Clinical Significance of Changes in Serum Proteins, Immunoglobulins, and Autoantibodies in Leprosy[1]

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Infection with *Mycobacterium leprae* induces changes in the humoral immune system which may involve the appearance of a number of aberrant responses (cryoglobulins, autoantibodies, biological false-positive test for syphilis) often associated with other diseases.

The serum electrophoretic profile has not shown consistent changes in different histological subtypes of leprosy. According to some reports total protein levels are altered, although in one parallel study of protein levels in polar lepromatous leprosy and tuberculosis patients, a comparable increase in total protein was found in both groups from the one population ([42]). Hypergammaglobulinemia is a multifactorial phenomenon. It may be nonspecific and secondary to the presence of other chronic infectious diseases within the population studied, secondary to liver involvement ([13]), or an artifact due to comparison with control groups who differ in social and general health status ([4, 37]).

The levels of individual immunoglobulin classes are frequently elevated and may be important in the clinical management of the disease ([31, 33]). Although these changes have been observed predominantly in patients with lepromatous leprosy, patients with tuberculoid disease may also demonstrate elevations in IgG ([8, 20, 24, 37]), IgA, and IgM ([5, 23]).

C-reactive protein (CRP) is an acute phase reactant mediating a similar range of reactions *in vitro* to the immunoglobulins ([29]).

CRP is elevated in 24%–97% of patients with lepromatous leprosy ([23, 36, 37, 40]), particularly during type 2 lepra reactions ([14]). Raised levels have also been found in patients with tuberculoid leprosy, but only in the minority (0%–26%) of cases ([34, 36, 37]). Levels of alpha-1 globulin may also be elevated during the acute phase response which is largely due to a rise in alpha-1 antitrypsin. Patients with lepromatous leprosy ([17, 42]) and the dimorphous variant of the disease ([17]) have shown significant increases in the alpha-1 globulin level, but this has not been confirmed by more recent studies ([18]).

Raised titers of autoantibodies (predominantly antithyroglobulin, antinuclear, antismooth muscle antibodies and rheumatoid factor) have been reported most commonly in lepromatous leprosy patients ([26]). These may well be epiphenomena; nevertheless, hypergammaglobulinemia is a common finding in autoimmune disease ([27]), and the clinical appearance of lepromatous leprosy patients in reaction has some similarity to that seen in vasculitis ([14]). Furthermore, leprosy patients may show serological changes that are characteristic of lupus—such as a biological false-positive test for syphilis, decreased circulating complement levels and autoantibodies to circulating white cells, red cells and platelets ([14, 15, 22]).

Studies to date have not resolved the possible significance of these changes. The current study measured multiple humoral factors in several groups of Australian Aboriginals from an area endemic for leprosy. The use of exposed but clinically unaffected controls (the family contacts) and stable treated patients from the same population allowed comparison of these two subpopulations that are identical for health, social, ethnic, and demographic features. The inclusion of a further group of European controls (the sporadic contacts) with differing health, socioeconomic, and ethnic

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status permitted investigation of the influence of other factors such as nonleprous infection on the results of humoral assays. Measurement of humoral factors in leprosy subgroups was also carried out in order to determine if humoral changes are associated with a subtype of the disease rather than putative presence of leprosy.

MATERIALS AND METHODS

Patients and controls. Patients and controls resided in the Northern Territory of Australia where leprosy is endemic. Its overall prevalence among Australian Aboriginals is 2.3% ([19]). Tests of humoral function were performed on three groups of subjects. The first consisted on noninfected European sporadic contacts of leprosy (N = 18) living within the leprosy-endemic area. They were healthy, had no relatives with leprosy at the time of study, and had been living in urban conditions in close contact with leprosy for over 2 years. This group had a mean age of 40.8 ± 12.1 years and included 11 males and 7 females. The second group was composed of Aboriginals who were family contacts of the leprosy patients (N = 58), lived in the same area, and had a first-degree family relative with inactive leprosy at the time of study. There were 22 males and 36 females with a mean age of 26.9 ± 11.1 years. The index cases (the third group) were Aboriginals either diagnosed on clinical grounds, using the Ridley-Jopling scale ([33]) with histological confirmation (N = 36; 23 males, 13 females), or on clinical grounds alone (N = 29; 14 males, 15 females). All members of this group had leprosy diagnosed between 12 and 40 years prior to the study (mean 13.3 ± 9 years).

Since the presence of active leprous infection is associated with a rapidly changing humoral and cell-mediated immunological state, all patients selected for this study were "inactive" at the time of testing, had a bacterial index (BI) of zero for at least 3 years, and had been fully treated or were receiving long-term maintenance antileprosy treatment (N = 9). This treatment consisted of diaminodiphenylsulfone (dapsone, DDS) 100 mg oral daily in 2 cases, and diacetyl DDS 225 mg intramuscularly every 6 weeks in 7 individuals. None of the leprosy patients were in reaction during or immediately before or after the study.

