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# Immunological status of histoid leprosy

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*Summary* The first report of the immunological profile in histoid leprosy revealed an impaired cell-mediated immunity because of the low percentage of early and total T-lymphocytes, and negative lepromin test. The humoral immunity, however, was greatly increased, this was shown by the increased percentage and absolute count of B-lymphocytes and the raised levels of IgG, IgA and IgM. Hypocomplementaemia (C3) was another significant complement.

## Introduction

Histoid is a unique expression of leprosy so far as the clinical, bacteriological and histopathological features are concerned. The immunological status of the entity is, however, not known. It was, therefore, thought worthwhile to investigate this aspect in detail, the observations made form the subject matter in this paper.

## Materials and methods

Twenty-three histoid leprosy patients, comprising 21 males and 2 females formed the subject material for the study. The diagnosis in each was established on detailed morphological characteristics.[1–4]

Fifteen millilitres of blood was collected under aseptic conditions. The technique of 'E' rosette[5] was used for quantification of T cells, while for B cells the method of 'EAC' rosette[6] was followed. A preliminary total and differential leucocytic count was done from each blood sample in order to obtain the percentage, and absolute count of lymphocytes. The lymphocytes were separated from the heparinized blood by using histopaque solution (Stock No. 1077-1,

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Sigma Chemical Company, P O Box 14508, St Louis, USA) containing Ficoll type 400 (5·7 g/100 ml) and sodium diatrizoate (9 g/100 ml) for gradient centrifugation. After cell separation, their viability was checked with 0·25% Trypan blue (A G Fluka, S F Buchs, Switzerland) and more than 90% of cells were confirmed to be viable. A final cell suspension between 1000 and 10,000 cells $mm^3$ was obtained. For T cell estimation, an equal quantity (0·2 ml) of adjusted lymphocyte cell suspension and 0·5% sheep red blood cells were mixed and incubated at 37°C for 15 min in two sterile glass test tubes. These were centrifuged at 1000 g for 3 min and kept at 4°C. Early and total rosettes were estimated after an incubation period of 2 and 24 h respectively. For estimation of B cells, equal quantities (0·2 ml) of lymphocyte cell suspension and erythrocyte amboceptor complement (EAC) were mixed and incubated at 37°C for 30 min in a sterile glass test tube. The B lymphocytes were counted immediately after incubation.

Immunoglobulins—IgG, IgA and IgM—were measured in the sera by radial immunodiffusion technique[7] using limit diffusion in agar gel. Undiluted sera were used for estimating IgA and IgM, while the serum was diluted 10 times with isotonic saline for IgG. Readings were recorded after 50 h for IgG and IgA and 80 h for IgM. The final value of IgG was calculated by the multiplication of the figure by 10, the dilution factor.

Complement component C3 was measured in the sera by single radial immunodiffusion technique[7] by limit diffusion in agar gel. Monospecific anticomplement C3 antiserum and its three reference standards were obtained commercially (Lallested Laboratories Inc., Lake Hazeltine Drive, USA) after a diffusion time of 48 h, followed by washing in isotonic saline for the same duration, the slide was stained by amido-black stain. It was then destained in rinsing solution, dried and the diameter of the precipitin rings measured. The reference curve was plotted for the values of the reference standard and the square of the ring diameters and the values were read from this curve.

For estimating circulating immune complexes (CIC), equal amounts of serum (0·2 ml) and 8% polyethylene glycol (PEG, mol. wt 6000, BDH, England) were mixed and the precipitate was separated by centrifugation in a clinical centrifuge. It was washed three times with 4% PEG. The composition of the precipitate was determined qualitatively by a double diffusion technique on agar gel using various monospecific anti-immunoglobulins and antihuman C3 and C4 antiserum.[8] Protein concentration in PEG precipitates was also estimated.[9]

Cryoglobulins were detected by keeping the fresh serum at 4°C for 7 days. The cryoprecipitate was harvested by centrifugation at 1500 g for 30 min at 4°C.[10]

A lepromin test—early (Fernandez) and late (Mitsuda)—was performed using lepromin A (armadillo derived, containing 40 million bacilli per ml, obtained from Dr W P Kirchheimer, Chief, Laboratory Research Branch, National Hansen's Disease Centre, Carville, USA, through WHO).

Ten normal individuals and 9 non-histoid, active lepromatous (LL) patients of corresponding age, sex and socio-economic background served as controls.

## Observations

*Histoid leprosy and healthy controls.* The basic haematological profile revealed that the mean percentage of lymphocytes was 33·1% ($P > 0·4$) and the mean absolute lymphocytic count was 2375·1 mm$^3$ ($P > 0·2$) which was statistically insignificant in comparison to controls. The mean absolute counts of early (825·01 mm$^3$) as well as total (1514·78 mm$^3$) T-lymphocytes also had insignificant deviation ($P > 0·4$). The mean percentage of early T-lymphocytes was 34·9% ($P < 0·001$), while the total T-lymphocyte percentage was 64·3% ($P < 0·005$). Both these values were statistically significant.

