th week and peaked at the 8th week, just at the time when BM basophils sharply return to the basal level. As shown in Table 1, egg deposition started around the 4th week, and egg maturation was confirmed from the 6th week after cercarial infection. The spleen neutrophils and eosinophils were concordant with the kinetics of spleen basophils. Both neutrophils and eosinophils showed progressive induction especially after the 6th week in only the mixed infection (Fig. 3c, d).

Cytokines expression in spleen of infected mice by quantitative real-time PCR:
Spleen cells from bisexual infection were tested for IL-4 and IFNγ expression by quantitative real-time PCR. As shown in Fig.4a, IFNγ expression reached a maximum level at the 7th week after infection. On the other hand, IL-4 ex-

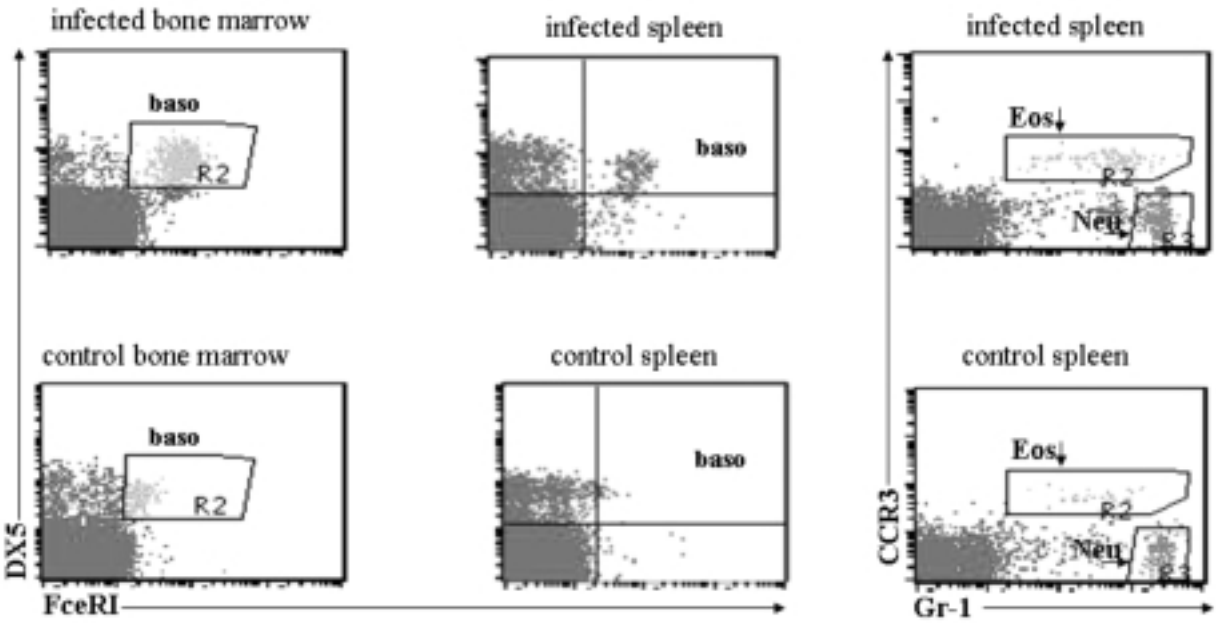

Fig. 2
FACS profiles of basophils, eosinophils and neutrophils. Basophils were identified as a cell population double positive for FcεRⅠ and CD49b, and eosinophils were double positive for Gr-1 and CCR3. Neutrophils were of Gr-1-positive but CCR3-negative.

