Reaginic and homocytotropic IgG antibody response of *Mastomys natalensis* in experimental infections of filarial parasites (*Litomosoides carinii, Dipetalonema viteae, Brugia malayi, B. pahangi*)

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Abstract. Reaginic and homocytotropic IgG antibodies in sera using passive cutaneous anaphylaxis (PCA) tests and antigen from *Litomosoides carinii* were followed in *Mastomys natalensis*, infected with *L. carinii, Dipetalonema viteae, Brugia malayi* or *B. pahangi*. Groups of animals with infections of various ages so as to cover a total infection period of up to 300 to 420 days post-infection (p.i.), depending on the species of parasites, were bled at 1- to 3-week intervals over periods of 50–112 days. In addition, intradermal tests were performed on animals infected with *L. carinii* to detect immediate type hypersensitivity. Reaginic antibodies were usually first detected in the 3rd week after infection. Thereafter, a marked increase of PCA titres was observed in the 4th week p.i., depending on the species of parasites, were bled at 1- to 3-week intervals over periods of 50–112 days. In addition, intradermal tests were performed on animals infected with *L. carinii* to detect immediate type hypersensitivity. Reaginic antibodies were usually first detected in the 3rd week after infection. Thereafter, a marked increase of PCA titres was observed in the 4th week p.i., leading to maximum titres 4 weeks after infection with *D. viteae* and *B. pahangi* and 6 weeks after *B. malayi* infection. Mean maximum titres were between 1:40 and 1:160. Following the peak response, titres decreased markedly until the beginning of patency in infections with *D. viteae, B. malayi* and *B. pahangi* whereas a constant course was observed at this time in animals infected with *L. carinii*. A further rise in PCA titres occurred in all infections around the beginning of patency, resulting in maximum reagin levels in *L. carinii* infections (mean titre 1:80) and moderate titres in the other infections. During early patency there was an inverse relationship between microfilaraemia density and levels of reaginic antibodies. However, in the phase of decreasing parasitaemia in *L. carinii* infected animals, microfilariae counts and PCA titres were directly correlated. Homocytotropic IgG antibodies showed relatively constant PCA titres of about 1:20 in *L. carinii* infected *Mastomys* throughout the observation period. In *D. viteae* infections they were demonstrated at 30 days p.i., reaching titres of about 1:40. *B. malayi* infected animals showed a maximum titre of 1:40 40 days p.i.. Thereafter, titres decreased continuously and homocytotropic IgG antibodies were absent at 110 days p.i.. High titres were observed at day 150 but thereafter sera were negative. *B. pahangi* infected animals showed moderate titres (1:5) 35 days p.i.. Thereafter, antibodies were found at low titres until 115 days p.i.. Intradermal reactions in *L. carinii* infected animals generally increased in size from days 30–60 but decreased when microfilariae appeared in the blood. After 180 days p.i. moderate skin reactions were observed until the end of the experiment 420 days p.i., regardless of whether adult worms could still be found or not.

Helminth infections in man and animals are usually accompanied by increased levels of homocytotropic antibodies (Jarrett 1973). However, little is known in general of the production pattern of these antibodies during the course of infection and the relationship of the response to different developmental stages. Limited studies are available on the time course of homocytotropic responses for experimental filarial infections (see Sadun et al. 1967; Hsu et al. 1974; Benjamin and Soulsby 1976; Gusmao et al. 1981; Hayashi 1982).
The multimammate rat *Mastomys natalensis* has proved to be a suitable host for various filarial parasites (see Zahner et al. 1980; Sänger et al. 1981). Recent studies on homocytotropic antibodies of this animal demonstrated the occurrence and characteristics of two types of homocytotropic antibodies, i.e. IgE and IgG (Zahner et al. 1987).

In the present experiments the pattern of reaginic and homocytotropic IgG responses were followed in four different filarial infections (*Litomosoides carinii*, *Dipetalonema viteae*, *Brugia malayi*) followed in four different filarial infections. Thick-test and skin-sensitizing antibodies were detected in sera and also in non-inoculated *M. natalensis* after inoculation with a filarial antigen (see Zahner et al. 1980; Stinger et al. 1981).

**Material and methods**

**Animals**

*Multimammate rats (M. natalensis)*, strain GRA Giessen, were conventionally bred at the institute and used for the investigation. The animals were kept in groups in polycarbonate cages and fed a diet developed for Syrian hamsters. Drinking water was available ad libitum.

**Experimental infections**

*Litomosoides carinii*. Male and female animals were infected at an age of 4–6 weeks by exposure to mites (*Ornithonyssus bacoti*), which had previously been fed on infected cotton rats with a high level of parasitaemia (Lämmler et al. 1968).

