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STAGE-SPECIFIC ANTIBODY RESPONSE OF MASTOMYS NATALENSIS DURING THE COURSE OF DIPETALONEMA VITEAE INFECTION

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Abstract. Stage-specific haemagglutinating (HA) and precipitin antibodies (PA) to infective larvae (L_3), adult worms and microfilariae (mf) have been demonstrated in sera of *Mastomys natalensis* during the course of *Dipetalonema viteae* infection using indirect haemagglutination (IHA) and Ouchterlony's gel-diffusion tests. L_3-specific HA antibody titre was shown to be quite low and lasted for shorter period whereas adult-specific antibody response was significantly higher and persisted for longer duration (beyond day 240 post exposure). No precipitin antibody to L_3 stage could be detected, however, significant adult-specific antibody was detected which ultimately tapered off by day 210 post exposure (p. e.). In contrast, mf-specific PA which appeared at the beginning of patency, never disappeared even at the late stage of infection. Mf-specific HA antibody appeared at early incubation period (day 15 p. e.) and after exhibiting two peaks, one in the midst of prepatent period (day 30 p. e.) and the other on day 180 p. e. persisted at low level during the late stage of infection when amicrofilaraemia developed.

Filarial parasites exist in the tissues/body cavities of vertebrate host for a considerable period. Though infective larvae (L_3) and other developing stages have lesser span of existence, adult worms and microfilariae coexist for a prolonged period. During their stay in the host, immune reactions are likely to develop against these life stages. Au et al. (1982) working with *Brugia pahangi* in cat observed development of antibody initially against L_3 followed by adult and then microfilariae (Mf.). Nevertheless cross reactive surface antigens were also shown to exist in mf and adults of *B. pahangi* (Philip et al. 1986). It is therefore worthwhile to understand the immunoglobulin response of host directed against different developmental and adult stages of the parasite which would be of help in stage specific diagnosis as well as to examine immunogenic nature of different life stages and their possible impact in development of resistance. The present communication reports the results of investigation on sequential changes in stage-specific haemagglutinating and precipitin antibody response of *Mastomys natalensis* during *Dipetalonema viteae* infection.

MATERIALS AND METHODS

Infection: Male mastomys were infected with *D. viteae* using the method of Worms et al. (1961). Three groups, each consisting of 7 male mastomys (6 wks old), were infected with 100, 50 and 25 L_3, respectively, obtained from freshly dissected infected ticks *Ornithodoros moubata*.

Assessment of microfilaraemia: Microfilaraemia of each animal was estimated by examining 5 cmm of tail blood taken initially on day 60 p.e. and thereafter every time while collecting blood for serologic investigations.

Preparation of *D.viteae* antigens:

Adult antigen

Adult worms of both sexes of *D. viteae* were recovered from infected mastomys. These were homogenized for 30 minutes in a tissue homogenizer and sonicated (Soniprep 150" MSE, England)

at 10 kcs for 10 minutes. Homogenate was centrifuged at 5000 rpm for 10 minutes and the supernatant was used as adult soluble somatic antigen.

Microfilarial (mf) antigen

Mf were isolated from infected blood by passing through millipore filter (5.0μ). The mf were sonicated at 10 kcs for 10 min centrifuged as above and soluble antigen was isolated and designated as of antigen.

Infective larval (L_3) antigen

L_3 were collected by dissecting infected ticks. These were homogenized and sonicated at 10 kcs for 10 minutes. The soluble part was isoleted by centrifugation and used at L_3 antigen.

Protein estimation: Protein content of antigen preparations was estimated by the method of Lowry et al. (1951).

Serum samples: Blood samples were taken for sera from the retro-orbital plexus of infected mastomys on day 15 and thereafter at fortnightly interval up to day 90. From day 90 onwards blood was collected at monthly interval up to day 270 p.e. Blood samples from age- and sex-matched identical healthy uninfected mastomys were also taken simultaneously as controls. Serum samples were stored at −20 °C till used.

