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Immune Complex and IgE Antibody Formation of the Rats Infected with *Brugia pahangi*. Evasive Mechanism of Filarial Worms from Host Immune Surveillance

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Abstract: Circulating immune complexes (CIC) and filaria parasite specific IgE antibody levels were investigated and compared each other in Lewis and Wistar rats infected with 100 infective larvae of *Brugia pahangi* by two different kinds of inoculation route; i. e., subcutaneous and intraperitoneal infection route. The precipitation of the sera of rats with 6% polyethyleneglycol detected CIC during the course of infections. IgE and indirect hemagglutination (IHA) antibody titers were also detected in the same sera from the infected rats. CIC formation was generally the same regardless of the infection routes, and there were also no appreciable differences in CIC formation between the microfilaremic and the nonmicrofilaremic groups during the course of the infections regardless of strains used. On the other hand, antifilarial IgE and IHA antibody titers varied significantly according to the infection routes and to the patent or nonpatent infection. Both titers of IgE and IHA antibody in the rats infected subcutaneously became always higher and persisted longer period than in the rats with the intraperitoneal infection regardless of the strains used, and both titers in the patent animals were always higher than those in the nonpatent animals especially after the appearance of microfilariae (Mf) in the blood of the infected rats of Lewis and Wistar strain. No correlation could be observed between these individual titers and CIC levels in these filarial infections. The possible role played by CIC in protective mechanisms to the filarial infection was discussed in the special references to the IgE antibody formation.

Key words: Immune complex, IgE antibody, Filarial infection, *Brugia pahangi*, Patent infection.

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INTRODUCTION

Raised levels of circulating immune complexes (CIC) are frequently found in the course of many infectious diseases. The CIC has been observed in clinical filariasis (Gajanana, BheemaRao and Manonmani, 1982; Prasad, Kharat and Harinath, 1983) and experimental animals (Karavodin and Ash, 1981), but the identity of antigens and antibodies involved in CIC has little been studied. On the other hand, helminths have been shown to have a unique ability to induce IgE antibody in man and experimental animals. We have reported that the unique ability to induce IgE antibody resulted in the allergen located in the helminths (Fujita, 1975), and we could obtain a highly purified allergen from *Dirofilaria immitis* (Fujita and Tsukidate, 1981). The allergen was located in the excretory and secretory products exhausted from the worm (Fujita and Tsukidate, 1982). This paper reports CIC and parasite-specific IgE antibody formation in the course of two different infections of *Brugia pahangi* in two strains of rats, and the comparison is made between levels of CIC present in circulation and IgE antibody formation.

MATERIALS AND METHODS

Experimental infections: A total 20 male Lewis rats as well as 20 male Wistar rats (Tarami Animal Lab., Nagasaki Japan) were used in two separate experiments. Infective larvae (L_3) of *Brugia pahangi* were obtained from *Aedes aegypti* (Liverpool strain) infected 14 days previously by blood meals on infected jirds. Each of 10 Lewis and Wistar rats were infected with 100 L_3's subcutaneously into the left inguinal region. In the other group of the 10 rats from each of Lewis and Wistar strain, one hundred of L_3's were administered intraperitoneally. Microfilariae (Mf) were examined either with blood from the orbital sinus or with fluid from the abdominal cavity at weekly intervals after infection.

Detection of circulating immune complexes (CIC): CIC were precipitated from the serum by 6% polyethyleneglycol (Wako Co. Ltd., Tokyo Japan) according to Creighton, Lambert and Miescher, 1975, with slight modification. The precipitated samples were centrifuged at 3,000 r.p.m.. The pellet was dissolved in the distilled water equal in volume to the initial volume of serum. The OD (280nm) was determined using an aliquot of the aqueous immune complexes solution diluted to 1/10 with 0.1N NaOH. Control noninfected rats sera were always employed in this experiment, and the amounts of CIC after infection were calculated from the difference between the test and control sera.

Passive cutaneous anaphylaxis (PCA) and indirect hemagglutination (IHA) test: Antifilarial IgE antibody titers were determined in PCA reaction (Fujita, 1975). Test sera were serially diluted with PBS and injected intradermally into normal indicator rats in 0.05ml volumes. Seventy-two hours later, these rats received 1.5mg of *D. immitis* adult antigen intravenously along with 1.0 ml of a 1% Evans Blue Solution. Thirty minutes later, animals were sacrificed and their skin was reflected to determine the positive bluing

reactions. Reactions greater than 0.5cm in diameter were considered positive, and results were expressed as the reciprocals of the greatest dilution of sera yielding positive reactions. The *D. immitis* antigen was used for these filaria parasite specific IgE antibody determinations because of the difficulty in obtaining adequate amounts of the homologous *B. pahangi* antigen. A part of sera was also tested by IHA test according to previous paper (Fujita, 1975).

