Basic Studies on Filaria and Filariasis

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Although Japanese parasitologists and physicians have published many papers on the epidemiology, clinical signs and symptoms, treatment, and control of filariasis, they also conducted basic research on filaria or filariasis using materials obtained from patients and animal models. This basic research includes morphology, immunology, physiology and pharmacology.

Recently, most papers are written in English and published in peer-reviewed international journals. However, decades ago, scientific papers were usually written in Japanese and published in local journals. Therefore, there are many valuable papers that have been hidden from foreign eyes. In this review article, the authors shed light on these hidden papers published years ago. The research subjects reviewed in this article are: (i) periodicity of microfilariae, (ii) immunology, (iii) mode of action of diethylcarbamazine, (iv) parasite-intermediate host relationship, and (v) others.

1 Studies on the periodicity of microfilariae

The periodicity of microfilariae, which was first reported by Manson in 1881 [1] in China, has long been one of the research subjects that parasitologists are interested in. It is universally accepted that the periodicity of microfilariae, or the circadian rhythm of microfilariae, is due to the periodic migration of microfilariae between the peripheral blood and the lung capillaries, that is, in the case of Wuchereria bancrofti in Japan, microfilariae were found in the finger-prick blood at night but not daytime, when they were confined to the lungs (nocturnal periodicity). The periodic migration of microfilariae between the two sites was verified by postmortem examination (Hayashi, 1925) [2], and by biopsy of various organs and tissues such as the liver, kidney and lung (Tsurumi and Takeda 1940; Hamada, 1958) [3,4], but the most impressive study was done by Kawasaki (1958) [5]. He used venous catheterization on 7 microfilaria carriers in Kagoshima at different times of day to find sites of microfilaria concentration. He counted the number of microfilariae in blood obtained from the cubital vein, femoral artery, right auricle, right ventricle, pulmonary artery, pulmonary capillary, hepatic vein and renal vein. His findings clearly showed that the pulmonary capillaries were the regular site of diurnal concentration of W.bancrofti microfilariae.

For Dirofilaria immitis, the periodic migration of microfilariae was supported by Kawakami and Nagasawa (1926) [6], Murata (1939) [7] and Shibata (1965) [8]. Some parasitologists studied the mechanisms of periodicity of microfilariae in relation to the circadian rhythm of the host, and others in relation to the response of microfilariae to external stimuli.

1.1 Effects of host rhythm on microfilarial periodicity

Attempts were made to alter the microfilarial cycle by subjecting the host to continuous light or continuous darkness. Sugamura (1921) detected microfilariae in the peripheral blood during the daytime when patients were kept in the dark [9]. Otsuji (1958) detected microfilariae in the peripheral blood late at night in summer, and early at night in winter when the sunset is hours earlier than in summer [10]. However, Era (1959) found only a few microfilariae in the peripheral blood during the day when patients worked in a coal mine during daytime for 20 days [11].

On the other hand, Era (1959) studied the circadian rhythm of D. immitis microfilariae in dogs forced to walk during the night and sleep during the day for 21 days. A significant fall in microfilaria count was found at night, and a rise in microfilaria count during the day [11]. In dogs infected with D. immitis, lowering the body temperature was followed by a remarkable fall in the count and a less marked circadian rhythm of microfilariae (Katamine et al., 1960) [12]. High pressure breathing and hyperventilation produced a significant increase in microfilaria count and less marked circadian rhythm of microfilariae (Shibata, 1965) [13].

Yoshida (1966) attempted to examine the circadian cy-

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circle of microfilariae of patients who traveled from Okinawa to Bolivia every week for 2 months [14]. Interestingly, the periodicity of microfilariae became adapted to the light/dark cycle of the location of the passenger ship.

Although other valuable experimental evidence was accumulated, it was not possible to identify the physiological factors in the hosts that caused the circadian cycle of microfilariae.