The mean percentage of B-lymphocytes was 21·9% while their mean absolute count was 517·7 mm$^3$. On statistical evaluation, a significant increase was observed in both the percentage ($P < 0·001$) and the absolute count ($P < 0·001$) in the studied group.

The mean levels (Table 1) of all the three immunoglobulins—IgG, IgA and IgM—were higher in the patients as compared to the controls, but this was statistically significant only in IgG ($P < 0·01$) and IgA ($P < 0·025$). These values are depicted in Figures 1 and 2.

The mean level of complement component was much lower in the patients and this too was statistically significant ($P < 0·001$).

The mean of total protein concentration (1·636 mg/ml) in CIC was significantly higher ($P < 0·001$) in the sera of the patients. The detailed analysis of

**Table 1.** Immunological profile: immunoglobulins, complement C3 and immune complexes

| Parameters | Patients | Healthy controls | Statistical evaluation |
|---|---|---|---|
| Immunoglobulin (mg/dl) | | | |
| IgG | 1623·28 ± 401·98 (725–2064) | 1225 ± 177·99 (975–1500) | S |
| IgA | 317·62 ± 109·71 (113–642) | 221·25 ± 37·56 (167·5–275) | S |
| IgM | 193 ± 79·72 (98–389) | 161 ± 33·23 (107·5–205) | n.s. |
| Complement C3 (mg/dl) | 76·19 ± 14·82 (50–102) | 146·05 ± 24·28 (117·5–188) | S |
| PEG precipitates (mg/ml) | 1·636 ± 1·293 (0·358–5·0007) | 0·097 ± 0·035 (0·052–0·164) | S |

Values are expressed as mean ± s.d. (range); S, significant; and n.s., not significant.

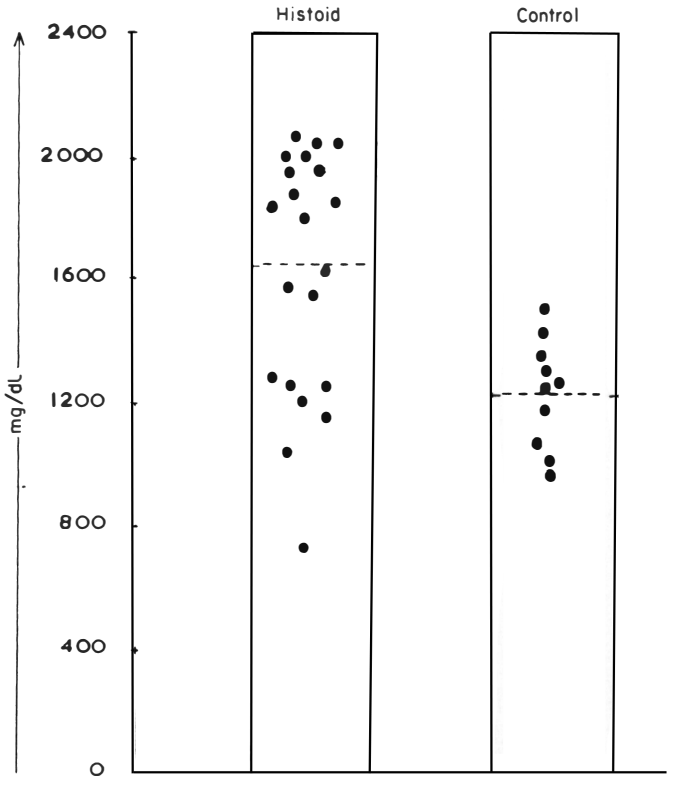


**Figure 1.** Scattergram of IgG, dotted line indicates the mean value.

PEG precipitates is shown in Table 2. The cryoprecipitate was observed in 9 (39·1%) patients and also 4 (40%) controls.

*Histoid leprosy and non-histoid active lepromatous patients.* Basic haematological profile revealed that although the total leucocytic count was statistically higher in histoid leprosy ($P < 0{\cdot}025$), the absolute lymphocytic count did not vary significantly ($P > 0{\cdot}04$) in the two groups. The percentages as well as the absolute counts of both early and total T-lymphocytes were significantly raised in histoid (Table 3). Furthermore, the percentage of B-lymphocytes was also raised significantly in the studied group ($P < 0{\cdot}025$). However, their absolute count did not reveal much variation ($P > 0{\cdot}4$). The levels of immunoglobulins IgG and IgA were significantly lowered in the histoid, while the levels of immunoglobulin IgM did not vary much in the two groups (Table 4). The complement C3 levels were grossly lowered in the studied group ($P < 0{\cdot}001$). Early and late lepromin test was uniformly negative in both the groups.

