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EOSINOPHIL HYPORESPONSE OF JIRDS INDUCED BY MICROFILARIAE OF *BRUGIA PAHANGI*

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Abstract. Male jirds (*Meriones unguiculatus*) were inoculated sc with 100 infective larvae of *Brugia pahangi*. After 16 weeks, the animals were reinoculated with a comparable number of organisms. Blood eosinophil responses during the 5 weeks subsequent to this attempt to reinfect were much lower than those of comparable naive animals, while the response to a heterologous infection (*Toxocara canis*) was comparable to that of controls. Mebendazole was given to infected animals for 2 weeks beginning 5 weeks (prepatent) or 16 weeks (patent) after infection. At comparable intervals after drug administration, the animals were reinoculated with infective larvae and the blood eosinophil response was measured over a 5 week period. The response in the animals treated during the prepatent period was higher than the untreated infected controls. Treatment during the patent period had no demonstrable effect. Jirds made artificially microfilaremic by intravenous inoculation of viable filaria before and after the standard infecting dose had a low eosinophil response to infective larvae.

A primary experience of jirds with the microfilariae of *B. pahangi* evokes an eosinophil response. Subsequent inoculation of larvae did not produce a comparable response.

Immunoregulatory events such as the mechanisms of antigen specific or non-specific lymphocyte unresponsiveness have been investigated in jirds with chronic *Brugia pahangi* infections.[1–4] Filarial parasites induce a variety of host immune responses in the course of infection in jirds, which may alter host susceptibility to subsequent infection.[5] Therefore, it is important to investigate the characteristics and capacity of immunological reactivity of jirds to the infection of brugian filariae.

Little is known about effector cell responses in jirds to brugian filariae. In our recent study,[6] high levels of blood eosinophilia were observed in jirds at the early phase of *B. pahangi* infection, but hyporesponsiveness was induced in the chronic phase of infection. Eosinophils as effector cells have been shown to play an important role in the protective response to secondary infections of parasites including of filariae.[7,8] In this study, eosinophil reactivity to the subsequent infections of *B. pahangi* and the possible mechanism of eosinophil hyporesponsiveness induced in the chronic *B. pahangi* infected jirds were investigated.

MATERIALS AND METHODS

Animals

Outbred male Mongolian jirds (*Meriones unguiculatus*) were maintained under conventional conditions. At the beginning of the studies they were 10–12 weeks of age.

Infection of parasites

Infective larvae (L3) of *B. pahangi* were obtained from *Aedes aegypti* fed on infected jirds 14 days earlier. For a primary infection, jirds were infected sc in the groin by 100 L3 suspended in 0.5 ml of Hanks' balanced salt solution (HBSS). For the challenge infection, jirds were infected with 100 L3 in the same manner on the scheduled days.

Toxocara canis embryonated eggs were obtained by the procedure of Sugane and Ohshima.[9] Jirds with chronic *B. pahangi* infected or uninfected age-matched control jirds were inoculated with 2,000 embryonated eggs orally using a stomach tube.

Implantation of microfilariae

Microfilariae (mf) were collected from jirds which had been inoculated ip with 300–400 L3 of *B. pahangi* 3 months previously.[10] The animals were anesthetized with ether and their peritoneal cavities were flushed with 10 ml of sterile HBSS. The peritoneal effusion was placed into a plastic dish (Sumitomo Bakelite Corp., Tokyo, Japan), and kept at 37°C for 30 min to remove peritoneal exudate cells. After centrifugation at 2,000 rpm at room temperature for 5 min, active mf were resuspended in sterile HBSS at a concentration of 4×10^5 mf/ml. Jirds were injected iv (penis vein) with 2×10^5 mf in 0.5 ml of HBSS for 4 times, 5 and 2 weeks before infection, the day of infection, and 2 weeks after infection.

Anthelmintic treatment

B. pahangi infected jirds were treated with 20 mg/kg body weight of mebendazole (MBZ) using a stomach tube for 14 consecutive days starting at 5 weeks (prepatent period) or at 16 weeks (patent period) of primary infection. Animals were checked for microfilaria in the blood weekly beginning 2 weeks after treatment until just before challenge infection. All animals used in the challenge infection were free from microfilaria in the circulation. Age-matched naive control jirds were treated with MBZ in the same manner.

Blood examination

Blood samples for examination were collected from the retroorbital plexus under ether anesthesia. Absolute eosinophil counts were performed using Hinkelman's diluting fluid in a Neubauer's improved hemocytometer.

