Status of Humoral Immune Responses in Leprosy[1]

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Although evidence is building up to suggest that there is a depression of cell mediated immune responses in patients with lepromatous leprosy ([5, 7, 20]) the status of humoral immune responses is not clearly defined. As in other chronic infections, increased levels of gamma globulins with decreased serum albumin have been described in lepromatous leprosy ([12, 19]). However, in one series of patients ([21]) no increase in gamma globulins was detected.

The level of three classes of immunoglobulins have been assessed by several workers, but observations have differed. Using capillary tube diffusion methods an increase in IgG, IgA and IgM was reported by Lim and Fusaro ([16]). These authors using semiquantitative methods of immunonelectrophoresis observed that the rise in IgM was peculiar to lepromatous leprosy and proposed this as a diagnostic criteria to distinguish it from tuberculoid leprosy ([17]). However, an increase in IgM fraction in cases of tuberculoid leprosy has also been reported by other workers ([1]). Sheagren *et al.* ([20]) did not find any change in IgM levels in lepromatous leprosy patients, the rise occurring in only IgG and IgA in their studies. Bonomo *et al.* ([2]) have described hypergammaglobulinemia in lepromatous leprosy patients, and an increase in the three classes of immunoglobulins and haptoglobulins. They found no alterations in IgA and IgM fractions in tuberculoid patients.

These variations could be due to several reasons amongst which are racial differences, the duration of the disease, the length and regularity of treatment, the inclusion of borderline (intermediate) cases in one group or the other etc. We have investigated the status of humoral immune responses in a North Indian group of leprosy patients. The cases chosen for this study were well characterized by clinical bacteriologic, histologic and serologic examination, and only patients in the two polar forms of leprosy (LL or TT) were taken. Careful studies to assess the status of humoral immune responses were also prompted by the indications ([23]) that there is a cooperation of thymus dependant lymphocytes (TDL) in the elicitation of the humoral response, and that there is a depression of the function of TDL cells in lepromatous leprosy ([4, 13, 24, 26]).

MATERIALS AND METHODS

A total of 38 patients were investigated. They were either tuberculoid (8) or lepromatous (30) leprosy cases. No dimorphous (borderline) patients were included. The diagnosis was made on clinical, bacteriologic and histologic grounds. Reactivity to lepromin was also tested in all subjects. Normal volunteers from the laboratory were used as controls. The following parameters were investigated:

Serum proteins and different classes of immunoglobulins. Total proteins were estimated by the Biuret method ([15]). Paper electrophoresis was done to assess the relative proportions of albumin and different fractions of globulins ([14]). Quantitative estimation of IgG, IgA, and IgM was done by radial diffusion in agar gel ([9]). The standards were very kindly provided by Dr. John Fahey of the U.S.A. National Institutes of Health.

Antibody activity of leprosy sera. Leprosy sera were tested against a variety of antigens. Lepromin was obtained from Chingleput Leprosy Sanatorium, Madras, India. PPD was obtained from WHO South-East Asia Regional Office, New Del-

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hi (courtesy Dr. J. de Vries). The presence of precipitating antibodies was tested by Ouchterlony double diffusion method. VDRL antigen, obtained from Veneral Diseases Research Laboratories, Calcutta, was used to determine whether serologic test for syphilis was positive. Hapten (dinitrophenol) BSA conjugate was prepared in the laboratory by the method of Eisen [8] and sera was tested for the presence of precipitating antibodies. The Rose Waaler test [27] was used to determine whether leprosy sera reacted with rheumatoid factor. Precipitating antibodies against antigens from mycobacteria were tested using both *Mycobacterium leprae* (bacilli separated from leproma) and *Mycobacterium tuberculosis* (H37Rv). Unsonicated saline extracts as well as extracts obtained by ultrasonic disruption of bacteria were tested.

Response to challenge with TAB antigen. The antigen used for immunization was obtained from Central Research Institute, Kasauli, and contained *Salmonella typhi* 1 million organisms/ml., *Paratyphi A* 500 organisms/ml. and *Paratyphi B* 500 organisms/ml. The immunization schedule used was 0.5 ml as the initial injection followed by 1 ml. of the antigen after an interval of a fortnight. Basal Widal titers were obtained before immunization. Subsequent titers at intervals of two weeks after the first and second injection were determined.