1.2 Response of microfilariae to external stimuli

Masuya and his colleagues conducted intensive studies on the mechanism of periodicity of microfilariae in relation to the response of microfilariae to external stimuli, especially to light. Masuya (1976) noted diffuse autofluorescence and numerous fluorescent granules in the body of the microfilariae of highly nocturnal species like W. bancrofti [15]. These granules were not found in non-periodic species (Setaria, Onchocerca, etc.). The comparison of the distribution and number of autofluorescent granules in the microfilarial specimens in 21 strains of 13 species collected from around the world revealed an approximate parallelism between the pattern of periodicity and the density of autofluorescent granules. It was concluded that the granules were acting as photoreceptors responsible for the negative phototaxis of microfilariae. Microfilariae of W. bancrofti had been reported to show negative phototaxis (Suganuma 1921) [9]. Masuya’s group chemically analyzed the granules of nocturnal W. bancrofti and found that they contain vitamin A and carotenoid substances.

1.3 Effect of diethylycarbamazine on microfilarial periodicity

Various drugs have been reported to exert an effect on microfilaria count or periodicity of microfilariae. Diethylcarbamazine (DEC) had a significant effect on the circadian cycle of microfilariae (Katamine et al., 1952) [16]. Administration of a small dose of DEC (0.02-0.1g) induced an immediate rise in microfilaria count during the daytime. DEC induced the highest count 5-15 min after oral administration of DEC and altered the defined circadian cycle of microfilariae (Tamura 1954; Iwamoto, 1971) [17,18]. Based on these findings, the DEC-induced rise in microfilaria count was used for the diagnosis of bancroftian filariasis during daytime (DEC provocation test).

Although many significant experimental findings were accumulated by Japanese parasitologists, the mechanism of microfilarial periodicity remained unknown. There are reviews by Katamine (1970), Katamine (1972) and Masuya (1993) [19-21]. Outside Japan, the “oxygen barrier theory” was proposed by Hawking (1975), who found that microfilariae flowed out of the pulmonary capillary barrier into the peripheral blood when the pO2 difference between the pulmonary artery and vein decreased to below 55 mmHg [22].

2 Immunological studies

The immunological studies conducted in Japan cover a wide range of research subjects. Fujita et al. (1983) [23] and Tajima et al. (1983) [24] found the interesting fact that prevalence of adult T cell leukemia (ATL) was geographically very similar to that of bancroftian filariasis in Japan. They showed the close relationship between the anti-filaria antibody titers and anti-ATL virus titers in the endemic area of filariasis. Sato (1991) proposed the hypothesis that filarial infection stimulates the augmentation of IL-2 receptor on T cells [25].

Extensive studies on filaria-induced modulation of host immunological reactions were conducted by Fujita and his colleagues. Since their papers were published in international journals, those papers are not reviewed in this article.

Some parasitologists dealt with the mechanism of killing of microfilariae and protective immunity against filarial infection. Kobayakawa et al. (1974) stressed the role of sensitized lymphocytes in the killing of microfilariae using the Litomosoides carinii-cotton rat model [26]. Their in vitro cytotoxicity study revealed a high mortality rate of microfilariae cultured in the presence of sensitized peritoneal exudate cells. The diffusion chamber study showed that sensitized splenic and peritoneal exudate cells had a significantly high microfilaricidal activity.

Hayashi et al. (1984a, 1984b) examined the effect of vaccination with radiation-attenuated infective larvae of Brugia malayi and B. pahangi on BALB/c mice [27,28]. Vaccinated mice showed 33.8-99.5 % reduction in recovered worms in the challenge infection as compared to the control, and passive transfer of protective immunity with serum and/or spleen cells from vaccinated mice to normal mice was successful. Moreover, Tanaka (1986) succeeded in the production of monoclonal antibodies that produced a significant microfilaria reduction in mice and promoted in vitro adherence of normal mouse spleen cells to microfilariae [29]. Hayashi et al. (1984c) stressed the role of the mononuclear phagocyte system (MPS) in DEC-induced clearance of microfilariae in the L. carinii-cotton rat model. DEC-mediated clearance of microfilariae was remarkably enhanced by the activation of MPS and depressed by the blockade of MPS [30].

3 Parasite-intermediate host relationship

It has been universally recognized that microfilariae exsheath in the midgut and invade the thoracic muscle of a
mosquito and that the rate of exsheathed microfilariae in the midgut has a close relationship with the susceptibility of mosquitoes to the filarial parasite. Japanese parasitologists have clarified significant facts in relation to the exsheathment of microfilariae, the invasion of microfilariae into the muscle and the susceptibility of mosquitoes.