Contact. Evidence of contact with the leprosy bacillus was assessed by the indirect immunofluorescent test of Abe ([3]) and a standard ELISA assay for IgG to sonicated *M. leprae* antigen and the phenolic glycolipid (PGL-I) subfraction of whole *M. leprae* antigen. In the ELISA, sera giving optical density (OD) readings greater than the third standard deviation above the mean were considered to be positive ([9]).

Electrophoretogram. Electrophoresis of serum proteins was performed using standard techniques ([43]) on a cellulose acetate strip, stained with ponceau S protein stain, and scanned in an automated densitometer (Gelman Instrument Co., Ann Arbor, Michigan, U.S.A.).

Immunoglobulins. Immunoglobulin levels were determined by radial immunodiffusion in agarose gel plates with antihuman immunoglobulins (Tri-Partigen; Behring Institute, West Germany).

C-reactive protein and autoantibodies. Latex agglutination was used to assay C-reactive protein (CRP) (CRP-Wellcotest; Wellcome Research Laboratories, Beckenham, England), autoantibodies to IgG (Rheuma-Wellcotest; Wellcome), and thyroglobulin (Thyro-Wellcotest; Wellcome). Sera were diluted 1:25 for the rheumatoid factor (RF) assay and 1:10 for measurement of antithyroglobulin antibodies, as recommended by the manufacturer. A positive result for CRP was reported if the level was greater than 0.6 mg/dl.

Statistical analysis. The results from male and female subjects were pooled, since analysis of the data failed to reveal any significant difference between the two. All groups did not differ significantly in age. No statistically significant correlations between age and any results were found on analysis, consequently all group results are given without cross-reference to age. Comparison between results was performed using Student's *t* test with pooled sample variances or Chi-squared test where indicated for immunoglobulins ([18]) and serum proteins; the latter having been shown previously to be normally distributed ([21]). All results are expressed as mean ± one standard deviation, unless otherwise stated.

TABLE 1. *Specific antibody response to* M. leprae—*serological evidence of contact.*

Group	No. sub-jects[a]	FLA-ABS		IgG ELISA	
		% Titer >1+[b]	Antibody titer[c]	Sonicate (% positive)	PGL-I (% positive)
European sporadic contacts	18	33	0.78 ± 0.71	Not Done	Not Done
Aboriginal family contacts	58	71	1.01 ± 0.49	78	64
Aboriginal leprosy patients					
Clinical diagnosis (BT-TT)	29	100	1.00 ± 1.41	91[d]	78[d]
Histological diagnosis	36	100	[e]	Not Done	Not Done

[a] Subjects' demography the same as shown in Table 2.
[b] Titer of ≥1+ regarded as definite evidence of contact.
[c] Mean antibody titer ± one standard deviation where titer of 1 = 1:40, 2 = 1:160, 3 = 1:640, 4 = 1:2560.
[d] N = 24.
[e] Mean antibody titer of LL = 2.17 ± 0.94; BL = 1.91 ± 0.30; BB = 2.50 ± 1.00; BT = 2.43 ± 0.79; TT = 1.00 ± 1.41.

RESULTS

Contact. Evidence of contact with the leprosy bacillus, assessed by the fluorescent antibody absorption test (FLA-ABS), was obtained in 41/58 (71%) of the family contact group (Table 1). The mean fluorescence titer was 1.01 ± 0.49. In the ELISA assay with *M. leprae* sonicate, 78% (45/58) of family contacts were positive for antibodies. This figure fell to 64% (37/58) when the PGL-I antigen was used in the ELISA assay. Sporadic European contacts of leprosy were only studied with the FLA-ABS assay. Six of the 18 individuals (33%) had a FLA-ABS titer of greater than 1+ fluorescence, which is regarded as definite evidence for the presence of antibodies. The mean antibody titer in this group was 0.78 ± 0.71.

Of the 65 patients studied all had serological evidence of anti-*M. leprae* antibodies using the FLA-ABS test. Figures for the ELISA assay were only available for 24 of the clinically diagnosed patients. In this group, 91% (22/24) were positive for IgG using *M. leprae* sonicate as antigen. When PGL-I was substituted as antigen, 78% (19/24) were positive for IgG.