Analysis of antigens in normal human serum and serum of patients with leprosy. Antibodies to pooled lepromatous leprosy sera were elicited in rabbits using Freund's complete adjuvant. Six weeks after inoculation immunoelectrophoresis was done both with normal sera and lepromatous sera and precipitin lines were developed using anti-normal human serum and antilepromatous leprosy serum.

RESULTS

Table 1 summarizes the level of total and differential serum proteins in patients with

TABLE 1. *Serum proteins in normal subjects and in leprosy patients.*

Category	No. cases	Total proteins (g./100 ml.)	Percentage globulins				Albumin
			α 1	α 2	β	γ	
LL	18	7.3 ± 0.27 (5.4 – 10)	5.7 ± 81	8.8 ± 0.96	17.4 ± 1.7	35.9 ± 2.5	30.8 ± 1.5
TT	5	7.0 ± 0.37 (6 – 8)	5.9 ± 0.90	8.4 ± 0.90	15.5 ± 2.7	29.3 ± 3.0	42.1 ± 2.7
Normal	8	6.6 ± 0.10 (6.4 – 7)	5.9 ± 0.86	8.8 ± 0.33	14.3 ± 1.5	22.8 ± 2.4	48.2 ± 2.0

TABLE 2. *Level of different classes of immunoglobulins in leprosy patients*

Category	No. of cases	IgG	IgA	IgM
		mg./ml.		
1. Lepromatous leprosy (LL)	30	14.35 ± 0.5 P 0.001	2.76 ± 0.05 P 0.001	1.23 ± 0.03 N.S.
2. Tuberculoid leprosy	8	9.7 ± 0.04 P 0.001	1.67 ± 0.10 N.S.	1.2 ± 0.10 N.S.
3. Normal	10	7.95 ± 0.11	1.71 ± 0.16	1.05 ± 0.06

TABLE 3. *Reactivity of immunoglobulins from patients with lepromatous leprosy with different types of antigens.*

Antigen tested	Method	No. of sera examined	Positive
1. Lepromin	Gel diffusion	25	Nil
2. PPD	Gel diffusion	25	Nil
3. VDRL antigen	Flocculation	25	Three
4. Rheumatoid factor	Rose Waaler	25	Nil
5. Hapten-BSA	Gel diffusion	18	Nil
6. DNA (calf thymus)	Gel diffusion	18	Nil

lepromatous (LL) and tuberculoid (TT) leprosy. Hypergammaglobulinemia was commonly found in lepromatous leprosy. There was also a concomitant depression in the level of serum albumin. In tuberculoid leprosy patients there was a marginal but statistically insignificant rise in gamma globulins.

Quantitative determination of various classes of immunoglobulins showed that there was a considerable increase of IgG in patients with lepromatous leprosy (Table 2). There was also a moderate rise in IgA and a marginal but statistically insignificant increase in IgM. The tuberculoid patients showed some increase in IgG levels. IgA and IgM were not altered.

As lepromatous leprosy sera showed consistently high IgG levels, and some increase in IgA as well as IgM, the possible antigenic stimuli capable of evoking this response were investigated. No precipitin reactions in Ouchterlony agar diffusion assays were obtained with lepromin, PPD, dinitrophenol conjugated to bovine serum albumin or with DNA. (The source of DNA was calf thymus DNA from Sigma). The Rose Waaler test for rheumatoid factor was also negative in all patients. Three patients showed positive VDRL tests. Infection with syphilis could not be excluded in these patients, though these may also indicate nonspecific reactions (Table 3). Precipitating antibodies against sonicated extracts of *M. leprae* (from nodules of LL patients) and *M. tuberculosis* H37Rv were detected (Table 4).

The capacity of leprosy patients for humoral antibody response is apparently not depressed. Both lepromatous and tuberculoid leprosy patients showed a positive antibody response to TAB antigen. The titers of antibodies against TH, AH and BH were well within the normal range. Figure 1 shows the mean titers obtained in normal subjects and in patients with lepromatous leprosy.