Serologic tests: The serological tests conducted were indirect haemagglutination (IHA) and Ouchterlony's (1949) double gel-diffusion (GD) test. Each serum sample was divided into two parts, one part was decomplemented by heating at 56 °C for 30 min. in a water bath and used in IHA test while the other part was kept as such and used in gel-diffusion reaction.

IHA test: The method of Boyden (1951) as adopted by Rao et al. (1977) was followed. Initially experiments were carried out to find out the optimal concentration of antigen derived from L_3, mf and adults for coating of sheep erythrocytes (SRBC) in IHA test.

Serum samples were also tested following treatment with β-mercaptoethanol.

Test of precipitin antibody: Ouchterlony's gel-diffusion technique (1949) as adopted by Chatterjee et al. (1978) was followed. Reciprocal of highest dilution giving positive precipitin reaction was expressed as precipitin titre. Here also three antigen preparations derived from adult, mf and L_3 were used to monitor corresponding stage-specific response. Normal control sera were kept as usual.

Both IHA and precipitin titres have been expressed as log 2 titre.

RESULTS

It was essential initially to determine the minimal concentration of stage-specific antigens required to react with sera of animals having the same life stage of the parasite. Concentrations of 2.47 mg/ml of adult, 1 mg/ml of L_3 and mf antigen proteins were found optimal for precipitin reaction while for IHA test, 75 μg/ml of adult and 50 μg/ml of L_3 and 25 μg/ml of mf antigen proteins were determined to be the minimal requirements.

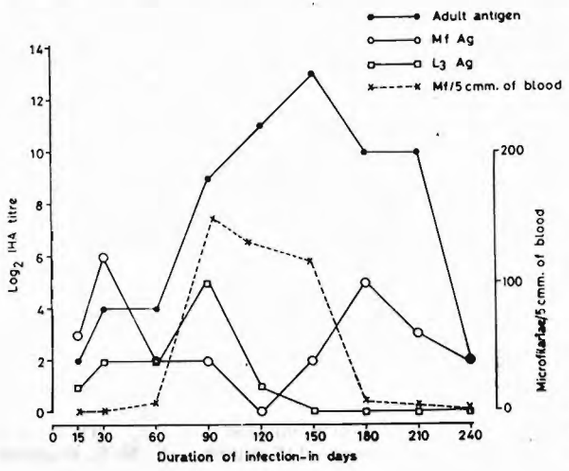

Fig. 1. Haemagglutinating antibody response of host to different life stage of *D. viteae*.

HAEMAGGLUTINATING (HA) ANTIBODY RESPONSE

Fig. 1 shows the details of stage-specific HA antibody during the course of *D. viteae* infection in mastomys.

i) **L_3-specific response**

L_3-specific IHA titre was detected (log_2 titre 1) on day 15 p.e. This level was slightly increased to 2 on day 60. The highest log_2 titre (5) directed against L_3 was found on day 90. Thereafter, the titre started receding abruptly reaching to 1 on day 120, and no HA antibody could be detected on day 150 p.e.

ii) **Adult-specific response**

Like L_3, adult-specific titre could also be detected from day 15 p.e. which increased steadily till day 150 when it was recorded as 14. Afterwards the titre came down to only 2 on day 240 p.e.

iii) **Mf-specific response**

Though mf-specific IHA titre could be recorded on day 15, it varied significantly throughout the course of infection. The highest titre (5—6) was recorded on day 30 and again on day 180 p.e. The mf-specific peak titre was, however, lower in comparison to adult-specific maximum titre. On day 240 p.e., the mf-specific titre was reduced to 2.

It was observed (Fig. 2) that the nature and intensity of HA antibody pattern did not alter (using adult antigen) when the amount of infective inoculum varied (i.e. 25, 50 and 100 L_3). It was also established in the present study that IHA reaction was due to IgM antibody as confirmed with β-mercaptoethanol treatment of serum samples.

PRECIPITIN ANTIBODY RESPONSE

Fig. 3 shows the details of precipitin antibody response against three important life stages of *D. viteae* in mastomys.

i) **L_3-specific response**

L_3 specific precipitin antibody titre could not be detected in infected animals at any stage during the course of 270 days of observation period.