Serum electrophoretic profiles. Levels of alpha-1 globulin were significantly higher in the patients with lepromatous leprosy (p < 0.05) and borderline tuberculoid leprosy (p < 0.001) than in noninfected family contacts. On the other hand, there was no difference between the levels in family and sporadic contacts who had markedly different levels of specific antibody (Table 1), which implies that alpha-1 globulin is not a reliable indicator of exposure to *M. leprae.*

Gamma globulin levels were higher in the noninfected Aboriginal family contacts than in the sporadic European contacts of leprosy (p < 0.001). All other patients showed no significant changes in gamma globulin levels when compared to noninfected contacts.

Immunoglobulins. Significantly higher levels of IgG were found in the family contacts of leprosy when compared to sporadic contacts (p < 0.001) as shown in Table 2. This was thought to reflect the higher rate of skin, respiratory, and gastrointestinal infection in the Aboriginal population from which the leprosy patients were drawn since the sporadic contact group were European individuals living in urban conditions; whereas the noninfected family contact group consisted of Aboriginal individuals living in rural conditions.

Patients with a clinical diagnosis of borderline and polar tuberculoid disease had significantly higher IgG levels than noninfected family contacts and sporadic contacts (p < 0.02).

Measurement of IgA concentrations yielded unexpected results. When compared to those of the noninfected family contact group, the IgA levels were significantly higher in the borderline lepromatous patients (p < 0.05) and the clinically diagnosed borderline and polar tuberculoid patients (p < 0.05) but not in the polar lepromatous cases. Furthermore, there was no significant difference in IgA levels between family and sporadic contacts as was the case

TABLE 2. *Immunoglobulins and autoantibodies in leprosy patients and controls.*

Subjects	No.	Mean age (yrs)	Immunoglobulins (mg/dl)[a]			CRP[b] %	RF[c] %	TA[c] %
			IgG (680–1500)	IgA (60–400)	IgM (50–320)			
Noninfected controls								
Sporadic (European)	18	40.8 ± 12.1	1891 ± 958	357 ± 170	167 ± 113	16.5	0	11.0
Family (Aboriginals)	58	26.9 ± 8.0	2682[d] ± 874	332 ± 142	139 ± 52	20.5	8.5	1.5
Leprosy patients (Aboriginals)								
LL	11	41.3 ± 19.4	3117 ± 968	409 ± 189	197 ± 116	54.5[e]	27.5	0
BL	12	44.4 ± 15.8	2650 ± 951	432[f] ± 140	157 ± 65	67.0[g]	0	8.5
BB	4	28.3 ± 24.5	3193 ± 1494	374 ± 134	148 ± 83	0	0	25.0
BT	7	32.6 ± 10.5	2646 ± 1287	385 ± 260	158 ± 43	57.0[f]	0	14.5
TT	2	24.0 ± 2.0	2785 ± 813	371 ± 188	68 ± 11	100.0	0	0
Clinical diagnosis								
BT-TT	29	45.4 ± 18.3	3241[e] ± 1024	403[f] ± 151	130 ± 64	31.0	7.0	7.0

[a] Results expressed as mean ± one standard deviation. Numbers in parentheses are normal ranges.
[b] Percentage of patients with C-reactive protein (CRP) level ≥0.6 mg/dl.
[c] Percentage of patients with rheumatoid factor (RF) titer of ≥1:25 and antithyroglobulin antibody (TA) titer of ≥1:10, respectively.
[d] $p < 0.001$, compared to sporadic contacts, two-tailed Student's t test.
[e] $p < 0.02$, compared with noninfected family contacts, two-tailed Student's t test.
[f] $p < 0.05$, compared with noninfected family contacts, two-tailed Student's t test.
[g] $p < 0.001$, compared with noninfected family contacts, two-tailed Student's t test.

with alpha-1 globulin, suggesting that IgA is a poor indicator of potential subclinical infection. All other immunoglobulin levels did not differ significantly between leprosy patients and the noninfected contact groups.

C-reactive protein. Detectable amounts of CRP (i.e., above 0.6 mg/dl) were present in a proportion of the subjects from all groups studied, except for patients with borderline leprosy. The incidence of positive tests was highest in the BL and LL patients (67% and 55%, respectively). BT patients also had detectable amounts of CRP in 57% of the individuals studied, which was significantly higher than that found in the noninfected contact groups (both sporadic and family contacts) ($p < 0.05$).

Autoantibodies. The incidence of autoantibodies was high in both leprosy patients and their noninfected contacts (Table 2). Analysis was performed for the presence of rheumatoid factor and antithyroglobulin autoantibodies and for the presence of either autoantibody in a given individual. The proportion of subjects with antithyroglobulin antibodies ranged from between 0% for patients with polar lepromatous or tuberculoid disease and 25% for BB patients. Furthermore, a relatively high number of sporadic contacts had positive titers. In the case of rheumatoid factor, 27.5% of lepromatous leprosy patients had measurable titers; none were detected in other infected subjects or in the sporadic contacts. By contrast, 8.5% of family contacts were positive. Differences between the incidence of autoantibodies in the leprosy-infected group and the noninfected contact group (sporadic or family individuals) did not reach statistical significance when compared in the Chi-squared test (Table 2).

