Mechanisms of Acquired Immunity against *Brugia pahangi*: Inflammatory Cell Responses and Antibody Formation Following the Challenge Infection in Mice Immunized with Naive Infective Larvae

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Abstract: The mechanisms of resistance of immunized BALB/c mice to intraperitoneally challenged *B. pahangi* were examined. Mice developed significant resistance to challenge infection by immunization with naive *B. pahangi* infective larvae. Remarkable resistance was already established at 5 weeks after immunization and persisted at least until 12 weeks. Resistance in immunized mice appeared at the early phase of challenge infection (5 days post−inoculation) and became more remarkable on day 10 and 15 post−inoculation. Immunized mice showed higher inflammatory cell (lymphocytes, macrophages and eosinophils) responses against *B. pahangi* challenge infection than control mice at 5 and 10 days post−infection. At that time, immunized mice showed high levels of anti−*B. pahangi* IgG and IgM antibodies. Moreover, many killed or weakened larvae encapsulated with inflammatory cells (macrophages and eosinophils were predominant) were observed in the peritoneal cavities of immunized mice. These results suggest that the significant resistance to challenge infection in mice immunized by naive *B. pahangi* infective larvae is due to developed inflammatory cell responses and coexistence of specific antibodies.

Key words: BALB/c mice, Acquired immunity, Inflammatory cells, Antibody

INTRODUCTION

Filariasis is one of the most important tropical diseases. In order to control the filarial infection, many workers investigated various species of laboratory animals so as to develop effective vaccines (Wong *et al.*, 1969, 1974; Rao *et al.*, 1977; Oothuman *et al.*, 1979; Denham, 1980; Storey and Al−Makhtar, 1982; Yates and Higashi, 1986; Chusattayanond

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and Denham, 1986; Kazura *et al.*, 1986; Cross and Hsu, 1987). In mouse−*Brugia* system, vaccination with irradiated or naive infective larvae succesfully induced high protection against *B. pahangi* or *B. malayi* (Hayashi *et al.*, 1984a, b; Carlow and Philipp, 1987). However, the effector mechanisms of acquired resistance to the infection of brugian filariae have not been fully elucidated. Recently, Nakanishi *et al.* (1989) reported that macrophages have an important role on the resistance to a primary *B. pahangi* infection in BALB/c mice. Thus the aim of the present study is to clarify the mechanisms that induce the acquired resistance against *B. pahangi* infection, especially inflammatory cell responses and antibody formation against *B. pahangi*.

MATERIALS AND METHODS

Animals

Inbred male BALB/c mice were raised in our laboratory by sib-mating under conventional conditions.

Preparation of antigen

B. pahangi adult worms were collected from the peritoneal cavities of the jirds which had been infected with 300−400 infective larvae (L3) 3 months before. Worms were washed with saline twice and with phosphate−buffered saline (PBS, pH 7.2) twice. They were homogenized in a glass homogenizer and extracted in PBS by continuous stirring overnight at 4℃. The extract was centrifuged at 12,000 rpm for 1 hr. After centrifugation, the protein concentration of the supernatant was measured by the method of Lowry *et al.* (1951).

Preparation of sera

Immunized or control male mice intraperitoneally *B. pahangi*−inoculated were bled on days 0, 5, 10 and 15 of infection.

Inoculation of parasites

B. pahangi L3 were obtained from mosquitoes (*Aedes aegypti*) which had been fed on microfilaremic Wistar rats 2 weeks previously. Mice were inoculated intraperitoneally with 50 L3 suspended in 0.5 ml of Hanks' balanced salt solution (HBSS).

Immunization and challenge infection

Mice, 7−8 weeks old, were immunized subcutaneously by 100 naive *B. pahangi* L3 in the left groin. Five, eight or twelve weeks later, immunized mice were challenged intraperitoneally with 50 homologous L3.