*Dipetalonema viteae*. The parasite strain was maintained in *M. natalensis* and the soft tick *Ornithodoros moubata* (Sänger and Lämmler 1979). Infected ticks were kept at 28°C and 80% relative humidity. Infective larvae were collected from ticks 30 days after exposure on infected hosts anaesthetized with Nembutal. The ticks were crushed in a few drops of Tyrode solution. Male animals were infected with tick-derived larvae at an age of 4–6 weeks by the subcutaneous injection of 50 third-stage larvae in the neck region.

*Brugia malayi*. The subperiodic strain of *B. malayi* was maintained in *M. natalensis* and *Aedes togoi*. Mosquitoes were kept at 26°C and 75% relative humidity. Mosquitoes were stunned by ether 11 days after infection, crushed on glass slides and a few drops of Tyrode solution were added. The larvae were isolated and concentrated with a Baermann funnel. Male *M. natalensis* were infected at an age of 6–8 weeks with 70 third-stage larvae by subcutaneous injection in the neck region (Sänger et al. 1981).

*Brugia pahangi*. Infective larvae were isolated from *Aedes aegypti* 11 days after an infective blood meal as described for *B. malayi*. Male Mastomys were infected at an age of 8 weeks by subcutaneous injection of 70 larvae in the neck region (Sänger et al. 1981).

**Blood and serum samples.** The blood for serum and microfilariae counts was collected by puncture of the retro-orbital venous plexus. The density of microfilaraemia in the peripheral blood was determined by counting chamber methods (Raether and Meyerhöfer 1967). In several groups of *D. viteae* infected animals parasitaemia was also stimulated before bleeding (Sänger and Lämmler 1979) by 10 mg dexamethasone/kg s.c. (group Dv 8) or 5 mg histamine/kg s.c. (group Dv 1).

Sera were isolated after clotting (1 h, room temperature) and centrifugation (15,000 g, 4 min) and stored at −40°C until use.

**Antigens**

*L. carinii* adult antigen. This antigen was used in the PCA and for intradermal tests. Freeze-dried worms were homogenized in distilled water for 2 min at 20,000 revs/min at 4°C, sonicated in an N₂ atmosphere at 4°C for 2 min and extracted with stirring at a concentration of 1 g dry matter to 100 ml distilled water. The homogenate was centrifuged (25,000 g, 30 min, 4°C) and the supernatant was lyophilized.

*L. carinii* microfilariae antigen. This antigen was used for the intradermal tests. Microfilariae were isolated after the method of Kimmig and Braun (1980). Larvae were washed 4 times (200 g, 10 min), suspended 10% in PBS (v/v) and sonicated as above. After extraction overnight at 4°C the homogenate was centrifuged (25,000 g, 30 min, 4°C). The supernatant was stored at −40°C.

**Passive cutaneous anaphylaxis (PCA)**

PCA was performed to evaluate levels of reaginic and homocytotropic IgG antibodies. Apart from *B. pahangi* infections, where pooled sera of groups were tested, reaginic antibodies were determined in individual serum samples. Studies for IgG antibodies used pooled sera of individual groups.

Since previous studies had shown that homocytotropic IgG cannot be demonstrated by PCA in sera containing intact IgE (Zahner et al. 1987), IgG titres were determined after separating it from IgE by ion exchange chromatography (Gorkow and Zahner 1985). DE52 (Whatman, Springfield, UK) was equilibrated with 0.01 M NaH₂PO₄ buffer (pH 8.0). After dialysis against the same buffer, 0.2 ml test serum was applied to a 1 × 6 cm column. Homocytotropic IgG was eluted with 15 ml of a 0.15 M NaH₂PO₄ buffer at pH 7.5. Samples were concentrated to the equivalent of a 1:2 dilution.

Skin-sensitizing antibodies were demonstrated in normal homologous recipients. Intradermal injections were done under Nembutal anaesthesia. Recipients were sensitized in duplicates with 0.05 ml of undiluted and two-fold diluted sera (reaginic antibodies) or in triplicates of the concentrated fractions (homocytotropic IgG) in PBS, starting from a 1:5 dilution. In all recipients PBS controls were included.

Recipients were challenged 5 h (IgG) or 72 h later (IgE) by intravenous injection of 2 mg of antigen in 0.4 ml of a 0.6% solution of Evans Blue in saline (per 100 g body weight) into the retro-orbital venous plexus.

Animals were killed 30 min later with chloroform. After skinning, the reaction was graded on a 0–4 scale. A “1+” reaction was taken as the end-point.

**Intradermal (active cutaneous anaphylaxis : ACA) tests**

Tests were only used on *L. carinii* infected Mastomys and appropriate controls. Animals were anaesthetized and injected intradermally with antigen in 0.05 ml PBS and PBS free of antigen as a control. After 5 min, 0.3 ml of a 0.6% solution of Evans Blue in saline was injected intravenously. The animals were killed after 30 min with chloroform and the reactions were graded on the internal surface of the skin.