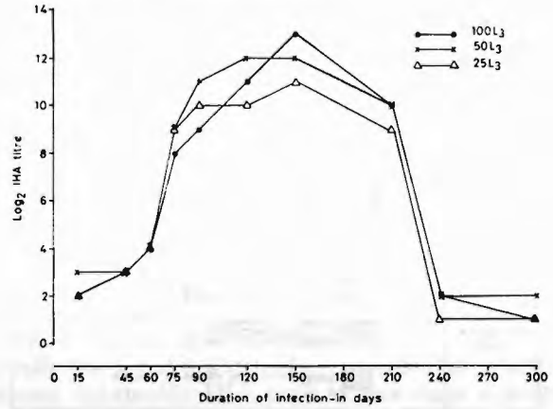

Fig. 2. Haemagglutinating antibody response of host to variable amount of L_3 exposure.

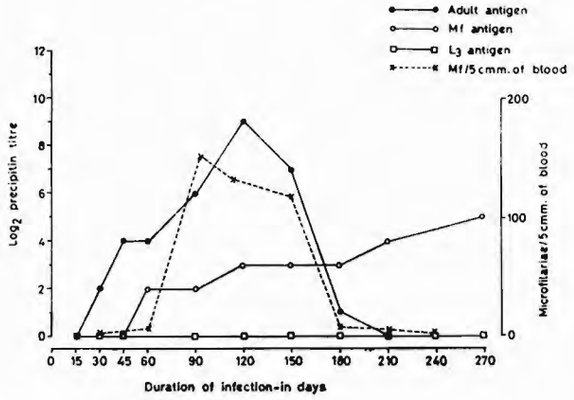

Fig. 3. Precipitin antibody response of host to different life stages of *D. viteae*.

ii) Adult-specific response

Adult-specific precipitin antibody response was significant. Initially, the titre could be detected on day 30 p.e.; thereafter it showed progressive rise reaching to peak level (9) on day 120 p.e. However, adult-specific titre totally disappeared on day 210 p.e.

iii) Mf-specific response

The precipitin antibody titre as detected with mf antigen was somewhat different. Antimicrofilarial antibody appeared at the beginning of patency, i.e. on day 60 p.e. The titre which was quite low (2) increased to 6 on day 270 p.e. The maximum intensity of microfilaraemia (recorded on day 90 p.e.), however, did not coincide with peak antimicrofilarial precipitin titre.

DISCUSSION

The present study which was directed to explore antibody response (haemagglutinating and precipitin types) of the host to different developmental and adult forms of filarial parasite and their possible immunogenic impact, has exposed certain facts. It may be recalled in this connection that filarial parasites undergo certain developmental changes before reaching adulthood in the vertebrate host. Amongst different life stages, L_3 and subsequent forms have comparatively shorter period of existence whereas the adult and mf continue to stay for prolonged period (Fig. 4). During the

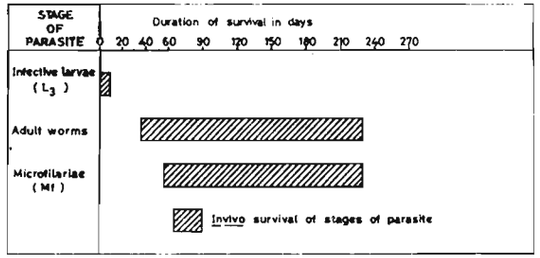

Fig. 4. Prevalence of different life stages of *D. viteae* in mastomys.

presence of various life stages immune responses of different natures and intensities are liable to be elicited besides certain common antigenic responses (Prusse et al. 1982). It is now known that antibody plays pivotal role in the host's immune mechanisms responsible for the clearance of at least one life stage, the mf from infected blood (Weiss and Tanner 1979).