Recovery of worms from peritoneal cavity

Worms were recovered from the peritoneal cavity of infected mice by the methods

described previously (Nakanishi, 1987). Briefly, the peritoneal cavity was flushed with 5 ml of heparinized HBSS. Worms were collected in a Petri dish and then the carcass was soaked in HBSS in a 50 ml tube for 2 hr. Living worms in a Petri dish and a tube were counted.

Differential counts of peritoneal exudate cells

Peritoneal exudate cells (PEC) were collected from the peritoneal cavity of immunized or control mice by flushing with 5 ml of heparinized HBSS. Total number of PEC at various time of infection was determined using an improved Neubauer's hemocytometer. Differential count was made by counting 200 cells in a smear stained with Giemsa.

Enzyme-linked immunosorbent assay (ELISA)

ELISA for detecting *B. pahangi*-specific IgG or IgM was carried out according to the method of Tanaka *et al.* (1983) using crude *B. pahangi* adult worm antigen (2.5 μg/ml). Peroxidase-conjugated rabbit anti-mouse IgG and IgM were commercially obtained.

Histopathology

Cell adhered worms collected from the peritoneal cavity of infected mice were fixed in 10% formalin, embedded in paraffin and sectioned at 4 μm. Sections were stained with haematoxylin and eosin or Giemsa solution.

RESULTS

Recovery of worms

Immunized and age-matched control mice were injected intraperitoneally with 50 L3. As shown in Fig. 1, significant differences in recovery rates of worms were observed between immunized and control mice. Recovery rates of worms on day 15 post-inoculation (PI) from mice immunized 5, 8 or 12 weeks before challenge infection were significantly lower than that from control mice ($P<0.001$).

To know the time when this difference become apparent, immunized and control mice were injected intraperitoneally with 50 L3 and their peritoneal cavities were subsequently examined at given intervals. As shown in Fig. 2, significant difference was readily observed 4 days PI ($P<0.05$) and this difference was more apparent at 10 and 15 days PI ($P<0.001$).

Peritoneal exudate cells

Inflammatory cell responses against *B. pahangi* infection was examined. Cell counts of peritoneal lymphocytes, macrophages and eosinophils of immunized mice became signifecantly ($P<0.001-P<0.05$) higher on day 5 and 10 PI than those of controls (FIg. 3).

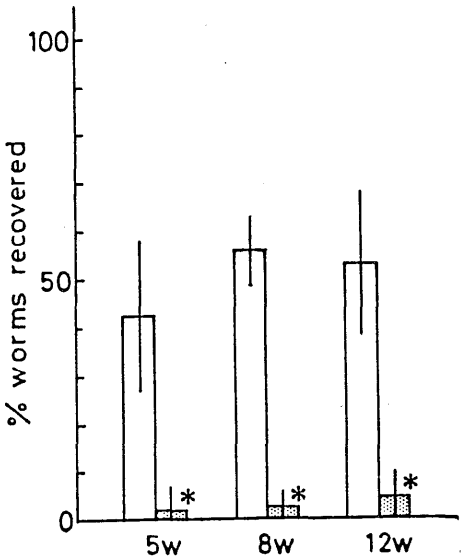

Fig. 1. Recovery rates of worms of naive control (open column) and immunized (dotted column) BALB/c mice at 15 days after intraperitoneal infection with 50 L3 of *B. pahangi*. Challenge infection was done at 5, 8 and 12 weeks after immunization. From left to right each column represents the mean from 11, 11, 5, 5, 11 and 10 mice, respectively. Vertical bars indicate the standard deviation. Significantly lower ($P<0.001$) recovery rates of worms than controls are indicated by an asterisk (*).