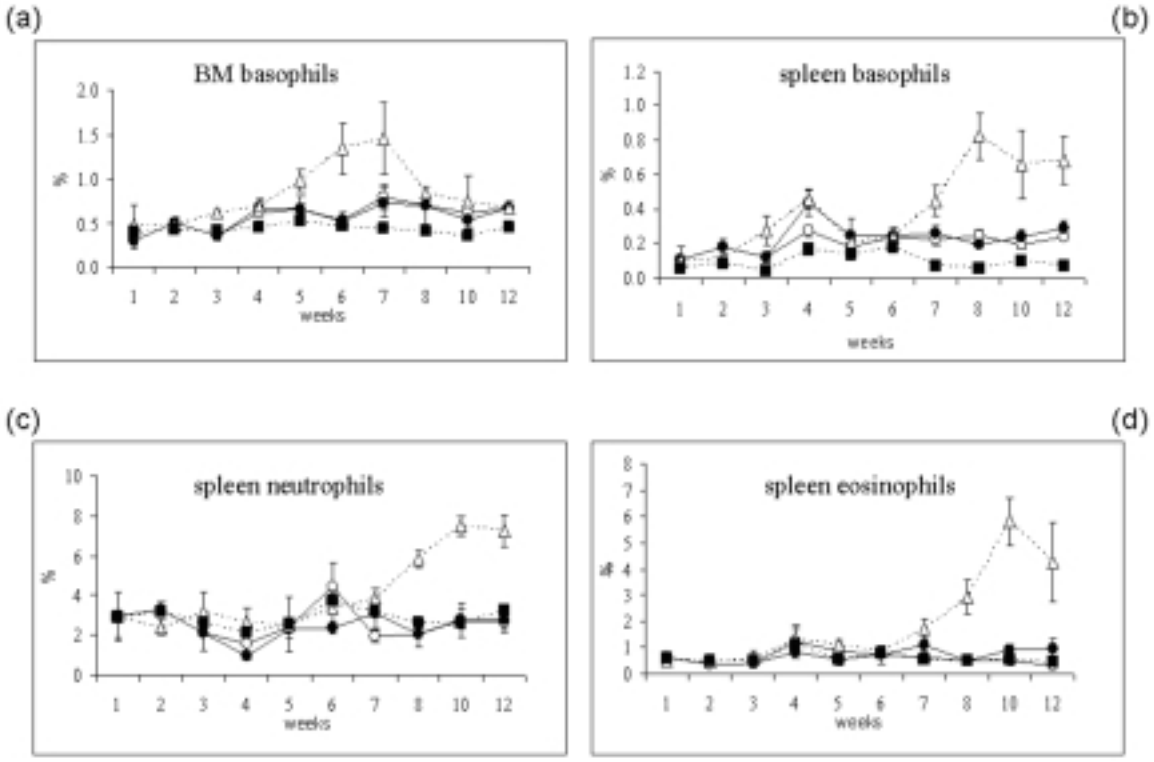

Fig. 3
Time course kinetics of basophils, eosinophils and neutrophils in (a) bone marrow and in (b)-(d) spleen during single-sex and bisexual infection are shown. ■: infection-free control, □: single sex male alone, ●: single sex female alone, and △: bisexual infection.

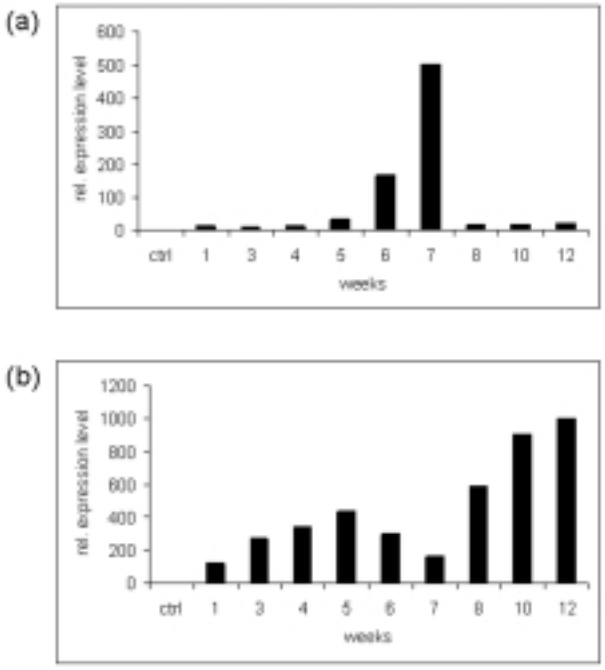

Fig. 4
IFNγ and IL-4 production measured by real-time PCR. Splenic cells from bisexual infection were tested for cytokine production by the method described in Materials and Methods. (a) IFNγ production and (b) IL-4 production are shown.

pression showed a bimodal pattern during infection. The first peak was observed around the 4th and 5th weeks and the second peak at the 8th week or later, both peaks being concordant with the two peaks of basophilia (Fig. 4b).