In order to see whether any new antigen-

TABLE 4. *Reactivity of immunoglobulins from patients with lepromatous leprosy with different types of antigens.*

Antigen tested	Method	No. of sera examined	Positive
1. Lepromatous biopsy extract			
i. Sonicated	Gel diffusion	5	5
ii. Unsonicated	Gel diffusion	5	Nil
	Complement-fixation	5	5
2. HRV 37			
i. Sonicated	"	5	5
ii. Unsonicated	"	5	Nil

ic components were present in leprosy patients, antibodies were elicited in rabbits against pooled sera from cases of lepromatous leprosy and pooled sera from five normal subjects. Immunoelectrophoretic patterns developed against antilepromatous leprosy serum did not reveal the presence of any additional precipitin line.

DISCUSSION

Hypergammaglobulinemia with little change in total serum proteins was commonly found in patients with lepromatous (LL) leprosy. There was a rise in globulins in some cases of tuberculoid (TT) leprosy, but the change was not statistically significant. The increase in globulins is accompanied by a decrease in serum albumin. Such alterations in serum proteins are not peculiar to lepromatous leprosy but are noticed in malnutrition and in a large number of chronic diseases ([3, 6, 10, 11]).

Three classes of immunoglobulins, namely IgG, IgA and IgM, were quantitatively estimated in the two categories of leprosy patients. There is a consistent and significant increase in IgG and IgA in patients with lepromatous leprosy. In patients with tuberculoid leprosy only IgG is raised with no significant change in IgA. The alteration in IgM was not found to be significant in either lepromatous or tuberculoid leprosy and thus does not appear to be generally applicable for the differential diagnosis of the two types of leprosy. Our results are in conformity with the observations of Sheagren *et al.* ([20]), but differ in many respects from those of Lim and Fusaro ([16, 18]).

The reasons for an increase in the level of immunoglobulins are not clear. These could be due to either an increase in the rate of synthesis of immunoglobulins or due to a decrease in their rate of catabolism. Attempts were made to define the possible antigens inducing the formation of antibodies in these patients. The sera gave positive complement fixation and precipitin reaction with sonicated extracts of *M. leprae* prepared from human lepromas. However, similar reaction was obtained with extracts from *M. tuberculosis* H37Rv. The precipitin lines in Ouchterlony agar plates gave evidence of identity of antigenic components in the two extracts. It would therefore appear that the antibodies in sera of leprosy patients are not specific for *M. leprae* but are common to both of these mycobacteria. It was further observed that the reaction was best observed with fresh sera. On cold storage (0-4°C) of sera the reaction was lost in many cases, suggesting the cold sensitive nature of these antibodies. Unsonicated saline extracts of *M. leprae* gave a positive complement-fixation test but not a precipitin reaction. Leprosy sera showed no reactivity to lepromin, PPD, DNP-BSA conjugates and DNA. The VDRL reaction was positive in three patients. It is possible that the high level of IgG (and raised IgA in LL leprosy) is a consequence of the antibodies elicited against some components of *M. leprae* in course of the prolonged stay of bacilli in the body. It is also likely that the nonspecific mycobacterial antigens are essentially acting as adjuvants enhancing the antibody synthesizing ability of these patients to various infections to which they are exposed in view of the poor socioeconomic conditions of most patients.

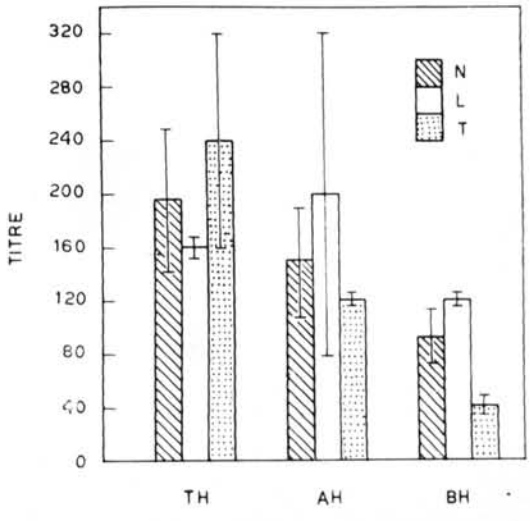

FIG. 1. Titers of antibodies against salmonella antigens. **N**: normal patients; **L**: Lepromatous leprosy patients; **T**: Tuberculoid leprosy patients; **TH**: S. typhi 'H' antigen; **AH**: S. paratyphi A "H" antigen; **BH**: S. paratyphi B "H" antigen.