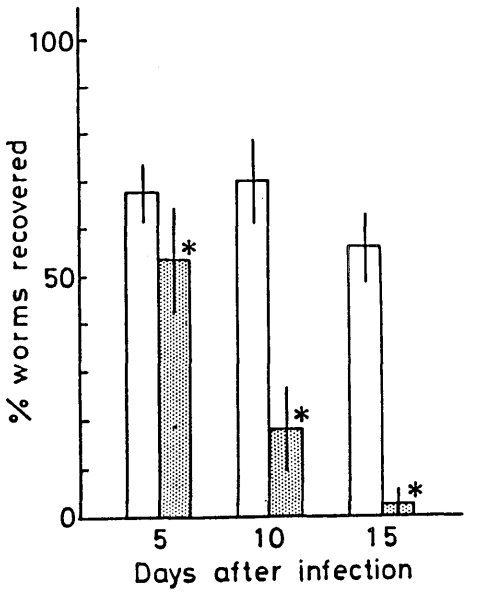

Fig. 2. Recovery rates of worms of naive control (open column) and immunized (dotted column) mice at various days after intraperitoneal infection with 50 L3 of *B. pahangi*. Challenge infection was done at 8 weeks after immunization. From left to right each column represents the mean from 7, 7, 7, 8, 5 and 5 mice, respectively. Vertical bars indicate the standard deviation. Significantly lower (from left to right, $P<0.05$, $P<0.001$ and $P<0.001$, respectively) recovery rates of worms than controls are indicated by an asterisk (*).

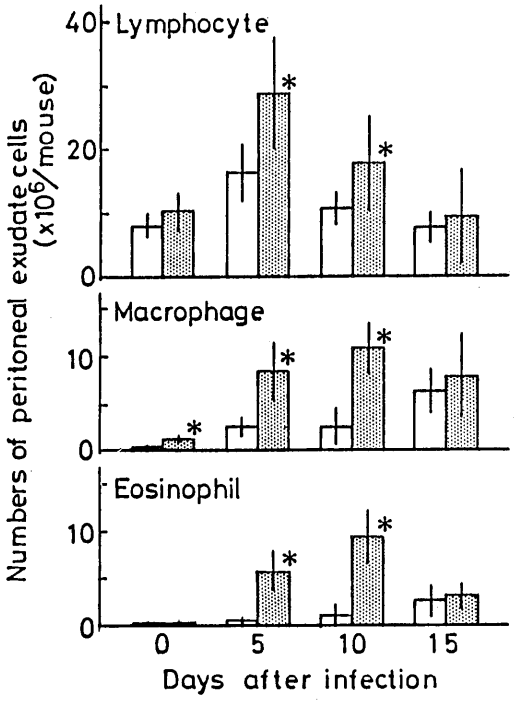

Fig. 3. Numbers of peritoneal exudate cells of naive control (open column) and immunized (dotted column) mice at various days after intraperitoneal infection with 50 L3 of *B. pahangi*. Challenge infection was done at 8 weeks after immunization. From left to right each column represents the mean from 3, 5, 7, 7, 6, 8, 5 and 5 mice, respectively. Vertical bars indicate the standard deviation. Significantly greater numbers of cells than controls are indicated by an asterisk (*). Lymphocyte: from left to right each asterisk represents $P<0.01$ and $P<0.05$, respectively. Macrophage: from left to right each asterisk represents $P<0.05$, $P<0.01$ and $P<0.001$, respectively. Eosinophil: each asterisk represents $P<0.001$.

Antibody levels

Kinetics of specific serum IgG and IgM antibody levels of *B. pahangi* were examined by using ELISA. Great differences in antibody titers to *B. pahangi* worm antigen were seen between immunized and control mice (Figs. 4 and 5). Both classes of antibody levels in immunized mice were significantly higher ($P<0.001-P<0.01$) than those in control mice during the observation period.

Histopathology of cell adhered worms

Cell adhered killed or weakened larvae were observed especially in the peritoneal cavity of immunized mice. Predominant adherent cells to larvae were macrophages and eosinophils (Fig. 6).