HA antibody as measured with adult antigen showed gradual increase in the titre reaching ultimately a peak level at the period coinciding with peak microfilaraemia (generally between days 90 and 120 p.e.). Interestingly, when the animals developed acquired resistance (day 240 p.e.) against circulating mf, adultspecific IHA titre went down to lowest level. An analysis of of the life stages of *D. viteae* (Fig. 4) reveals that adult worms come into existence around day 45 p.e., whereas certain percentage of them start dying around day 180 p.e. The peak adultspecific IHA titre (13) which was built up on day 150 p.e., appears to be due to the death of macrofilariae (adult worms). The death of adults which started from day 180 p.e., was completed around day 240 p.e., when adult-specific IHA titre also dwindled to its lowest ebb. The death of adult worms was also preceded by attainment of peak level of adult-specific precipitin antibody around day 120 p.e. Thus both agglutinating and precipitin antibodies were raised to the highest level before the onset of death of adult worms.

It was also interesting to observe that when adult-specific precipitin titre totally disappeared on day 210 p.e., IHA titre persisted, though at a low level, up to the present observation period (day 240 p.e.). Persistence of low adult-specific IHA titre for prolonged period could partly be due to mf-specific reaction following the use of antigen derived from adults including females which also contained mf in their uteri.

Of the two antibodies assessed, adult-specific IHA titre persisted throughout the course of infection (from day 15 p.e. to 240 p.e.) whereas precipitin antibody lasted for shorter period (from day 30 p.e. to 180 p.e.). As participating immunoglobulin in IHA titre test was of IgM class (as evidenced by β-ME treatment), the demonstration of filaria-specific IgM will possibly be helpful in diagnosing the disease from early to late stage of infection.

The HA antibody as detected with mf antigen revealed certain interesting as well as intriguing results. There were apparently two peaks of agglutinating antibody titre of which the first one appeared at an early stage of infection (day 30 p.e.) which slowly disappeared during the next 90 days. The second peak was detected on day 180 p.e. The levels of both the peaks were almost equal. However, unlike the first peak, a low IHA titre (2) persisted for more than day 240 p.e. If the course of microfilaraemia is taken into account in relation to mf-specific IHA titre, it would be evident that first peak of titre appeared almost in the midst of incubation period (total incubation period being 55 ± 5 days). This was perhaps due to antigenic cross-reactions between infective larvae or their subsequent developmental stages and mf. The peak level of microfilaraemia generally occurred around day 90 to 120 p.e. when quite low mf-specific HA antibody could be detected. This could partly be due to usual "shift" from IgM to IgG response (Roitt 1980). The other possibility could be the formation of immune complexes due to the prevalence of excess of mf antigen. Earlier, the antibody titres detected in human subjects as well as in experimental animals with high microfilaraemia, were low or absent (Soulsby et al. 1975; McGreevy et al. 1980; Dissanayake and Ismail 1980).

The second peak of mf-specific IHA titre, as observed on day 180 p.e., coincided with developing amicrofilaraemia which was total around day 240 p.e. It may be mentioned that adult worms, especially the females which also contained mf, started dying around day 180 p.e. The second mf-specific IHA peak at this stage appears

to be a secondary response (Carpenter 1975) following death of gravid female worms.

The development of precipitin antibody, as detected with mf antigen, was different from HA antibody response. The initial appearance of precipitin antibody coincided with the appearance of mf in circulation. The mf-specific precipitin titre showed a slow but progressive rise without any peak in between which differed from adult-specific precipitin titre where there was a peak followed by total disappearance of titre. The mf-specific IHA and precipitin antibody titres persisted even after the disappearance of that life stage, whereas adult-specific IHA titre remained at a very low level and the precipitin titre totally vanished in due course. Nevertheless, the intensity of adult-specific precipitin titre was much higher than mf-specific titre. Working with *Litomosoides carinii* infection in cotton rat, Fujita and Kobayashi (1969) suggested that stimuli for the production of antibody in filarial infection were generated principally by adult worms rather than by L_3 or mf. This finding in the present context appears to be more applicable so far as the intensity of adult-specific antibody titre is concerned.

Antibody response to the L_3 stage was the weakest of all life stages of *D. viteae*. No precipitin antibody titre to L_3 could be detected throughout the course of infection which could be due to short persistence of this life stage of parasite in the host. However, positive precipitin titre specific to adult worms as detected on day 30 might be due to cross-reactions with L_4 or pre-adult stages.