Basophils in BM, lung, liver and spleen of mice intravenously injected with S. mansoni *eggs:*
We tested to see whether eggs play a major role in the basophil induction observed in the model of intravenous injection of parasite eggs. As shown in Fig. 5, basophil induction was driven by eggs from the 8th day in the absence of adult worms and continued until the 12th day in the three organ tissues analyzed, although stimulation was still apparent beyond the 12th day in BM.

Detection of cytokines produced in the spleen of mice intravenously injected with S. mansoni *eggs:*

In view of the fact that the pattern of IL-4 expression closely resembled basophil kinetics in the spleen (Fig. 3b and Fig. 4b), we examined the role of splenic basophils in IL-4 production in the egg injection model by performing cytoplasmic staining. As shown in Fig. 6, splenic basophils expressed a high level of IL-4 production from the 8th day. Considering the expression level of IL-4 between basophils

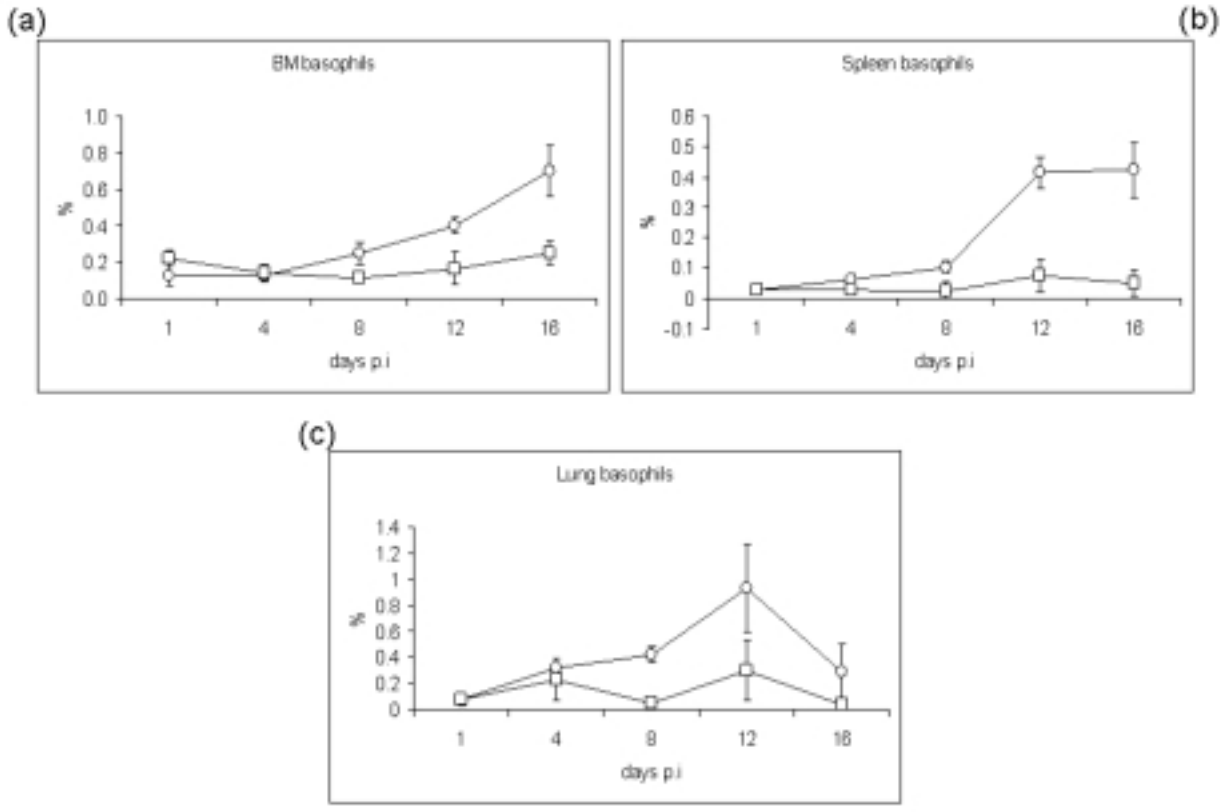

Fig. 5
Induction of basophils in the egg-injection model. Basophils were increased in number from the 8th day and later in three organs (bone marrow (a), spleen (b) and lung (c)) tested by FACS analyses. ○: Injection of *S. mansoni* eggs, □:beads-injection control.