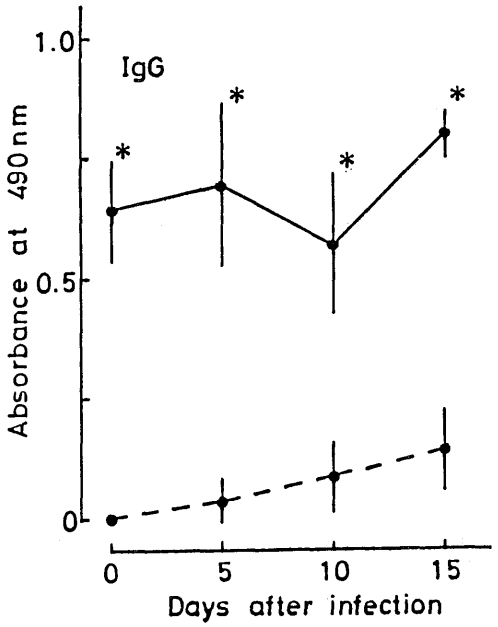

Fig. 4. Anti−*B. pahangi* IgG levels of naive control (− −●− −) and immunized (——●——) mice at various days after intraperitoneal infection with 50 L3 of *B. pahangi*. Challenge infection was done at 8 weeks after immunization. Values are the mean absorbance (experimental − background) at 490nm and standard deviation from 3 to 7 mice. Significantly higher (from left to right, $P<0.01$, $P<0.01$, $P<0.001$ and $P<0.001$, respectively) levels of antibody than controls are indicated by an asterisk (*).

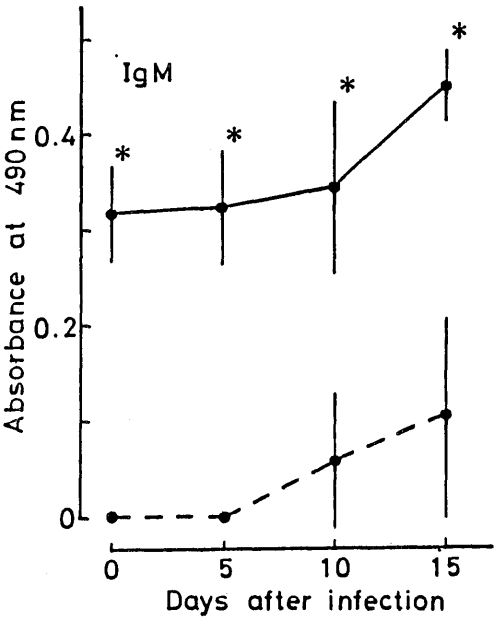

Fig. 5. Anti−*B. pahangi* IgM levels of naive control (− −●− −) and immunized (——●——) mice at various days after intraperitoneal infection with 50 L3 of *B. pahangi*. See legend to Fig. 4.