Aoki (1971a 1971b) recorded the fact that microfilariae of *W. bancrofti* exsheath *in vitro* on a clot of blood or agar plate [31,32]. When microfilariae were placed on the agar or clot, they immediately started to move about within the sheath, breaking the anterior tip of the sheath and completing the exsheathment. These observations strongly suggest that the exsheathment of microfilariae in the midgut of mosquitoes is triggered when the movement of microfilariae is severely restricted by the formation of a clot of blood.

Yamamoto *et al.* (1983) reported that many sheathed microfilariae passed through the midgut wall and invaded the muscle [33]. Their additional experiments revealed that the sheathed larvae successively developed to the infective larvae when they were artificially inoculated into the muscle of mosquitoes.

Yamamoto *et al.* (1985) and Kobayashi *et al.* (1986) reported that the susceptibility of mosquitoes to filarial worms was directly related to melanization or encapsulation of microfilariae in the muscle of mosquitoes [34,35]. When *B. malayi* microfilariae were digested by *Armigeres subalbatus*, most of them were subjected to melanization. When *B. pahangi* microfilariae were digested by the same species of mosquitoes, they were not encapsulated and successively developed to infective larvae. Kobayashi *et al.* (1991) devised an *in vitro* method by which melanization of microfilariae was quantitatively assessed, and they revealed that the exsheathed *B. malayi* microfilariae were easily encapsulated by the hemolymph of *A. subalbatus*, while only a few exsheathed *B. pahangi* were encapsulated [36].

4 Mode of action of diethylcarbamazine

The mode of action of DEC is a research subject that has challenged many Japanese parasitologists over the years. The papers cited in this review describe the exciting results regarding the mode of action of DEC. However, these papers have gone mostly unnoticed outside Japan, because they were published in Japanese.

Fujimaki (1956, 1958) examined the serum concentration of DEC in patients infected with *W. bancrofti* using the method of Luberan, which requires a large volume of blood for a test, and determined the minimum concentration of DEC effective against microfilariae of *W. bancrofti* [37,38]. Sakuma *et al.* (1967) examined the distribution of DEC in the body of mice and rats that were intraperitoneally injected with 'H-DEC [39]. The autoradiography showed that DEC was rapidly distributed to various organs, the highest radioactivity being observed at 20 min after injection. Although DEC was excreted rapidly from the kidney and stomach wall, relatively high radiation was found to remain in the muscle, brain, lungs, salivary glands, lymph nodes, etc.

Maeda (1968) and Harada (1970) made electron microscopic observations on the spermatogenesis, oogenesis and fertilization of *D. immitis*, and reported a novel finding that DEC inhibited spermatogenesis of *D. immitis* [40,41]. Shigeno *et al.* (1983) proposed the prophylactic action of DEC against the filarial worm, that is, by using a *B. pahangi* and jird model, they revealed that DEC was effective against the 3rd and 4th stages of *B. pahangi* [42].

5 Other studies

Prof. D. Katamine, Nagasaki University, and Prof. B. S. Seo, Seoul National University, conducted the Japan-Korea Joint Research Project on brugian filariasis in Cheju Island, Korea over a period of 3 years (1970-1972). The project realized distinguished results in the area of epidemiology and control of brugian filariasis. The other valuable results obtained by the project are as follows.

Domestic cats were successively infected with *B. malayi* in Cheju Island, Korea (Nakajima *et al.*, 1976) [43]. The establishment of a *B. malayi*-cat model encouraged the Japanese parasitologists to carry out comparative studies on *B. malayi* and *B. pahangi*. Aoki *et al.* (1976), using scanning electron microscopy, demonstrated a unique hook and 3-4 spines at the anterior tip of microfilaria of both species of filarial worm [44]. Sakamoto (1980) examined the lymphatic pathology of infected cats using lymphography and a histopathological method [45]. The pathologies observed at the acute stage of infection were dilatation or tortuosity of lymphatics, leakage or stasis of contrast medium in lymphatics etc. The chronic pathologies were collateral formation, aiastomoses of lymphatics etc. DEC induced a strong inflammatory reaction in lymphatic vessels. Sakaguchi *et al.* (1982) examined the chromosomes of *B. malayi* and *B. pahangi* [46].

Acknowledgement

This paper is revised from Asian Parasitology Vol.3 Filariasis in Asia and Western Pacific Islands, 131-136 by The Federation of Asian Parasitologists in 2004.
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