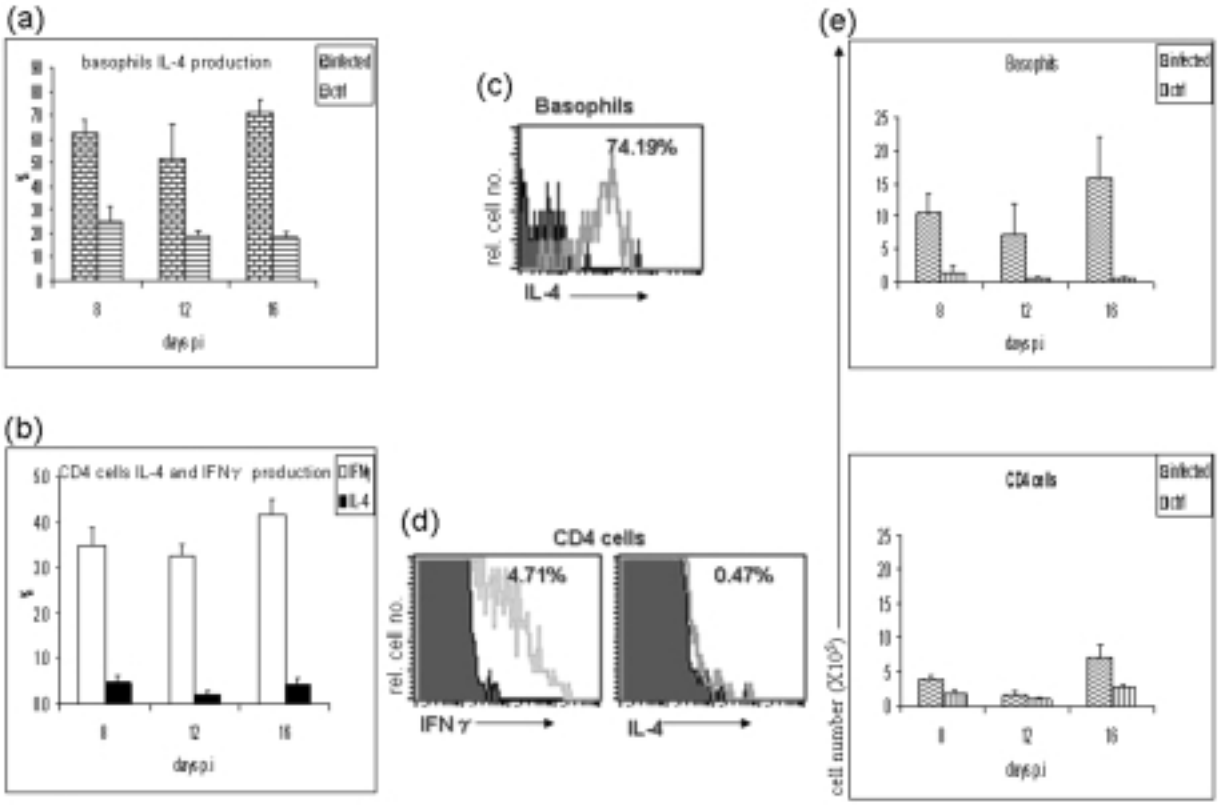

Fig. 6
Basophils induced in the egg-injection model produced a high level of IL-4 measured by cytoplasmic staining by FACS. (a) Splenic basophils with IL-4 production were increased in ratio in egg-injected mice at the 8th day and after. (b) $CD4^+$T cells in the spleen of egg-injected mice produced dominantly IFNγ rather than IL-4. (c) Intensity of IL-4 production was evaluated by FACS. Representative results of basophils at the 16th day is shown. Activated basophils shifted to strong positive for cytoplasmic IL-4. (d) Intensities of cytoplasmic IFNγ and IL-4 of $CD4^+$T cells from egg-injected mice at the 16th day are shown. (e) Actual cell number of IL-4-producing basophils and $CD4^+$ T cells are shown. Basophils with IL-4 production were higher in number than $CD4^+$ T cells with IL-4 production.