The ability to form antibodies in a normal manner by leprosy patients is shown by their positive response to known antigens (Fig. 1). These observations are fur-

ther supported by the fact that appreciable porportions of lymphocytes in lymph node biopsies of patients with lepromatous leprosy carry immunoglobulin determinants as revealed by fluorescence given with fluorescein tagged antihuman globulins ([25]). The normalcy of humoral immune responses in patients with lepromatous leprosy is of interest in spite of the fact that the cell mediated immune responses in untreated cases of lepromatous leprosy (LL) are appreciably depressed ([22]).

SUMMARY

Hypergammaglobulinemia with lowering of serum albumin was found in 30 patients with lepromatous leprosy. IgG and IgA were found to be raised significantly in lepromatous leprosy (LL) cases, while in patients with tuberculoid leprosy (TT) only IgG levels were increased with no significant changes in IgA. There was no statistically significant alteration in IgM levels in tuberculoid or lepromatous leprosy patients.

Sera from leprosy patients gave positive complement fixation and precipitin reactions with extracts of *Mycobacterium leprae* and *Mycobacterium tuberculosis* (H37Rv). Antibody titers in response to TAB (typhoid-paratyphoid vaccine) injections were in the same range in lepromatous leprosy patients as in normal control subjects. Humoral immune responses are essentially normal in patients with lepromatous leprosy in spite of a depression of cell mediated immune responses.

RESUMEN

En 30 pacientes con lepra lepromatosa se encontró hipergamaglobulinemia con disminución de la albúmina sérica. Se encontró que la IgG y la IgA eran significativamente más elevadas en casos con lepra lepromatosa (LL), mientras que en pacientes con lepra tuberculoide (LT) solamente los niveles de IgG estaban aumentados, sin que hubieran cambios significativos en la IgA. No hubo alteración estadísticamente significativa de los niveles de IgM ni en los pacientes con lepra tuberculoide ni en los con lepra lepromatosa.

Los sueros de los pacientes con lepra dieron resultados positivos a las pruebas de fijación de complemento y de precipitina con extractos de *Mycobacterium leprae* y *Mycobacterium tuberculosis* (H37rv). Los títulos de anticuerpos contra inyecciones de TAB (vacuna tífica-paratífica) estuvieron dentro del mismo rango en los pacientes con lepra lepromatosa y en los sujetos controles normales. Las respuestas inmunológicas de tipo humoral son esencialmente normales en pacientes con lepra lepromatosa a pesar de la depresión de las respuestas de inmunidad por células.

RÉSUMÉ

Chez 30 malades souffrant de lèpre lépromateuse, on a observé une hypergammaglobulinémie, avec abaissement du taux d'albumine du sérum. On a trouvé que les IgC et les IgA présentaient une augmentation significative dans les cas de lèpre lépromateuse (LL), alors que chez les malades présentant une lèpra tuberculoïde (TT), on notait une augmentation des taux d'IgC sans modification significative dans les IgA. On n'a pas enregistré de modification statistiquement significative des taux d'IgM, que ce soit chez les malades souffrant de lèpre tuberculoide ou chez ceux présentant une lèpre lépromateuse.

Les échantillons de sérum provenant de malades de la lèpre donnaient des réactions positives pour la fixation du complément et pour les précipitines, lorsqu'on les faisait réagir avec des extraits de *Mycobacterium leprae* ou de *Mycobacterium tuberculosis* (H37rv). Les titres d'anticorps obtenus en réponse à des injections de TAB (vaccin typhique-paratyphique) étaient du même order de grandeur chez les malades souffrant de lèpre lépromateuse que chez les individus témoins normaux. Les réponses immunologiques humorales étaient en règle générale normales chez les malades présentant une lèpre lépromateuse, et ceci malgré une dépression dans les réponses immunologiques survenant à l'intervention des cellules.

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