Immunosuppression in bancroftian filariasis

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Summary

Immunological function in Filipino patients with bancroftian filariasis, manifested as either asymptomatic microfilaraemia or chronic obstructive disease, was compared with that found in healthy control subjects living in the same area. As a group, patients with filariasis had raised serum IgG levels, impairment of antibody responses to tetanus and typhoid vaccines, and suppression of delayed hypersensitivity skin reactions to heterologous antigens. This immunosuppression in filariasis may result from antigenic competition and may contribute to the development of secondary infections.

Introduction

Immunological responses have been shown to be impaired in human protozoal infections such as malaria (McGregor & Barr, 1962; Greenwood et al., 1972) and Gambian trypanosomiasis (Greenwood et al., 1973). The effects of helminthic infections on humoral and cell-mediated immunity to heterologous antigens have been studied principally in animal models. In trichinosis, allograft rejection (Svet-Moldavsky et al., 1970; Ljungstrom & Huldt, 1977), antibody formation in response to sheep red cells (Faubert & Tanner, 1971; Ljungstrom & Huldt, 1977) and to Japanese B encephalitis virus (Cypess et al., 1973) and the production of plaque-forming cells to sheep erythrocytes (Faubert & Tanner, 1974; Lubiwiek & Cypess, 1975) have all been shown to be suppressed. Similarly, immunodepression has been observed in animal filarial infections. Hamsters and gerbils infected with Dipetalonema viteae had impaired antibody responses to bovine serum albumin and reduced splenic plaque-forming cells to sheep red cells (D'Alesandro & Klei, 1976). Moreover, the responsiveness of spleen cells to T lymphocyte mitogens was suppressed in animals infected with Brugia pahangi (Portario et al., 1976).

This investigation was undertaken, therefore, to determine whether chronic infection with the lymphatic-dwelling worm, Wuchereria bancrofti, produced immunosuppression in human filariasis. We have investigated humoral and cellular immune responses to unrelated antigens in patients with asymptomatic microfilaremia or chronic obstructive filarial disease and compared them with the responses of healthy, uninfected individuals living in the same community.

Materials and methods

Patients and control subjects from villages in the adjacent provinces of Sorsogon and Catanduanes, Republic of the Philippines were studied. Infection with nocturnally periodic W. bancrofti is highly endemic in this low-lying coastal region (Estrada & Basio, 1965) with many patients having evidence of chronic obstructive disease. Filarial transmission is facilitated by cultivation of abaca (Musa textilis) for the production of Manila hemp; larvae of the principal vector (Aedes policulus) breed in the leaf axils of the plant (Cabrera & Arambulo, 1973). Each subject was examined and a clinical history taken using protocol B recommended for filariasis surveys by the World Health Organization (1974). Blood samples were taken by venepuncture in the evening and one ml of anticoagulated blood examined after Nuclepore(R) filtration (World Health Organization, 1974). 32 patients had chronic obstructive filariasis with hydrocele and/or elephantiasis of the legs (97% males; mean age 51 ± 13 years). Nine subjects had asymptomatic microfilaraemia (100% males; mean age 49 ± 12 years). 38 subjects had neither microfilaraemia nor evidence of obstructive disease (92% males; mean age 45 ± 14 years).

Serum levels of immunoglobulins (lg) G, A and M were measured with Behringwerke immunodiffusion plates. Standard solutions were obtained from Behringwerke.

Subjects were immunized with tetanus toxoid (Commonwealth Serum Laboratories, Melbourne, Vic.), 0.5 ml subcutaneously (s.c.) and typhoid vaccine (CSL), 0.1 ml s.c. Blood was collected at the time of immunization and again two weeks later. Haemagglutinating antibodies to tetanus toxoid and precipitating antibodies to Salmonella typhi H antigen were measured as previously described (Forbes, 1971). Patients were considered to fail to respond to immunization when no antibodies were detectable.