and $CD4^+$T cells, we observed an increase in the number of basophils with a higher intensity of IL-4 production than those in $CD4^+$ T cells (Fig. 6a-e). The ratio of IFNγ-producing $CD4^+$ T cells was higher compared with IL-4 at all time points tested (Fig. 6b). By calculating the number of basophils with a potentials for IL-4 production, it seems likely that basophils were dominant in IL-4 production in the egg-injection model (Fig. 6c).

DISCUSSION

Schistosoma mansoni infection is prominently characterized by Th2 response accompanying elevated response of IgE and eosinophils [32, 33]. In recent times, basophils, quantitatively the least of the granulocytes, are rapidly being acknowledged to play a role in immunoregulation in some helminth infections [17]. Schistosome egg antigen (SEA) components in the presence of some reagents stimulate basophils to produce IL-4 *in vitro* [34, 35]. However, the mechanism of basophil stimulation *in vivo* has not yet been elucidated. The recent development of reagents and tools for identifying basophils [19, 25] enable researchers to explore whether schistosome eggs are responsible for the induction of basophilia during the course of *S. mansoni* infection as well as to determine whether basophils induced by the parasite egg play any key role in immunoregulation.

Considering the possibility that schistosome eggs are involved in the induction of basophilia, we conducted a comparative study using single- and mixed-sex infection. Adult worms were present in both situations, while only mixed-sex infection produced eggs. Although growth retardation of worms is suggested in single-sex infection [36], mice susceptibility to infection and worm maturation in the single-sex situation were comparable to those in mixed-sex infection in the present study. The lack of any apparent splenomegaly in the single-sex infection indicates that

worms were not major players in the pathological lesion. Also, the failure of eggs produced at the 4th and 5th weeks in the mixed-sex infection mice to show any granuloma may be consistent with reports that produced eggs require a substantial period to mature and to elicit an immune response. These findings were confirmed by the 6th week and beyond, as both splenomegaly and granuloma correlated well with egg maturation and production over the period, signifying the important role of parasite eggs in disease pathology [37].

The fact that basophils are the least of all the leukocytes is shown by our results. During the course of infection, basophils in BM and spleen were highly elevated at a specific time point in bisexual infection. Although the cell frequency increasing at this time point appears to be small, it was confirmed in our repeat experiment (data not shown). Interestingly, the modest basophil induction in BM and spleen appearing before and just at egg deposition suggests that adult worms could, in part, exert an effect on the induction of basophilia [38]. However, the highly significant BM basophilia from the mixed-sex infection as compared to the marginal elevation in single-sex infection ($p<0.05$), implies that eggs enhance induction to a higher degree than worms alone. This was clarified in the spleen where only mixed-sex infections showed marked basophilia after the modest 4th week peak. The marginal basophilia induced by worms is probably due to site inhabited and immune evasiveness in the host [6].

More concrete evidence for mature eggs being a direct trigger for basophilia was observed in the egg-injection model. After intravenous injection of parasite eggs, splenic basophils were highly induced. Those basophils showed high a level of IL-4 production, while $CD4^+$ T cells in the spleen produced only a faint level of IL-4 at the same time point. This confirms that mature eggs of *S. mansoni* are responsible for inducing basophilia with the potential for IL-4 production.

Conspicuously, the peak level of basophils was far lower in the spleen than in the BM, which was, however, consistent with earlier reports [13, 17]. Furthermore, time points of induction were different as a drastic decline in BM basophils was concurrent with a rapid increase in spleen basophils, suggestive of a feed back mechanism to avoid overproduction probably fatal to the host. The splenic eosinophilia and neutrophilia coincided with the splenic basophilia. This gives further support to suggestions that BM basophils express or release factors which may have facilitated their stimulation [39] and recruitment [40]. Reports published elsewhere [22, 29, 38] indicate that basophils produce IL-4 which up-regulates expression of VCAM-1 on endothelial cells and eotaxin synthesis, thereby enhancing the influx or recruitment of other leukocytes into the affected tissues. This might be supported by another study showing that depletion of basophils diminished eosinophil infiltration at the site of inflammation [4]. It may also be consistent with a report by Wedemeyer et al. that basophils played a role in immunoregulation in both innate and adaptive responses [41]. Our observations strongly suggest that eggs are main inducers of basophilia and that the egg-driven basophilia may be involved in the regulation of pathology of schistosome infection.