## Discussion

The results of our study are indeed intriguing, because for the first time they focus attention on the immunological status of histoid leprosy. It is quite apparent that

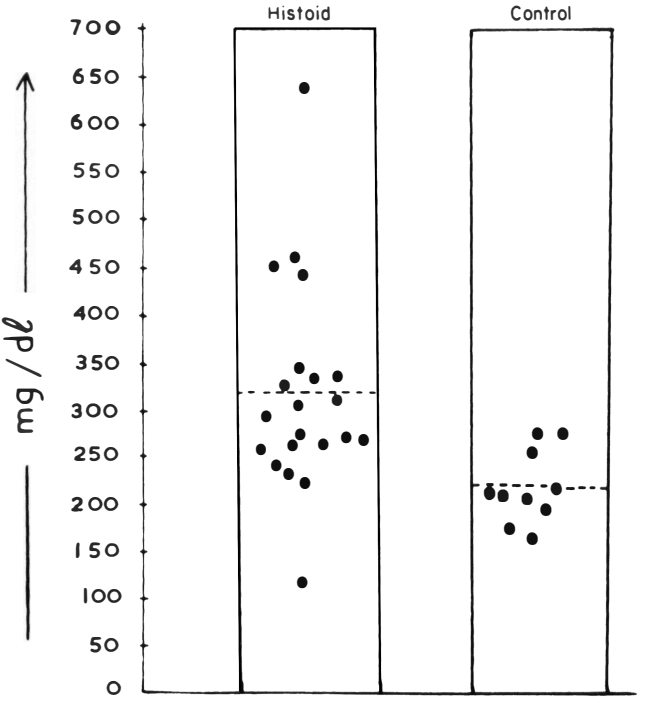


**Figure 2.** Scattergram of IgA, dotted line indicates the mean value.

**Table 2.** Analysis of polyethylene glycol (PEG) precipitates

| Components | Patients | Healthy controls |
|---|---|---|
| IgG | 21 | 6 |
| IgA | 3 | 1 |
| IgM | 16 | 3 |
| C3 | 1 | 3 |
| C4 | 13 | — |

the cell-mediated immunity was considerably impeded in this entity. This was revealed through the low percentage of early and total T-lymphocytes. It was complemented by negative early and late lepromin test. Furthermore, when results of our study were compared with those of non-histoid active LL, it was found that there was a relative increase in cell-mediated immunity in the studied group which may suggest an attempted focalization of the lesion to only limited regions of the body in histoid leprosy.

The humoral immunity too was considerably increased in histoid leprosy. It

**Table 3.** Immunological profile: T and B lymphocytes

| Parameters | Patients | Active LL controls | Statistical evaluation |
|---|---|---|---|
| Early T cells | | | |
| Percentage | 34·9 ± 4·5 (25–40) | 29·7 ± 7·3 (20–45) | S |
| Absolute count | 825·01 ± 254·1 (537·4–1344) | 618·1 ± 243·6 (269–1080) | S |
| Total T cells | | | |
| Percentage | 64·3 ± 5·7 (50–72) | 55·2 ± 10 (40–70) | S |
| Absolute count | 1514·7 ± 427·9 (940·8–2402·4) | 1163·6 ± 444·3 (538–1824) | S |
| B cells | | | |
| Percentage | 21·9 ± 2·9 (16–25) | 18·9 ± 7·2 (10–30) | S |
| Absolute count | 517·7 ± 142 (336–813·2) | 504·9 ± 328·3 (162–896) | n.s. |

Values are expressed as mean ± s.d. (range); S, significant; and n.s. not significant.

**Table 4.** Immunological profile: immunoglobulins and complement C3

| Parameters | Patients | Active LL controls | Statistical evaluation |
|---|---|---|---|
| Immunoglobulins (mg/dl) | | | |
| IgG | 1623 ± 401·98 (725–2064) | 1714·4 ± 373·9 (1210–2700) | S |
| IgA | 317·62 ± 109·71 (113·5–642) | 405·3 ± 75·9 (131–350) | S |
| IgM | 193·48 ± 79·7 (98–389) | 236·1 ± 42·8 (170–265) | n.s. |
| Complement C3 (mg/dl) | 76·19 ± 14·82 (80–102) | 189·4 ± 75·4 (110–280) | S |

Values are expressed as mean ± s.d. (range); S, significant; and n.s., not significant.

was reflected as the increased percentage and absolute count of B-lymphocytes and also by the increased levels of IgG, IgA and IgM. IgG and IgA in particular, were much raised. Interestingly, the findings in non-histoid active lepromatous (LL) patients were more or less identical except for a conspicuous rise in the levels of immunoglobulins IgG and IgA which are largely complementary to our preceding observations (*vide supra*). In addition, a gross hypocomplementaemia (C3) was another salient feature in histoid.

The immunological profile may, thus, indicate that histoid leprosy is a relatively stable component of multibacillary leprosy.

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