The HA antibody specific to L_3, as detected on day 15 p.e., was the lowest in comparison to mf or adult stages. Unlike precipitin antibody, IHA titre (specific to L_3) showed a progressive rise up to day 90 p.e., after which there was a sudden fall in titre, which totally vanished on day 150 p.e. Thus HA antibody response to L_3 stage was the shortest amongst different stagespecific responses.

From the present information it is apparent that different life-stages of filarial parasite do excite immune response of host. However better knowledge on stage specific filarial antigens is needed not only to understand immunopathology of the disease but also to have antigens for immunodiagnosis as well as to explore stage-specificity of immune response to parasites. The pioneering work of Wong (1964) showed the presence of antimicrofilarial antibodies in the serum of dogs resistant to *D. immitis* microfilariae. The IgG response to different life-stages of *B. pahanoi* was also investigated by Tomisto et al. (1983). Very recently the possible role of stage-specific homocytotropic antibody in hypersensitivity reaction to and protective immunity against *D. viteae* in mastomys has been elaborated by Singh et al. (1987).

In conclusion it may be said that the intensity of antibody response to adult parasite is highest in comparison to other important life stages. On the other hand, mf-specific antibodies, though not prevalent in high concentration, persist for the longest time. Nevertheless, L_3-specific antigenic stimulus is the weakest, possibly due to their short stay in the host. The different antibodies also appear to play some role in the elimination of parasitaemia.

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СПЕЦИФИЧЕСКАЯ ДЛЯ ОТДЕЛЬНЫХ СТАДИЙ ИММУННАЯ РЕАКЦИЯ У *MASTOMYS NATALENSIS* В ТЕЧЕНИЕ ЗАРАЖЕНИЯ ФИЛЯРИЕЙ *DIPETALONEMA VITEAE*

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Резюме. Специфические для отдельных стадий гемагглютинирующие (НА) и преципитирующие (РА) антитела против инфекционных личинок (L_3), половозрелых червей и микрофилярий (mf) были обнаружены в сыворотке *Mastomys natalensis* в течение заражения филярией *Dipetalonema viteae* с помощью тестов непрямой гемагглютинации и диффузии через гель (реакция Оухтерлони). Титры L_3-специфических НА антител были совсем низки и встречались более короткое время, тогда как титры специфических для половозрелых экземпляров антител были гораздо выше и встречались продолжительное время (более чем 240 дней после заражения). Преципитирующие антитела у личинок L_3 не встречались, но у половозрелых экземпляров были обнаружены специфические антитела, которые исчезли до 210 дней после заражения. В отличие от этого, Mf-специфические РА, которые появились в начале заражения, никогда не исчезли, даже ни в поздней стадии заражения. Mf-специфические НА антитела были обнаружены в начале инкубационного периода (на 15 день после заражения) и позднее, образуя два пика: в середине препатентного периода (на 30 день после заражения) и на 180 день после заражения. Низкий титр этих антител переживал до поздней стадии заражения, когда появилась микрофиляремия.

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R. G. Soboleva (Ed.): Nasekomye i kleshchi Dal'nego Vostoka imeyushchie mediko-veterinarnoe znachenie. (Medically important insects and mites of the Far East). *Publ. House Nauka, Leningrad 1987, 311 pp., 146 Figs. Price 3.50 R.*

The publication summarizes the contemporary knowledge about two groups of arthropods important for human and veterinary medicine: Insecta and Acarina. Both large chapters of the text are introduced by general characteristics, morphology of adults, and methods of conservation and determination. The individual systematic units of different level are uniformly treated, with data on their morphology, biology and harmfulness. A great attention is devoted to the correct determination of the parasites. Keys are given mostly for adults or for females only, and when necessary also for larvae, accompanied by numerous figures of very good quality. The text closes with an index of Latin names of insects and another one of mites.

The book is a collective work of 16 specialists from various institutes of the USSR. Carefully edited, it reflects a great progress achieved in the studies of parasitic insects and mites in the Far East during the last 25 years. Although oriented regionally, it may very well serve as a useful handbook for practice also in other countries.

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