



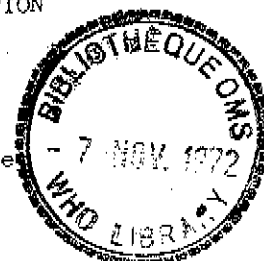
SERUM PROTEINS IN FILARIASIS OF THE LYMPHATIC SYSTEM CAUSED BY  
WUCHERERIA BANCROFTI VAR. PACIFICA

INDEXED

ELECTROPHORETIC ANALYSIS AND QUANTITATIVE IMMUNOCHEMICAL DETERMINATION  
 OF A, M, G AND E IMMUNOGLOBULINS<sup>1</sup>

by

J. P. Moreau,<sup>2</sup> G. Cuzon, G. Pichon, D. Outin-Fabre and J. Lagraule  
 with the technical assistance of N. Lefèbre and S. Lee Sang



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<sup>1</sup> Work carried out by the Section des Laboratoires de Recherches, Institut de Recherches médicales "Louis Malardé", Papeete, French Polynesia (Director: J. Lagraulet).

<sup>2</sup> Assistant, Hôpitaux des Armées.

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The pathogenesis of the clinical manifestations of lymphatic filariasis is still largely unknown and gives rise to considerable controversy, although it seems to be universally conceded that immunopathological processes are involved. However, the very nature of these processes needs elucidation. A possible first approach to the problem is to examine the serum proteins of persons suffering from filariasis. In this study we used two methods of attack: electrophoretic analysis and determination of immunoglobulin levels. The relative isolation of the islands of French Polynesia and the fact that only one type of human filariasis exists in this territory makes the study of particular interest.

## 1. ELECTROPHORETIC ANALYSIS

### 1.1 Material and methods

#### 1.1.1 Collection of sera

This study was carried out on a group of 30 filariasis sufferers with microfilaria counts ranging from 5 to 200 microfilaria per 20 mm<sup>3</sup> of blood. None of these subjects with positive findings of microfilariae in the blood (Mf+) had ever been treated with diethylcarbamazine and none showed chronic lesions. A second group of 30 clinically healthy subjects with no microfilariae in the blood (Mf-) was used for control purposes, although there was no way of proving by any biological test that they had never been infected.

All subjects had been born in French Polynesia, although their ethnic origins varied.

#### 1.1.2 Strip electrophoresis

Two microlitres of the serum were applied at a time to 30 x 160 mm strips of cellulose acetate. Migration was carried out in Elphor reservoirs using a veronal/veronal sodium buffer of pH 8.6 and took place under a steady voltage of 200 V with the initial current adjusted to 0.4 mA per cm. The migration time was 65 minutes. The strips were stained with Ponceau red S, destained with a mixture of ethyl acetate and acetic acid (3 vol./7 vol.) and analysed by integrator.

#### 1.1.3 Disc electrophoresis in polyacrylamide gel

Ornstein's (1964) original technique was used with some modifications. The sera were placed in stock gels of pH 6.7. The separation gels had a 7% concentration of acrylamide and a pH adjusted to 8.9. Migration was carried out in a Shandon reservoir at +4°C with a steady current of 5 mA per column applied for an hour. Voltage varied from 80 to 240 V between the start and end of migration, the leading edge of which was located with bromophenol blue. On removal, the gels were stained with amido black. Fixing and staining were carried out in a 10% solution of acetic acid.

## 1.2 Results

Strip electrophoresis showed the albumin percentages to be 61.9 for Mf+ subjects and 71.7 for Mf- subjects (Table 1). On the other hand, Mf+ subjects had a higher globulin fraction. There was no change in the alpha-1 and 2 fractions, but Mf+ subjects had slightly more beta-globulins, the percentage being 8.1 as opposed to 5.5 for Mf- subjects. However, the most pronounced difference between the two groups appeared in the gamma-globulin fraction, which was 21.1% for Mf+ subjects and only 13.6% for Mf- subjects. This difference has a very high level of significance ( $P < 10^{-9}$ ).