Statistical analysis

Student's *t*-test was used for statistical analysis. Data were considered significantly different from each other at $P < 0.05$.

RESULTS

Blood eosinophil responses to the subsequent homologous infections in the chronically (>16 weeks) *B. pahangi* infected jirds were compared to those in the age-matched naive jirds. The eosinophil response of chronically infected animals was remarkably lower than that of naive animals as shown in Figure 1. The eosinophil reactivity of chronically *B. pahangi* infected jirds to heterologous infection of *T. canis* was comparable to that of control animals (Fig. 2).

To determine whether macro- and/or microfilaricidal treatment affect eosinophil response to challenge infection of *B. pahangi* in the infected jirds, MBZ treatments were performed at a prepatent period or a patent period. Experimental protocols are summarized in Table 1. *B. pahangi* infected jirds of Group A were treated with MBZ weeks 5–7 of the primary infection, and they did not become microfilaremic until week 21 of the primary infection. At week 16 of the primary infection, infected MBZ-treated jirds, infected untreated jirds, and age-matched controls were sc challenged with 100 L3 of *B. pahangi*. At this time, infected MBZ-treated jirds and infected untreated jirds of Group A harbored 1 ± 1 ($n = 3$) and 33 ± 10 ($n = 3$) adult worms, respectively. Infected jirds of Group B were treated with MBZ weeks 16–18 of the primary infection. From week 23 of the primary infection (5 weeks after the final MBZ treatment), the mf count (85 ± 39 mf in an average of 20 μl blood before treatment) began to gradually decrease, and mf had completely disappeared by 15 weeks after treatment. At 38 weeks of primary infection, infected MBZ-treated jirds, infected untreated jirds, and age-matched controls were challenged with 100 L3 in the same manner as Group A. At this time, infected MBZ-treated jirds and infected untreated jirds of Group B harbored 18 ± 9 ($n = 3$) and 35 ± 11 ($n = 3$) of adult worms, respectively. As shown in Figure 3a, anthelmintic treatment at the prepatent period caused an elevation in the eosinophil response of jirds, whereas no significant change was observed after the treatment at the patent period, or chronic stage of infection (Fig. 3b).

To confirm the effect of mf on the suppressed eosinophil response of jirds, 2×10^5 of intact mf were implanted into naive jirds 4 times by iv injections. Artificially induced microfilaremic jirds (10–35 mf in an average of 20 μl blood throughout the observation period) showed significantly ($P < 0.05$) lower eosinophil response than that of control jirds (Fig. 4).

DISCUSSION

The present study clearly shows that mf of *B. pahangi* are capable of reducing eosinophil re-

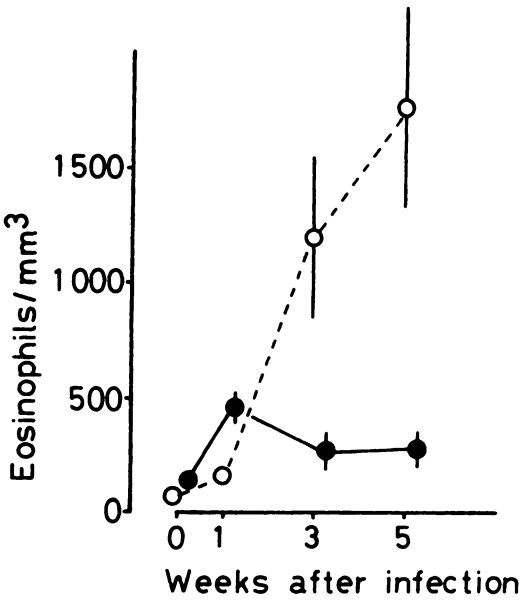

FIGURE 1. Eosinophil responses of chronically (>16 weeks) *B. pahangi* infected jirds (●, n = 8) and age-matched control jirds (○, n = 7) to the infection with 100 *B. pahangi* L3. Values are averages; vertical bars indicate SEM.