Antibodies to pooled Escherichia coli antigens were measured as described by Webster et al. (1974).

Delayed hypersensitivity reactions to intradermal injections of 0.1 ml Candida albicans (Dermato-
Table I—Immunoglobulin levels (grammes per litre) in control subjects, patients with obstructive filariasis and in subjects with asymptomatic microfilaraemia

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>IgG Mean ± S.D.</th>
<th>IgA Mean ± S.D.</th>
<th>IgM Mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects</td>
<td>33</td>
<td>22.3 ± 4.7</td>
<td>3.15 ± 1.18</td>
<td>1.77 ± 0.70</td>
</tr>
<tr>
<td>Obstructive filariasis</td>
<td>26</td>
<td>26.4 ± 6.8</td>
<td>3.16 ± 1.18</td>
<td>1.66 ± 0.71</td>
</tr>
<tr>
<td>Asymptomatic microfilaraemia</td>
<td>9</td>
<td>25.5 ± 6.5</td>
<td>3.49 ± 1.39</td>
<td>2.11 ± 1.13</td>
</tr>
</tbody>
</table>

phyton O(R), 1:100, Hollister-Stier Laboratories, Spokane, Wash.), mumps skin test antigen (Eli Lilly, Indianapolis, Ind.) and Streptokinase-streptodornase (Varidase<sup>(R)</sup>, Lederle, Pearl River, N.Y.) diluted in 0·9% saline to streptokinase 10 units and streptodornase 2·5 units per ml were measured at 48 hours. Induration of 5 mm or more in diameter was classified as a positive reaction. Patients were considered to have a negative response if they failed to react to at least one antigen.

Results

Immunoglobulin levels

Serum Ig levels are shown in Table I. Serum IgG levels were elevated in patients with obstructive filariasis (P<0.01, "t" test) and in patients with asymptomatic microfilaraemia as compared with control subjects. Serum IgA and IgM levels did not differ significantly among the various groups.

![Fig. 1. Titres of antibody to tetanus toxoid before and after immunization. Each solid line represents two patients and each broken line one patient, with the dot on the left being the preimmunization titre and that on the right the postimmunization titre. Titre is log<sub>2</sub> of reciprocal of highest dilution at which haemagglutination was observed.](image)

Antibody responses

Eight of 31 control subjects (26%) responded to immunization with tetanus toxoid. Only one of 35 patients with either form of filariasis (3%) responded to tetanus immunization (Fig. 1); this suppression was statistically significant (P<0.025, χ<sup>2</sup> with Yates correction). Similarly, there was a suppression of antibody responsiveness to S. typhi in patients with filariasis. 25 of 31 control subjects (81%) had antibody titres of one in 40 or more two weeks after immunization compared with only 15 of 26 patients with obstructive filariasis (58%); this difference was statistically significant (P<0.05, χ<sup>2</sup>, single tail). While typhoid antibody could be detected in every control subject, no antibody at all could be detected in three patients with obstructive filariasis.

There were no differences in levels of antibody to E. coli between the two groups. The geometric mean E. coli antibody titre in 33 control subjects was one in 28 (range one to 690) compared with one in 17 (range 0·5 to 580) in 35 patients with filariasis.

Delayed hypersensitivity skin reactions

All 38 control subjects who were tested had delayed hypersensitivity skin reactions to at least one of candida, mumps or streptococcal antigens. This responsiveness was seen in eight of nine subjects with asymptomatic microfilaraemia (89%) and 28 of 32 patients with obstructive filariasis (88%). The number of control subjects and patients with either form of filariasis who reacted to none, one, two or three antigens is shown in Table II. The suppression of reactivity in patients with filariasis is statistically significant (P<0.01, χ<sup>2</sup>, Brandt & Snedecor’s formula).