TABLE 1. ELECTROPHORESIS ON CELLULOSE ACETATE STRIP

|     | Albumins |     | Globulins |      |         |      |      |      |       |     |
|-----|----------|-----|-----------|------|---------|------|------|------|-------|-----|
|     |          |     | Alpha-1   |      | Alpha-2 |      | Beta |      | Gamma |     |
|     | M*       | S*  | M         | S    | M       | S    | M    | S    | M     | S   |
| Mf+ | 61.9     | 6.5 | 1.03      | 0.05 | 5.9     | 1.07 | 8.1  | 1.81 | 21.1  | 4.5 |
| Mf- | 71.7     | 3.5 | 1.04      | 0.04 | 5.6     | 1.48 | 5.5  | 1.03 | 13.6  | 2.5 |

\* M = Mean

S = Standard deviation

Electrophoresis in polyacrylamide gel gave a maximum of 22 observed discs for all the sera examined. The average number of discs was 17.6 among Mf+ subjects and 14.7 among Mf- subjects. In the post-albumin zone, the average number of discs was 6.4 for Mf+ subjects and 5.1 for Mf- subjects. The difference was much more pronounced in the gamma-globulin zone, where Mf+ subjects had an average of six discs and Mf- subjects only three.

### 1.3 Discussion

#### 1.3.1 Strip electrophoresis

This technique showed an increase in beta and gamma-globulins. Alpha-1 globulins, which usually increase during the processes of cellular necrosis, and alpha-2 globulins, which generally increase during acute or chronic inflammatory processes, had the same values as in the healthy subjects. Inflammatory processes are thus unimportant or non-existent in filariasis sufferers with microfilariae in the blood. A survey of filariasis cases with elephantiasis would probably give different results, but it is well known that such cases have no microfilariae in their circulating blood.

The rise in globulins thus principally relates to those fractions with which the antibody globulins migrate. These results confirm the findings of the only other electrophoretic analysis made on filariasis sufferers in an area which has only one species of human filaria. In Madagascar in 1965, Dodin et al. (1965) noted that the globulin levels in five cases of lymphatic filariasis caused by *W. bancrofti* were higher than the mean levels found among the indigenous population. A study carried out by Benex & Deschiens (1960) in tropical Africa showed an increase in gamma-globulins compared with the usual mean values. Alpha-2 globulins were also shown to increase slightly, but the survey included filariasis cases at all stages of development.

#### 1.3.2 Disc electrophoresis in polyacrylamide gel

This technique for separating proteins is much more sensitive than the preceding one, since only 0.1 micrograms of protein are needed to produce a visible disc. On the other hand, it is much more difficult to interpret, as the number of discs varies with the genetic variations of certain serum proteins, haptoglobin and Gc globulin in particular. These variations have been studied with the help of disc electrophoresis by Peacock et al. (1965).

In the post-albumin zone, Peacock considered that the positions of the discs 2, 3 and 4 were related to variations in Gc. In our study, no differences in the distribution of these discs were found between the Mf+ and Mf- groups.

On the other hand, the Mf+ group had more discs in the gamma-globulin zone, where the discs for the haptoglobin genetic types 2-2 and 2-1 are found. However, without a benzidine test (Smithies & Walker, 1955), it is practically impossible to identify the genetic types, for two reasons. Firstly, there is interference from the discs a, b and c, which may or may not be present. Secondly, the number of discs seen depends on the absolute amount of each fraction contained in the serum. Now, although the volume of serum is constant, the amounts of the various protein fractions vary. This may explain why the number of discs observed for Mf+ subjects, whose sera had a higher globulin content, was greater on average than the number observed for Mf- subjects.

In conclusion, electrophoretic techniques need to be supplemented by an immunochemical study if the globulins responsible for the increase in gamma-globulins are to be identified.

## 2. QUANTITATIVE IMMUNOCHEMICAL DETERMINATION OF A, M, G AND E IMMUNOGLOBULINS

### 2.1 Material and methods

Twofold serial dilutions of the sera were used. In the IgE determination, sera were lyophilized to give concentrations of 4/1 and 2/1. A microtechnique of the Ouchterlony double diffusion test in agar was used for the determination. 2.5 ml of a 1.5% solution of pure agar in a 0.15 M phosphate buffer of pH 7.1 was run on to 7.5 x 2.5 cm slides. Wells 2 mm in diameter were cut around a central well, the distance between the holes being 6 mm. The test sera were placed in the outer wells. The central well contained specific monovalent anti-Ig A, M and G rabbit sera (Institut Pasteur, Paris) or anti-Ig E goat sera (ICN). Diffusion time was 16 hours in a moist chamber at  $+25^{\circ}\text{C} \pm 2$  and this was followed by washing in saline for 24 hours. The slides were then dried in an oven at  $37^{\circ}\text{C}$  and stained with amido black.