RESULTS

Parasitology

Two separate experiments were carried out in each strain of Lewis and Wistar rats. In one of these, rats were given a single subcutaneous inoculum of $100L_3$'s. The interesting finding common to two strains of rats was the fact that regardless of the strains used for inoculum, only 20% of animals became microfilaremic, the remainder never developing patent (i, e., microfilaremic) infection. However, in animals which became Mf positive, the prepatent period varied depending on the strains, being 9−10 weeks in Lewis strain and 11−12 weeks in Wistar strain. The microfilarial density varied slighly for rats of both strains, ranging 30 to 240 Mf/ml of blood in Lewis and 30 to 60 Mf/ml in Wistar strain. On the other hand, in the experiment of the intraperitoneal infection, no animals from both strains became microfilaremic.

CIC levels during filarial infection

Sequential serum samples from Lewis and Wistar strain of rats infected with L_3's subcutaneously or intraperitoneally and age and sex matched controls were individually analysed for CIC. Figure 1 shows the mean values of CIC of both strains in two different infection groups; i. e., the subcutaneous and the intraperitoneal infection groups. It can be seen that the difference of CIC formation in both infection groups was prominent only in the initial phase in the rats infected of Lewis strain, namely the amount of CIC of the

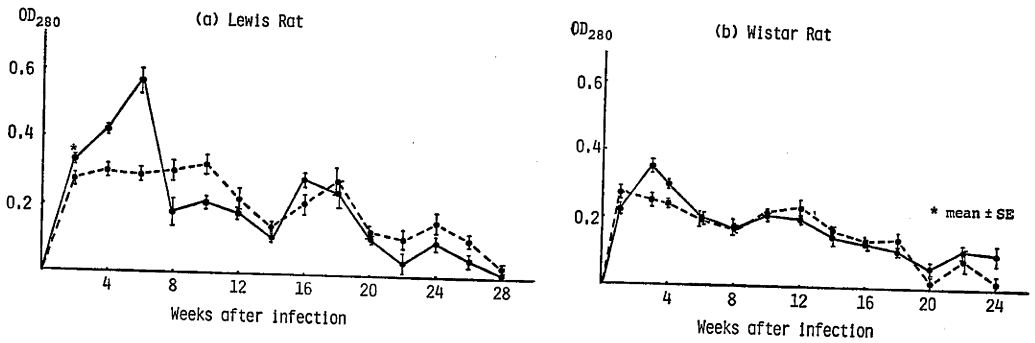

Fig. 1. CIC of the Lewis (a) and the Wistar (b) rats infected with *B. pahangi* subcutaneously (——) or intraperitoneally (……).

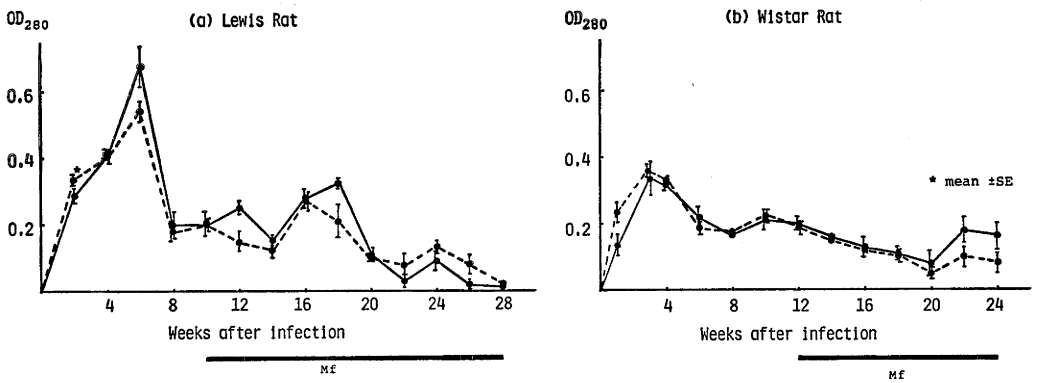

Fig. 2. CIC of the Lewis (a) and Wistar (b) rats with the patent infection (——) or with the nonpatent infection (……).

subcutaneous infection group became larger than that of the intraperitoneal infection group in Lewis rats. CIC formation in the other phase of infection was almost the same regardless of the strain used and of infection route. Then, comparison of CIC formation was made between the microfilaremic rat group and the nonmicrofilaremic rat group in both Lewis and Wistar strain. As shown in Figure 2, amount of CIC in two strains was completely same between the two groups; there were no appreciable differences in CIC formation between the microfilaremic and the nonmicrofilaremic group during the course of infections regardless of the strains used. The general trend include a peak in CIC at 5 weeks after infection in Lewis strain and 4 weeks in Wistar strain, and a gradual decline in CIC levels to 8−13 weeks in Lewis and to 5−8 weeks in Wistar and then a slight increase. This second rise in CIC levels in infected animals was not as high as the initial peak in any strain of rats.