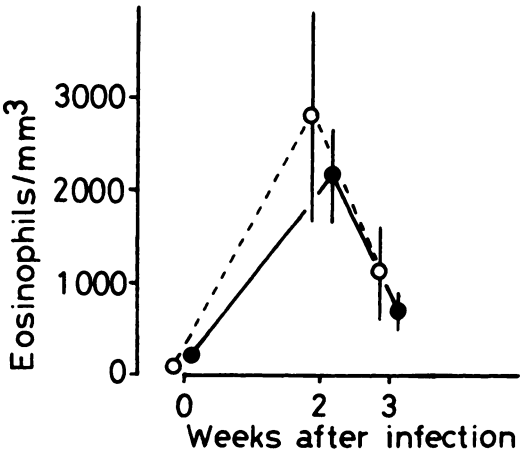

FIGURE 2. Eosinophil responses of chronically (>16 weeks) *B. pahangi* infected jirds (●, n = 5) and age-matched control jirds (○, n = 5) to the infection with *T. canis*. Values are averages; bars indicate SEM.

sponse of *B. pahangi* infected jirds. The results reported here strongly suggest that eosinophil hyporesponse to subsequent homologous infection in chronically *B. pahangi* infected jirds is caused by mf in the circulation.

Continuous peripheral blood eosinophil response was observed in chronically *B. pahangi* infected (microfilaremic) Wistar rats.[6] The eosinophil response of rats in the patent phase of *B. pahangi* infection is mainly caused by mf, because it disappeared after a microfilaricidal treatment with diethylcarbamazine. However, eosinophil response of jirds at the same stage of infection was very weak, despite the microfilaremic condition.[6] The eosinophil response of chronically infected jirds was weak not only to mf but also to L3 of *B. pahangi* (Fig. 1). It is likely that the weak eosinophil response to micro/macro filariae was caused by species-specific

TABLE 1

Protocols of mebendazole (MBZ) treatment and challenge infection

Weeks after primary infection	0	5–7	16	38
Group A	Primary infection 100 L3/sc	MBZ treatment 20 mg/kg × 14 days	Challenge infection 100 L3/sc	
Infected MBZ-treated	Yes	Yes	Yes	
Infected untreated	Yes	No	Yes	
Uninfected controls	No	Yes	Yes	
Group B	Primary infection 100 L3/sc		MBZ treatment 20 mg/kg × 14 days	Challenge infection 100 L3/sc
Infected MBZ-treated	Yes		Yes	Yes
Infected untreated	Yes		No	Yes
Uninfected controls	No		Yes	Yes

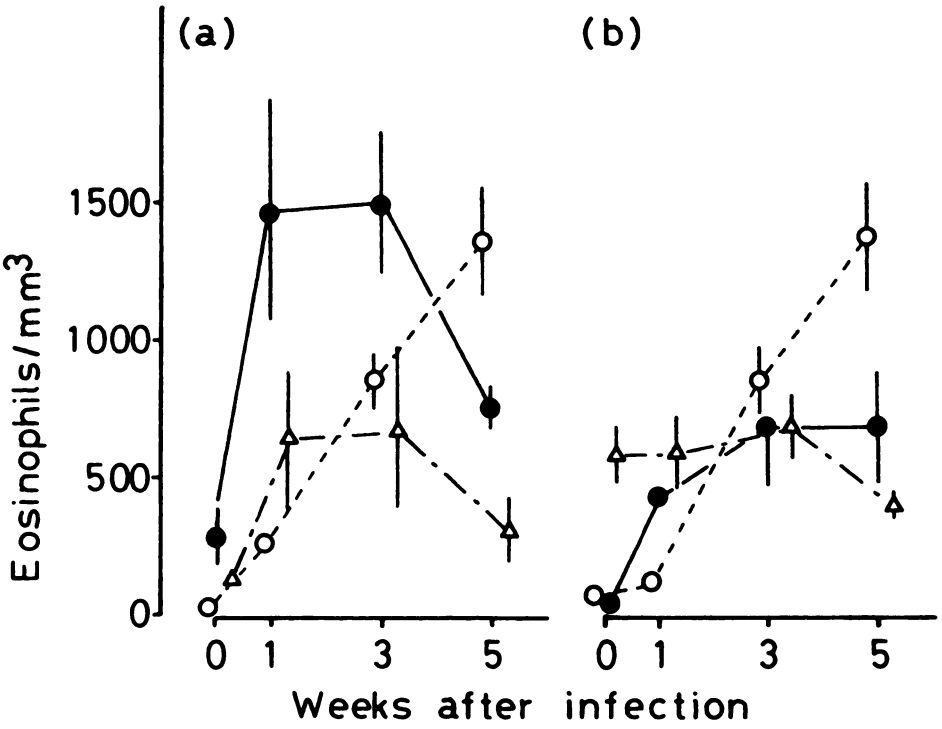

FIGURE 3. (a) Eosinophil responses of *B. pahangi* infected jirds treated with MBZ 5–7 weeks (prepatent period) of primary infection (●, n = 9), infected untreated jirds (△, n = 6), and age-matched control jirds (○, n = 10) to the challenge infection with 100 *B. pahangi* L3. Values are averages; bars indicate SEM. (b) Eosinophil responses of *B. pahangi* infected microfilaremic jirds treated with MBZ 16–18 weeks of primary infection (●, n = 8), infected untreated jirds (△, n = 8), and age-matched control jirds (○, n = 6) to the challenge infection with 100 *B. pahangi* L3.