It has already been reported that polarization of $CD4^+$ T cells to Th2 type profiles as well as B cell synthesis of IgE and IgG1 coincides with egg production [8] and that IL-4 plays a central role in all these events. The source of initial IL-4 continues to be a subject of debate. Williams et al. reported that IL-4 was first detected from spleen cells depleted of T cells at the egg production stage [38]. Despite the number of cells present in the spleen, our results suggest the involvement of spleen basophils in the Th1/Th2 conversion, because basophils were capable of a high level of IL-4 production in the initial phase of stimulation by the mature eggs (Fig. 6). This is consistent with earlier studies showing that the level of IL-4 production is much higher in basophils than in $CD4^+$ T cells [42]. The marked elevation of BM basophils at the 7th week in the mixed-sex infection model suggests that basophils are one of the major sources of IL-4, which is necessary to initiate the polarization of $CD4^+$ T cells to Th2 cells as well as the B cell class switch to IgE and IgG [20, 43].

In conclusion, the present study adds to earlier works postulating that stimulation of egg antigen to the host immune system is the main drive inducing basophils in the infected hosts. Even though the question of which cell population is responsible for providing IL-4 for Th2 polarization in the acute phase of infection has been controversial, our results point to basophils as the initiator and most probable candidate cell for immunoregulation. Further studies are underway to obtain additional evidence by preparing a new *in vivo* experimental model of *S. mansoni* infection.

ACKNOWLEDGEMENTS

The authors wish to thank Ms. Hato for her assistance in preparing the manuscript. This study was supported in part by a Grant-in-Aid from the Japan Society for Promotion of Science (#21406008 and #19790305), a grant from the Ministry of Health, Welfare and Labour (2008 and 2009), and Japan International Cooperation Agency.