Filaria specific IgE and IHA antibody responses according to infection route

There were two distinct peaks in the IgE antibody response in the subcutaneous infection group of both Lewis and Wistar strains. However, in the intraperitoneal infection

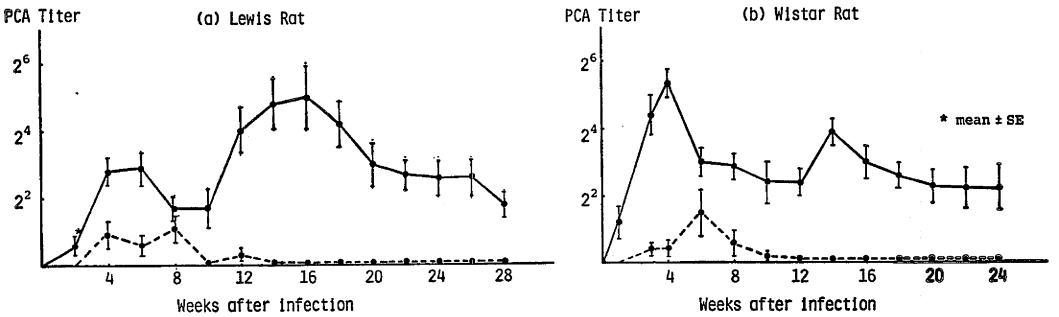

Fig. 3. Filaria specific IgE antibody responses of the Lewis (a) and the Wistar (b) rats infected subcutaneously (——) or intraperitoneally (……).

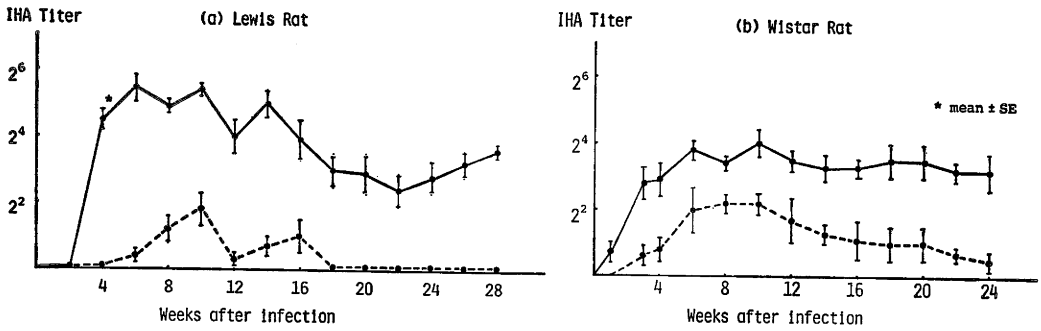

Fig. 4. IHA antibody responses of the Lewis (a) and the Wistar (b) rats infected subcutaneously (——) or intraperitoneally (……).

group, only one lower slight peak in the intial phase of the infection was observed, as shown in Figure 3. In IHA antibody response, higher antibody titers were always observed in subcutaneous infection group than those in intraperitoneal infection group in both strains of rats. The IHA titers of the Lewis rats infected intraperitoneally appeared only a slight rise, but those of the Wistar rats infected intraperitoneally showed relatively higher titers but always lower than those of the subcutaneous infection group of the same strain of rats (Fig. 4).

Filaria specific IgE and IHA antibody responses of the patent or the nonpatent infection

Two distinct peaks emerged when the development of filaria specific IgE antibody was examined in the microfilaremic and in the nonmicrofilaremic infection of Lewis and Wistar rats, as shown in Figure 5. Once microfilaremia became established in the patent animals, however, antifilarial IgE antibodies were produced much more and these persisted at higher levels than those of nonpatent animals for the duration of the experiment. An