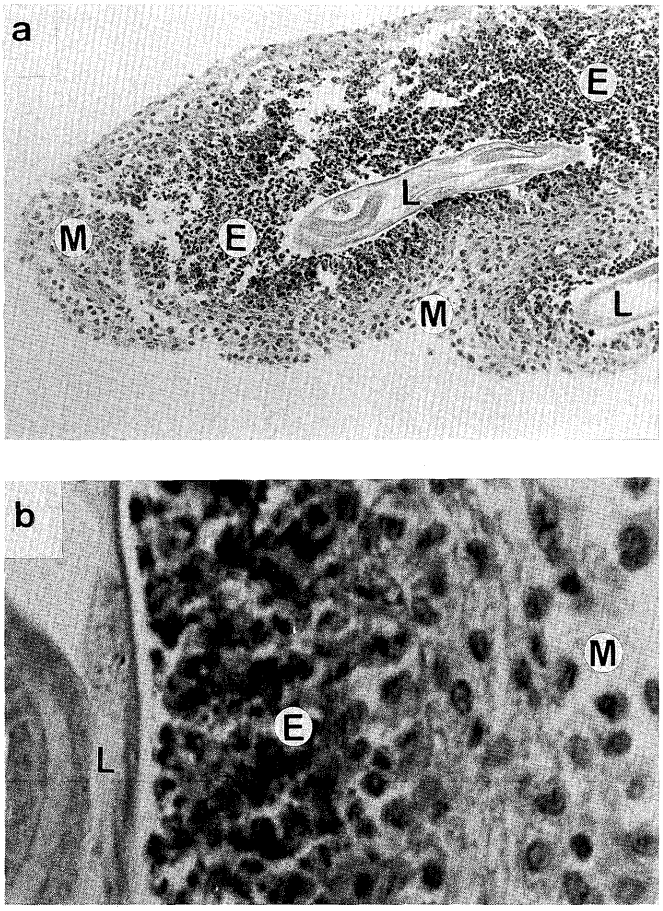

Fig. 6. Typical pictures of a larval worm encapsulated with peritoneal exudate cells. L=larva. M=macrophages. E=eosinophils. H & E. (a): ×125. (b): ×640.

DISCUSSION

The present results clearly indicate that immunized mice show higher resistance and higher inflammatory cell (lymphocytes, macrophages and eosinophils) responses against *B. pahangi* infection than normal control mice. Moreover, these mice showed high level of anti−*B. pahangi* antibodies (IgG and IgM). The time of appearance of high resistance to *B. pahangi* challenge infection in immunized mice corresponded with the onset of marked inflammatory cell responses. In addition, a number of macrophages and eosinophils adhered to killed or weakened larvae. These results suggest that high resistance to *B. pahangi* challenge infection in immunized mice could be attributed to high inflammatory cell responses (lymphocytes, macrophages and eosinophils) and coexistence of high titer of anti−*B. pahangi* antibodies (IgG and IgM), because macrophages and eosinophils have been identified as important effector cells for filarial worms (Haque *et al.*, 1982; Chandrashekar *et al.*, 1985, 1986; Mackenzie *et al.*, 1985; Nakanishi *et al.*, 1989) and antibody−dependent

cell−mediated cytotoxicity have been shown to be one of the major killing mechanisms not only for filariae (Weiss and Tanner, 1979; Mehta *et al.*, 1981) but also for other parasites (Butterworth, 1984). Recently, we demonstrated that macrophages have a major role rather than eosinophils which have minor roles on the resistance to a primary *B. pahangi* infection (unpublished data). However, eosinophils are known to be important effector cells for parasite especially in the acquired resistance (Mahmoud *et al.*, 1975). Therefore, the role of eosinophils on the resistance to *B. pahangi* challenge infection should be further clarified.

As to the mechanisms of inflammatory cell responses are concerned, many kinds of cytokines are known to have regulatory roles (Ewan and Yoshida, 1979; Rocklin *et al.*, 1980). In this study, numbers of lymphocytes and macrophages in peritoneal exdates of immunized mice were significantly greater than those of controls. These cells are capable of producing myelopoietic cytokines such as colony stimulating factors, which are able to act not only as myelopoietin (Metcalf, 1986) but also as functional modifier (Wing *et al.*, 1985; Reed *et al.*, 1987) for macrophages.

It has been reported that T cells have an important role on the passive transfer of protective immunity in helminth infections (Ogilvie and Jones, 1968; Phillips *et al.*, 1975; Riedlinger *et al.*, 1986; Lammas *et al.*, 1987). High protection against *B. malayi* infection is also able to be transfered with spleen cells of vaccinated mice (Hayashi *et al.*, 1984b). However, it is unclear whether the protective event mediated by transfered lymphocytes is in the same manner as the immunized mice proved in this study or not. Therefore, inflammatory cell responses and antibody formations against *B. pahangi* in mice received lymphocytes of immunized mice is now under investigation.

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