REFERENCES

1. World Health Organization (WHO). Report on the Re-

gional Workshop on comprehensive approaches for schistosomiasis control. Alexandria, June 1993, WHO-EM
2. Southgate VR. Schistosomiasis in the Senegal River Basin: before and after the construction of the dams at Diama, Senegal and Manantali, Mali and future prospects. J Helminthol 1997; 71: 125-132.
3. Chitsulo L, Engels D, Montresor A, Savioli L. The global status of schistosomiasis and its control. Acta Tropica 2000; 7: 41-51.
4. Phillips SM, Linette GP, Doughty BL, Byram JE, von Lichtenberg F. In vivo T cell depletion regulates resistance and morbidity in murine schistosomiasis. J Immunol 1987; 139: 919-926.
5. Pearce EJ, MacDonald AS. The immunobiology of schistosomiasis. Nat Immunol 2002; 2: 499-511.
6. Grzych JM, Pearce E, Cheever A, Caulada Z.A, Caspar P, Hieny S, Lewis FA, Sher, A. Egg deposition is the major stimulus for the production of Th2 cytokines in murine schistosomiasis mansoni. J Immunol 1991; 146: 1322.
7. Hirata M, Fukuma T. Cytokine regulation in experimentally -induced *Schistosoma japonicum* egg granuloma formation. Parasitol Int 2003; 52: 341-349.
8. Pearce EJ, Kane CM, Sun J, Taylor JJ, McKees AS, Cervi L. Th2 response polarization during infection with the helminth parasite *Schistosoma manosoni*. Immunol Rev 2004; 201: 117-126.
9. Capron M, Capron A. Effector function of eosinophils in schistosomiasis. Mem Inst Oswaldo Cruz 1992; 87: 167-172.
10. Noben-Trauth N, Hu-Li J, Paul WE. IL-4 secreted from individual $CD4^+$ T cells acts in an autocrine manner to induce Th2 differentiation. Eur J Immunol 2002; 32: 1428-1433.
11. Haas H, Falcone FH, Schramm G, Haisch K, Gibbs BF, Klaucke J, Poppelmann M, Becker, Gabius HJ, Schlaak M. Dietary lectins can induce in vitro release of IL-4 and IL-13 from human basophils. Eur J Immunol 1999; 29: 918-927.
12. Shinkai K, Mohr, M, Locksley RM. Helper T cells regulate type 2 innate immunity in vivo. Nature 2000; 420: 825-829.
13. Seder RA, Paul WE, Dvorak AM, Sharkis SJ, Kagey-Sobotka A, Niv Y, Finkelman FD, Barbieri SA, Galli , Plaut M. Mouse splenic and bone marrow cell populations that express high-affinity Fce receptors and produce interleukin 4 are highly enriched in basophils. Proc Natl Acad Sci USA 1991; 88: 2835-2839.
14. Arock M, Merle-Beral H, Dugas B, Ougas B, Ouaaz F, Goff LL, Vouldoukis I, Mencia-huerta JM, Schmitt C, Leblond-Missenard V, Debre P. IL-4 release by human leukemic and activated normal basophils. J Immunol 1993; 151: 1441-1447.
15. Gibbs BF, Haas H, Wolff H, Grabbe J. Early IgE-dependent release of IL-4 and IL-13 from leukocytes is restricted to basophils: a comparison with other granulocytes and mononuclear cells. Inflamm Res 2000; 49 (Spl. 1): S9-S10.
16. Gessner A, Mohrs K, Mohrs M. Mast cells, basophils and eosinophils acquire constitutive IL-4 and IL-13 transcripts during lineage differentiation that are sufficient for rapid cytokine production. J Immunol 2005; 174: 1063-1072.
17. Min B, Prout M, Hu-Li J, Zhu J, Jankovic D, Morgan ES, Urban JF, Dvorak AM, Finkelman FD, LeGros G, Paul WE. Basophils produce IL-4 and accumulate in tissues after infection with a Th2-inducing parasite. J Exp Med 2004; 200: 507-517.
18. Gibbs BF. Human basophils as effectors and immunomodulators of allergic inflammation and innate immunity. Clin Exp Med 2005; 5: 43-49.
19. Oh K, Shen T, Le Gros G, Min B. Induction of Th2 type immunity in a mouse system reveals a novel immunoregulatory role of basophils. Blood 2007; 109: 2921-2927.
20. Sokol CL, Barton GM, Farr AG, Medzhitov RA. Mechanism for the initiation of allergen-induced T helper type 2 responses. Nature Immunol 2008; 9: 310-318.
21. Karasuyama H, Mukai K, Tsujimura Y, Obata K. Newly-discovered roles for basophils: a neglected minority gains new respect. Nat Rev Immunol, 2009; 9: 9-13.
22. Costa JJ, Weller PF, Galli SJ. The cells of the allergic response. Mast cells, basophils, and eosinophils. JAMA 1997; 278: 1815-1822.
23. Marone G, Trigiani M, de Paulis A. Mast cells and basophils: friend as well as foes in bronchial asthma? Trends Immunol 2004; 26: 26-31.
24. Falcone FH, Haas H, Gibbs BF. The human basophil: a new appreciation of its role in immune responses. Blood 2000; 96: 4028-4038.
25. Obata K, Mukai K, Tsujimura Y, Ishiwata K, Kawano Y, Minegeshi Y, Watanabe N, Karasuyama H. Basophils are essential initiators of a novel type of chronic allergic inflammation. Blood 2007; 110: 913-920.
26. Tsujimura Y, Obata k, Mukai K, Shindo, H, Yoshida N, Nishikado H, Kawano Y, Minegishi Y, Shimizu T, Karasuyama H. Basophils play a pivotal role in immunoglobulin -G-mediated but not immunoglobulin-E-mediated systemic anaphylaxis. Immunity 2008; 28: 581-589.
27. Denzel A, Maus UA, Gonmez M R, Moll C, Niedermeier M, Winter C, Maus R, Hollingshead S, Brile, DR, Kunz-Schughart LA, Talke Y, Mack M. Basophils enhance immunological memory responses. Nature Immunol 2008; 9: 733-742.
28. Schramm G, Gronow A, Knobloch J, Wippersteg V, Grevelding CG, Galle J, Fuller H, Stanley RG, Chiodini PL, Haas H, Doenhoff MJ. IPSE/alpha-1: a major immunogenic component secreted from *Schistosoma mansoni* eggs. Mol Biochem Parasitol 2006; 147: 9-19.
29. Wynn TA, Eltoum I, Oswald IP, Cheever AW, Sher A. Endogenous interleukin 12 (IL-12) regulates granuloma formation induced by eggs of *Schistosoma mansoni* and exogenous IL-12 both inhibits and prophylatically immunizes against egg pathology. J Exp Med 1994; 179: 1551-1561.
30. Smithers SR, Terry RJ. The infection of laboratory host with cercariae of *Schistosoma mansoni* and the recovery of adult worms. Parasitology 1965; 55: 695-700.
31. Pinelli E, Brandes S, Dormans J, Fonville M, Hamilton CM, Der GJ. *Toxocara canis*: effect of pulmonary pathology and cytokine expression in BALB/c mice. Exp Parasitol