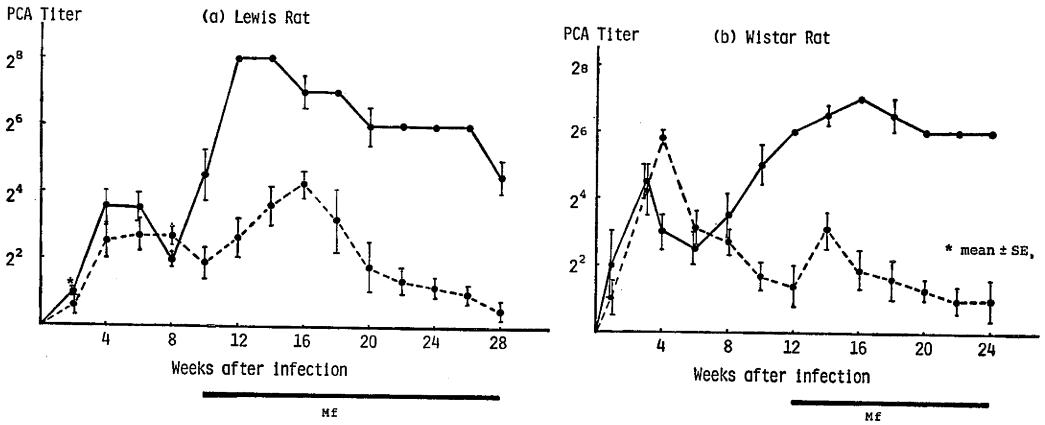

Fig. 5. Filaria specific IgE antibody responses of the Lewis (a) and the Wistar (b) rats with the patent infection (——) or with the nonpatent infection (……).

essentially identical pattern of IgE antibody development was found in both Lewis and Wistar strains, although initial peak of IgE antibody responses of Wistar rats were relatively higher, but second peak were slightly lower or almost same than those of Lewis rats. the titers in patent animals were always higher than those of the nonpatent animals especially after the appearance of microfilariae in the blood, probably because the subcutaneously inoculated L_3's became adult worms, and these worms continued to exhaust the allergen in the patent hosts and the allergen stimulated high and long lasting IgE antibody in the hosts.

Figure 6 showed the IHA antibody responses of the microfilaremic and of the non-microfilaremic rats of both strains. As shown in Figure 6, animals with patent infections demonstrated persistently higher or increasing IHA antibody titers while those not developing patency showed a progressive falloff after 10 or 12 weeks after infection.

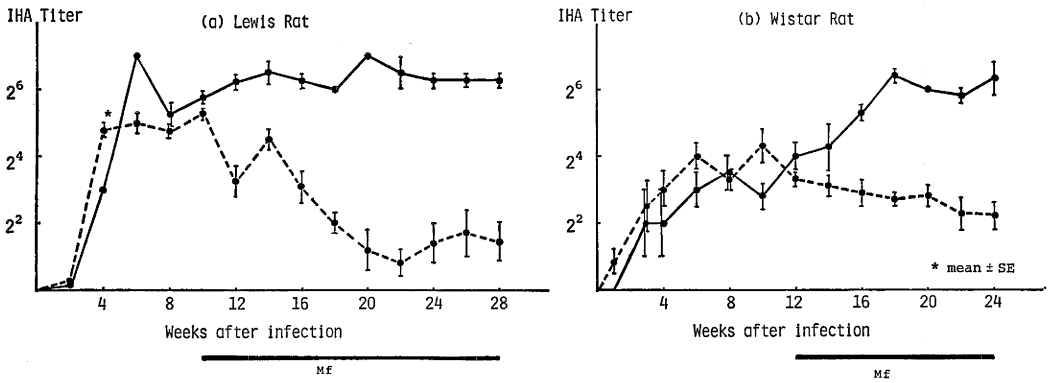

Fig. 6. IHA antibody responses of the Lewis (a) and the Wistar (b) rats with the patent infection (——) or with the nonpatent infection (·····).

Correlation of CIC levels to IgE and IHA antibody levels

The same sample of the serum drawn for the analysis of CIC in two different kinds of the infected animals of both strains was also analysed for IgE for IHA antibody titers. Rank correlation test showed no correlation between these individual antibody titers and CIC levels.

DISCUSSION

The ability of the filaria parasite to successfully evade the host's immune mechanisms throughout a long course of infection is a subject of considerable immunological interest (Fujita, 1984). The filarial parasite have been shown to exert strong immunoregulatory effects upon the host. Immunosuppressive responses of both celluler and humoral immunity have been reported in both experimental animals (Lammie and Katz, 1983) and human infections with filaria (Ottesen, Weller and Heck, 1977). Piessens *et al.* (1982)

reported that the human lymphatic filariasis induced much amount of antigen specific suppressor T lymphocyte, while Weler (1978) showed that the filaria infections reduced the activity of the helper T cell function in the hosts. Despite these observations, the immunoregulatory mechanisms responsiblle for alternations in immunologic reactivity during filaria infections remain poorly defined.

