Protective Immunity against Multiple Challenges of *Brugia pahangi* in Mongolian Gerbils Induced by Drug-Abbreviated Infection

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ABSTRACT. Protective immunity against multiple challenge infections was examined in Mongolian gerbils after a drug-abbreviated infection with *Brugia pahangi*. The gerbils treated with mebendazole (MBZ) during the late prepatent period (7–9 weeks of postinfection) were challenged with 5 inoculations of 50 infective larvae of *B. pahangi* at 4-week intervals. The worm burden was significantly reduced 68.6% (19.0 in average number) to that of controls (60.6) and was accompanied with enhanced eosinophil responses 1 week after each challenge. MBZ-treated gerbils suppressed microfilaremia almost completely after the challenge infections.—KEY WORDS: *Brugia pahangi, Meriones unguiculatus*, protection.

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In the Mongolian gerbil-*B. pahangi* system we have recently proved that complete chemotherapy during the prepatent period successfully induced protective immunity to secondary infection [7] which goes with previous reports of *Dirofilaria immitis* infection in natural hosts [1, 5]. If such treatment can induce long-term protective effect against repetitive infections, chemotherapy might be one of the leading candidates in controlling filariasis. In this study, therefore, the protection of gerbils with chemically-abbreviated *B. pahangi* infection against homologous multiple infections was examined.

Outbred male Mongolian gerbils (*Meriones unguiculatus*) were maintained under conventional conditions. At the beginning of the experiments the animals were 10–12 weeks of age. Infective larvae (L3) of *B. pahangi* were obtained from *Aedes aegypti* fed on microfilaremic gerbils 14 days earlier. Gerbils were immunized by subcutaneous (s.c.) infection into the groin with 100 L3 suspended in 0.5 m*l* of Hanks' balanced salt solution (HBSS). For the multiple challenge infections, gerbils were infected 5 times at 4-week intervals with 50 L3 beginning at 15 weeks after the anthelmintic treatment.

The gerbils (immunized group) were treated with 20 mg/kg body weight of mebendazole (MBZ) using a stomach tube for 14 consecutive days starting at 7 weeks (prepatent period) of primary infection [7]. The animals were checked for microfilariae (mf) in the peripheral blood weekly beginning 2 weeks after treatment until just before challenge infection. All animals used in the challenge infection were free from mf in the circulation. Age-matched naive gerbils (control group) were also treated with MBZ in the same manner.

Blood samples for examination were collected from the retro-orbital plexus under ether anesthesia. Absolute eosinophil count was performed using Hinkelman's diluting fluid in a Neubauer's improved hemocytometer. Microfilarial count was made in 5 wet films containing 20 μl aliquot of blood each (total 100 μl).

Necropsy was performed at 10 weeks after the final challenge infection according to the procedure of Denham *et al.* [4] with a slight modification. In short, at necropsy the gerbils were anesthetized by ether and exsanguinated from retro-orbital plexus, then killed by over-dose of ether. The worms were sought in the lymphatics, heart, lungs, testes, kidneys, pleural cavity and peritoneal cavity and in soakings of the various parts of the body and skin in HBSS.

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

Table 1 shows the microfilarial counts of the immunized and those of control gerbils after challenge infections. The immunized gerbils which were treated during the prepatent infection (7 to 9 weeks postinfection) showed no mf until 19 weeks after the first challenge infection. At the necropsy, 2 out of 6 immunized gerbils showed only 2 mf/100 μl blood in average. In contrast, control gerbils first showed mf at 10 weeks after the first challenge, then their mf counts increased gradually. All control gerbils were mf positive (597 mf/100 μl in average) at necropsy. Worm burdens of both groups of gerbils were compared as shown in Table 2. The immunized gerbils harbored 19.0 ± 11.4 worms, and showed 68.6% reduction ($P<0.001$) compared to controls (60.6± 13.1). Although exact count of mf in the uterus of female worms recovered from both groups was not made because of destruction of uteruses, all worms have mf. Eosinophil responses to *B. pahangi* infections in both groups are shown in Table 3. Eosinophil counts were made 1 week after each challenge infection. The eosinophil responses against challenge infections were higher in immunized group than in control group ($P<0.05$).

These results clearly show that continuous protective immunity to multiple challenge infections was induced by

Table 1. Microfilarial counts after challenge infection

Weeks after the 1st challenge infection	Mean mf count/100 μl blood	
	Immunized gerbils (n=6)	Control gerbils (n=5)
0	0	0
10	0	105
13	0	245
16	0	440
19	0	880
26 (at autopsy)	2	597

Table 2. Worm recovery at necropsy

Groups	No. of worms (Mean±SD)	Mean recovery rate (%)	% worm reduction
Immunized gerbils (n=6)	19.0±11.4	7.6	68.6*
Control gerbils (n=5)	60.6±13.1	24.2	

* $P<0.001$.

Table 3. Eosinophil counts after challenge infection

Challenge infection	Number of eosinophils/μl blood (Mean±SD)	
	Immunized gerbils (n=6)	Control gerbils (n=5)
1st	1894±1052*	257± 73
4th	1764± 945*	825±308
5th	1425± 976*	588± 25

Eosinophil count was made 1 week after each challenge infection.
* $P<0.05$.

an appropriate timing of chemotherapy on gerbils with primary *B. pahangi* infection. The gerbils treated during the prepatent period not only elicited 69% reduction of worm burden after 5 serial challenges of a total 250 L3 but also suppressed microfilaremia almost completely during the study period. Since the female worms recovered from immunized animals also have mf in their uteruses, mf could be produced in the circulation. It has been revealed that immunized gerbils were protective against artificially transferred-mf almost completely [7], therefore, produced mf might have been disappeared rapidly from circulation. Similarly, protective immunity to *D. immitis* has been successfully elicited by means of chemically-abbreviated infection in natural hosts [1, 5]. Chusattayanond and Denham [2] reported that treatment of *B. pahangi*-infected gerbils with subcutaneous injection of flubendazole could induce protection against challenge infection by the synergistic effect of chemoprophylaxis and host's immunity. However, the effector mechanisms of these protective responses have not been clarified. In this experiment, immunized gerbils by using MBZ-abbreviated infection showed elevated eosinophil responses 1 week after each challenge infection. Since Yates and Higashi [17] reported eosinophil mediated killing of *B. pahangi* in the gerbils which were immunized by an infection with irradiated larvae, in this protective event enhanced eosinophil response might have a role.

Eosinophil response to homologous challenge infection is suppressed in the chronically *B. pahangi*-infected gerbils, and this suppression is prevented by an appropriate chemotherapy in the prepatent period of primary infection [6]. Eosinophil response is regulated mainly by interleukin 5, a T cell-derived lymphokine [15]. Therefore, MBZ treatment during the prepatent period of this study might has prevented T cell unresponsiveness to the challenge infections. T cell-dependent antigen specific immunosuppression in filariasis is assumed as the induction of suppresser T cells [11, 13] or the anergy of T cell populations [12]. T cell unresponsiveness in filariasis has been reported to be closely related to the appearance of mf in the circulation [9–13], and a suppressor factor was detected in the mf of *B. malayi* [14]. Therefore, amicrofilaremia induced by drug-abbreviated infection might be an important state in gerbils for protection against challenge infection. With regards to this, in gerbil-*Brugia* system, irradiated larvae [3, 15], but not naive one [4, 8], are capable of inducing certain protection probably because of differences in the course of infection with both larvae. Naive larvae are able to mature in the host and reproduce mf but not irradiated ones.

In conclusion, this model might be useful for the development of more effective immunizing methods.

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