2007; 115: 76-82.
32. Sher A, Fiorentino D, Caspar P, Pearce E, Mosmann T. Production of IL-10 by $CD4^+$ T lymphocytes correlates with down regulation of Th1 cytokine synthesis in helminth infection. J Immunol 1991; 147: 2713-2716.
33. Capron M, Capron A. Immunoglobulin E and effector cells in schistosomiasis. Science 1994; 264: 1876-1877.
34. Falcone HF, Dahinden CA, Gibbs BE, Noll T, Amon U, Hebestreit H, Abramamsen O, Klaucke J, Schlaak M, Haas H. Human basophils release interleukin-4 after stimulation with *Schistosoma mansoni* egg antigen. Eur J Immunol 1996; 26: 1147-1155.
35. Schramm G, Falcone FH, Cronow A, Haischi K, Mamat U, Doenhoff MJ, Olivera G, Galle J, Dahinden CA, Haas H. Molecular characterizationof an interleukin-4-inducing factor from *Schistosoma mansoni* eggs. J Biol Chem 2003; 278: 18384-18392.
36. Shaw JR, Erasmus DA. *Schistoma mansoni*; an examination of the reproductive status of females from single sex infection. Parasitol 1981; 82: 121-124.
37. Warren KS. The secret of the immunopathogenesis of schistosomiasis: In vivo models. Immunol Rev 1982; 61: 189-213.
38. Williams ME, Kullberg MC, Barbieri S, Caspar P, Berzofsky JA, Seder RA, Sher A Fc epsilon receptor-positive cells are a major source of antigen-induced interleukin-4 in spleens of mice infected with *Schistosoma mansoni*. Eur J Immunol 1993; 23: 1910-1916.
39. Mukai K, Matsuoka K, Taya C, Suzuki H, Yokozaki H, Nishioka K, Hirokawa K, Etori M, Yamashita M, Kubota T, Minegishi Y, Yonekawa H, Kayasuyama H. Basophils play a critical role in the development of IgE-mediated chronic allergic inflammation independently of T cells and mast cells. Immunity 2005; 23: 191-202.
40. Chensue SW, Terebuh PD, Warmington KS, Hershey SD, Evanoff HK, Kunkel SL, Higashi GI. Role of IL-4 and INFg in *Schistosoma mansoni* egg-induced hypersentivity granuloma formation: Ochestration, relative contribution, and relationship to macrophage function. J Immunol 1992; 148: 900-906.
41. Wedemeyer J, Tsai, M, Galli SJ. Roles of mast cells and basophils in innate and acquired immunity. Curr Opin Immunol 2000; 12: 624-631.
42. Mitre E, Taylor R, Kunofcik J, Nutman TB. Parasite antigen-driven basophils are a major source of IL-4 in human filarial infections. J Immunol 2004; 172: 2439-2445.
43. Min B, Le Gros G, Paul WE. Basophils: a potential liaison between innate and adaptive immunity. Allergol Int 2006; 55: 99-104.