On the other hand, parasitic infection is known to induce a profound deregulation of the otherwise highly regulated IgE system. The role of this IgE in parasitic diseases even now is not entirely clear, though good correlations have been made between IgE production and resistance to infection (Rousseaux-Prevost *et al.*, 1978, Dessein *et al.*, 1981, Gusmao, Stanley and Ottesen, 1981). For example, Gusmao *et al.* (1981) studied the IgE antibody response of the Lewis rats infected with *B. pahangi*. In their study, while those rats destined to resist infection developed early specific IgE against the parasite, none of the animals in which patent infection was eventually established showed a similar IgE antibody response. From these findings, they concluded, though they did not prove, that IgE antibodies played a role in protecting the animals from acquiring filarial infection, and supported the notion that the ability to produce such antibodies may be important in protective immunity to helminths. However, in the present study, similar results could not be obtained. Rather adverse findings to those of Gusmao *et al.* were shown in the present study as mentioned in the results. The author has the notion that IgE response may be also related to the evasive mechanism of filarial worms from host immune surveillance (Fujita, 1984).

A number of experimental systems have shown that CIC can provoke a variety of immunological effects, including inhibition of T- and B-lymphocyte functions and interference with antigen presentation on the surface of macrophage. In recent years, increased interests have been attributed to the role of CIC in the chronic parasitic diseases including filariasis. CIC are thought to be responsible for the associated immunophathology and may also contribute specific host defence mechanism, as in experimental schistosomiasis IgE containing CIC could successfully stimulate macrophage mediated cytotoxity against schistosomulae (Capron *et al.*, 1977).

The present study was designed to examine the levels of CIC formed during two different infections and their relationship to the IgE antibody formation in the various stages of the filarial life cycle, from the entry of infective stage larvae to the development and persistence of pateint infection. This study demonstrated that CIC were present with almost the same levels in the sera of animals with filarial infections by either subcutaneously or intraperitoneally inoculation in both strains of Lewis and Wistar rats, and also showed that CIC levels were independent either to the microfilaremic or the nonmicrofilaremic infections. These individual animals displayed difinite trends in peak complex activity during 3 to 6 weeks after infections, and the peak of CIC rapidly decreased to the same period when inoculated infective larvae became adult worms. The immune complexes demonstrated here in experimental filarial infections may be acting as immunoregulators of the host re-

sponse but they appeared not to play a important role for the host to be the patent or the nonpatent status.

This findings of a dynamic pattern of CIC levels during the course of the filarial infections may be useful for the analysis of the filarial defense mechanisms, although understanding of their true significance will depend on their further experimentation. Characterization of the antigenic composition of the complex and determination of relative antigen or antibody excess at critical points in the infection would necessary to dissolve the problems whether CIC provide a true modulatory effect or are simply a by-product of this infection. These problems are currently being studied in our laboratry.

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フィラリア感染ラットの免疫複合体形成と IgE 抗体産生．フィラリア虫体の免疫回避機構との関連について

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フィラリア虫体は，宿主の免疫機構を巧妙に免れる術を備えている．今回著者は，鼠径皮下および腹腔内という2つの異なった感染経路で *Brugia pahangi* を感染させた2系統のラットにおいて，流血中に存在する免疫複合体の形成状況を調べ，同時にそれらの宿主に産生された IgE および IHA 抗体を測定して相互に比較し，虫の免疫回避機構の一端を知ろうとした．

免疫複合体の形成状況を経時的に観察すると，ラットの系統によって量的な差異があるものの，感染経路や虫の感染状況とはほとんど無関係に形成されることがわかった．すなわち，免疫複合体は，Wistar 系ラットに比べ，Lewis 系ラットにおいて幾分多量に形成された．しかし，それらは感染経過と共に徐々に減少し，虫が完全に成熟すると形成されなくなることが，2系統ラットの鼠径皮下および腹腔内の各々の感染で観察された．また，免疫複合体の形成は，Mf 検出ラット群および未検出ラット群において本質的な差が見られなかった．

一方，IgE 抗体や IHA 抗体は，感染経路や虫の成育状況によって大きく左右されることがわかった．すなわち，虫の成育状況が比較的良好な鼠径皮下感染群は，腹腔内感染群に比べ，それぞれの抗体をより高価に産生した．また，Mf 検出群と未検出群とを比較すると，初期の抗体産生はほとんど同じ値であったが，中期から後期にかけては，はるかに Mf 検出群で高い抗体価を産生することが，Lewis および Wistar 系ラットにおいてそれぞれ観察された．

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