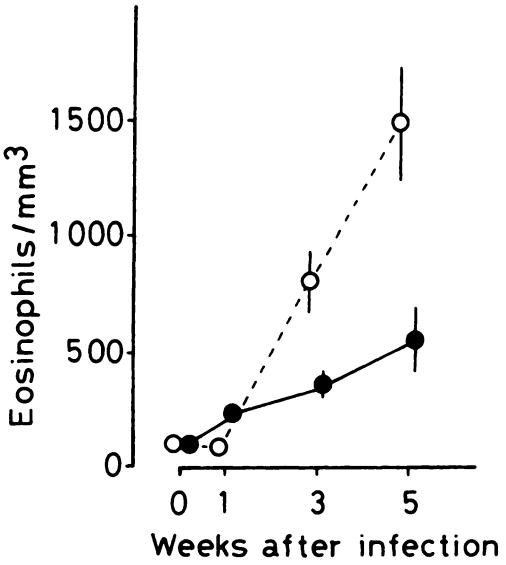

FIGURE 4. Eosinophil responses of *B. pahangi* mf transferred jirds (●, n = 5) and age-matched control jirds (○, n = 9) to the infection with 100 *B. pahangi* L3. Values are averages; bars indicate SEM.

(antigen specific) suppressive immunoregulation, but not by an essential disorder such as depletion or unresponsiveness of myeloid stem cells, because eosinophil response to the heterologous infection with *T. canis* of chronically *B. pahangi* infected jirds was comparable to those of age-matched control animals. Moreover, the results of anthelmintic treatment (Fig. 3a, b) suggest that the suppressive eosinophil response already induced by circulating mf of *B. pahangi* in chronically infected jirds is difficult to restore even by a successful microfilaricidal treatment. Anthelmintic treatment with MBZ for adult worms in a patent period is incomplete; thus, the effect of adult worms on eosinophil reactivity of chronically infected jirds should be further clarified.

Antigen specific[3,4] or nonspecific[1,2] immunoregulatory events have been investigated in chronically *B. pahangi* infected jirds. Antigen nonspecific lymphocyte unresponsiveness was mediated by adherent cells and appeared at an early phase of infection.[2] Antigen specific unre-

sponsiveness was mediated by suppressor T cells, appeared at a patent phase, and was most probably caused by circulating mf.[3,4] Such mf dependent immunosuppression has been reported in human filariasis.[11,12] Recently, a *B. malayi* mf derived suppressor factor has been demonstrated.[13]

Regarding mechanisms of eosinophil response in parasite infection, Basten and Beeson[14] found that a humoral factor from lymphocytes takes part in peripheral blood eosinophil responses of rats with trichinellosis. Afterwards, a wide range of humoral mediators, such as eosinophilopoietin,[15] eosinophil colony-stimulating factor (E-CSF),[16,17] or eosinophil differentiating factor,[18] were reported. Recently, it has been reported that interleukin 5 (IL-5) with or without IL-3, granulocyte-CSF, and granulocyte/macrophage-CSF, has an important role for proliferation, maturation, functional modification, or maintenance of mouse eosinophils.[19] These humoral factors that might regulate eosinophil response were mainly produced by T lymphocytes. Real mechanisms of eosinophil response in jirds still remain unclear, however, *B. pahangi* mf mediated lymphocyte unresponsiveness[3,4] might have caused a reduction of lymphokine production which consequently affected the eosinophil response of jirds.

Eosinophils have been identified as important effector cells for parasites by many workers.[7] Furthermore, a role of eosinophils in the killing of *B. malayi* worms in vaccinated jirds has been demonstrated.[8] Thus, the effect of mf induced eosinophil hyporesponses or enhanced response by the anthelmintic treatment on the susceptibility to the secondarily challenged L3 of *B. pahangi* in jirds warrants investigation.

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