### 2.2 Results

The limiting level taken for each serum was that of the highest dilution giving a precipitin arc. The IgA, IgM and IgG results were plotted on logarithmic probability paper with the dilutions taken in decreasing order as abscissae and the cumulative serum totals as ordinates. This enabled the mean level of each Ig to be determined for the two groups investigated (Table 2).

TABLE 2. MEAN IMMUNOGLOBULIN LEVELS

|     | IgA  | IgM  | IgG   |
|-----|------|------|-------|
| Mf+ | 1/59 | 1/9  | 1/590 |
| Mf- | 1/46 | 1/12 | 1/340 |

Karber's method was used for the statistical analysis of the results. The mean IgA level was 1/59 for Mf+ subjects as opposed to 1/46 for Mf- subjects (the difference is not significant). The IgM levels were 1/9 for Mf+ subjects and 1/12 for Mf- subjects (the difference is significant,  $P = 0.01$ ).

The difference was most marked in the IgG results, where Mf+ subjects showed a level of 1/590 as opposed to 1/340 for Mf- subjects. The Mf+ subjects thus had 1.7 times more IgG globulin and this difference had a very high level of significance ( $P < 0.0001$ ).

As far as IgE globulins were concerned, none of the 30 Mf- sera gave visible precipitin arcs. On the other hand, 18 Mf+ sera gave precipitin arcs, nine from sera concentrated fourfold, six with sera concentrated twofold, two with pure sera and lastly, one with a dilution of 1/2.

There appears to be no correlation between IgE levels and the degree of microfilaraemia.

### 2.3 Discussion

Mf+ subjects showed a considerable increase in IgG globulins. These globulins alone caused the rise in gamma-globulin detected by electrophoresis, especially in view of the fact that the Mf- subjects showed slightly higher IgM levels.

This IgG increase might be taken to be due to constant calls on the immunological system throughout the course of this long-term parasitosis. However, the stimulation does not seem to be very strong, since the level is only 1.7 times higher than the Mf- level.

The most interesting result was that for the IgE globulins. The normal level is known to be from 100 to 700 nanograms/ml. Such concentrations are thus well below the threshold for detection of antibodies by precipitation in agar, which is 5 micrograms/ml. For this reason, this technique is rarely used in the study of IgE globulins and recourse is had to autoradiographic techniques such as the RISA (radio immunosorbent assay) test of Wide & Porath (1966) or the RSRD (radio-active single radial diffusion) test of Rowe (1969).

To increase the sensitivity of the precipitation-in-agar technique we used in this study, we concentrated sera fourfold by lyophilization (no higher concentration was feasible, even after elimination of the serum lipids). Under these conditions, we obtained precipitates with 18 Mf+ sera. Using the lower limit of sensitivity of the technique as a basis for calculation, the IgE levels in the sera could be estimated approximately as over 1250 nanograms for nine subjects, over 2500 for six, over 5000 for two and over 10 000 for the last.

In this way, concentration by lyophilization alone enabled IgE globulin to be detected by the diffusion in agar technique in 18 filariasis cases with positive findings of microfilariae in the blood.

Johansson et al. (1970) have shown that parasitic infections, in addition to asthma and atopic dermatoses, cause an increase in IgE globulins. Lymphatic filariasis is no exception. Manifestations of the immediate hypersensitivity type have long been observed in this disease. It would seem that IgE globulin must be recognized as playing a part in the pathogenesis of such manifestations.

### 3. CONCLUSION

Mf+ subjects who had no chronic clinical symptoms and had never been treated showed a definite increase in gamma-globulins. A more sensitive analysis using disc electrophoresis in polyacrylamide gel confirmed the increase in globulins, but no characteristic protein discs could be identified by this technique in the Mf+ group.

Immunological analysis showed an increase in IgG and IgE globulins. There seems no doubt that IgE globulins play a part in manifestations of the immediate hypersensitivity type observed in lymphatic filariasis. This is not to say that they are the only factors responsible. Indeed, as seen above, Mf+ subjects also had increased IgG globulins, some fraction of which may be anaphylactic antibodies of the heterocytotrophic type.

SUMMARY

Electrophoretic analysis of the sera from subjects with lymphatic filariasis caused by Wuchereria bancrofti var. pacifica, with positive findings of microfilariae in the blood, who showed no chronic lesions and had never been treated, revealed a marked increase in gamma-globulins. Analysis of the immunoglobulins showed the increase to be in the IgG and IgE globulins.

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