![Table II—Number of delayed hypersensitivity skin reactions to a battery of three antigens in control subjects and in patients with filariasis (asymptomatic microfilaraemia and obstructive disease combined)](image)

Discussion

These studies have shown a number of abnormalities of immunological function in patients with bancroftian filariasis. These include elevation of serum IgG levels, impairment of antibody responsiveness on immunization and suppression of delayed hypersensitivity skin reactions to nonfilarial antigens.

Serum IgG levels are commonly raised in populations living in tropical as compared with temperate climates (Turner & Vollcr, 1966; Rowe et al., 1968; Grove et al., 1975). This is presumably due to frequent exposure to the antigenic stimuli of helminths, protozoa, fungi, bacteria and viruses (Wells, 1968). Serum IgG
levels were even higher in our patients with filariasis than in control subjects. This may reflect greater stimulation of the immune system by filarial worms lying in close proximity to antibody-producing cells in the lymph nodes.

Antibody production is impaired by intercurrent infection (Lee et al., 1971; Forbes, 1971) and malnutrition (Jou & Good, 1972), both of which are common in tropical populations. The antibody response to tetanus toxoid in our control subjects was suppressed when compared with that normally seen in Caucasians, but was similar to our observations in Papua New Guinean subjects (Grove et al., 1975). Nevertheless, there was a significantly greater impairment of antibody response to tetanus toxoid in patients with filariasis. Further evidence that this group of patients has impaired ability to produce specific antibody is the significantly reduced antibody titres after immunization with S. typhi. This suppression of specific antibody responses may result from antigenic competition in which the antibody response to one antigen is reduced by prior contact with a second, unrelated antigen (Adler, 1964). Chronic stimulation by filarial worms may therefore interfere with the humoral immune response to heterologous antigens.

Delayed hypersensitivity skin reactions to a battery of antigens offer perhaps the simplest and most effective clinical method of testing the integrity of the cell-mediated immune system. Normal subjects, both in temperate and tropical areas, should react to at least one of the three antigens used (Grove et al., 1975). There was a significant suppression of delayed hypersensitivity skin reactions in patients with filariasis. This impairment of cell-mediated immunity may be analogous to the suppression of splenocyte responsiveness to T cell mitogens shown in experimental B. pahangi infections by Portaro et al. (1976). There was no relationship between the presence or absence of delayed skin reactivity and serum IgG levels or antibody responses after immunization.

The elevation of serum IgG levels, impairment of specific antibody production and suppression of delayed hypersensitivity skin reactions were seen in both patients with asymptomatic microfilaraemia and in those with chronic obstructive disease. These findings are consistent with the observations of D'Alesandro & Klei (1976) who found, in experimental D. viteae infections that all infected animals expressed some degree of immunosuppression which could not be related to the degree or duration of microfilaraemia.

Patients with bancroftian filariasis are particularly prone to develop cutaneous infections. Although other factors such as lymph stasis and skin folding are undoubtedly important, suppression of the immunological defence mechanisms may contribute to the development of secondary infections in these patients.

Our studies have demonstrated generalized immunosuppression with impairment of humoral and cell-mediated immunity against non-filarial antigens. Otterson et al. (1977) have shown recently that there is also a specific cellular immune unresponsiveness in human filariasis. They found that lymphocyte transformation in response to stimulation with filarial antigens was grossly reduced in chronic filariasis, particularly in children with persistent microfilaraemia.

The mechanisms by which specific and non-specific immunity are suppressed in bancroftian filariasis remain obscure; they may include the activity of suppressor lymphocytes, serum inhibitors of cell-mediated immunity, interference with macrophage function, the presence of immune complexes, disruption of lymphocyte traffic or the production of lymphotoxins by the parasite (Ogilvie & Wilson, 1976). Similarly, the pathogenetic significance of immunosuppression in filariasis is also unclear. It may simply be an epi-phenomenon. On the other hand, immunosuppression is a common feature in parasitic infections and may have evolutionary significance for the parasite by providing a means whereby the organism can evade the host's immune response (Ogilvie & Wilson, 1976).

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