DISCUSSION

Activation of the humoral immune system occurs in all types of leprosy, albeit to varying degrees. It may take the form of specific antibody production to *M. leprae* [3, 44] or nonspecific changes in acute phase reactants (Table 3) and immunoglobulins (Tables 2 and 4). Superimposed on these changes are the effects of the activity of an individual leprosy patient's disease [1, 38]. The patients studied here all had stable, treated, nonreactional disease with a BI = 0 for at least 3 years. Treatment remained unchanged for the 12 patients before or at

TABLE 3. *Serum proteins in leprosy.*[a]

Authors	Leprosy type	No. subjects	Total protein	Albumin	Globulins			
					$\alpha 1$	$\alpha 2$	β	γ
Sehgal, *et al.* ([37])	LL	22	I	D	—	—	—	I
	TT	50	I	D	—	—	—	I
Tamblyn, *et al.* ([41])	LL	50	I	—	I	I	—	—
Jha, *et al.* ([19])	LL	18	—	—	—	—	—	—
	TT	5	—	—	—	—	—	—
Gupta, *et al.* ([16])	LL	20	—	D	I	I	D	I
	D	15	—	D	I	I	—	D
	TT	22	—	—	—	I	D	—
Gupta, *et al.* ([17])	LL	28	D	D	—	—	I	I
	BL	8	—	—	—	—	—	—
	TT	24	—	D	—	—	I	—
Current study[b]	LL	11	—	—	I	—	—	—
	BL	12	—	—	—	—	—	—
	BB	4	—	—	—	—	—	—
	BT	7	—	—	I	—	—	—
	TT[c]	31	—	—	—	—	—	—

[a] I = Increased significantly when compared to controls; D = decreased significantly when compared to controls;
— = no change between levels in patients and controls.
[b] Results of patients compared with those of noninfected family contacts of leprosy.
[c] Tuberculoid leprosy diagnosed on clinical grounds only in 29/31 individuals.

any stage during the study. In view of this, the activity of clinical leprosy could be eliminated as a source of variability in the results. The use as controls of noninfected European sporadic contacts from a socio-economic background different from the patients as well as healthy Aboriginal family contacts of patients with confirmed leprosy and with comparable demographic features allowed more precise interpretation of the significance of the observed changes in humoral immunity within the patient population.

Evidence of contact with the *M. leprae* bacillus was obtained by using two assays for specific *M. leprae* antibodies. Both assays were sensitive and together identified all individuals with leprosy (Table 1). Those noninfected Aboriginal individuals in close social contact with the index cases of leprosy (Aboriginal family contacts) had a higher incidence of antibody positivity than those European individuals with more distant social contact. These findings are in keeping with previous conclusions based on studies of cell-mediated immune function in contacts of leprosy index cases ([16]). Furthermore, the presence of elevated levels of specific antibody in leprosy-infected and noninfected Aboriginals means that the degree of contact between the individuals

studied and their index cases was eliminated as a variable when interpreting the significance of changes observed in nonspecific parameters of humoral immunity.

The electrophoretic profile of the patients showed an elevated level of alpha-1 globulin in borderline tuberculoid and lepromatous

TABLE 4. *Studies of immunoglobulin levels in polar LL patients.*[a]

Authors	No. subjects	IgG	IgA	IgM
Lim and Fusaro ([22])	82	I	—	—
Tamblyn, *et al.* ([41])	13	D	—	I
Bullock, *et al.* ([10])	158	I	I	I
Jha, *et al.* ([19])	30	I	I	—
Malaviya, *et al.* ([24])	50	—	—	—
Kreisler, *et al.* ([21])	32	—	—	—
Gupta, *et al.* ([17])	28	I	—	I
Saha, *et al.* ([33])	37	I	I	I
Sengupta, *et al.* ([36])	41	I	I	—
Ahmed, *et al.* ([4])	13	I	I	I
Current study[b]	11	—	—	—

[a] I = Increased significantly when compared to controls; D = decreased significantly when compared to controls; — = no change between levels in patients and controls.
[b] No significant difference between polar lepromatous patients and controls. (The controls were uninfected contacts of leprosy patients from the endemic area in the current study.)

leprosy patients. A similar finding has been reported previously in dimorphous patients from a different population ([17]).

Gamma globulin levels were higher in the noninfected Aboriginal family contact group than in the noninfected European sporadic contact group, but they were not elevated in all leprosy groups studied when compared to family contacts. This suggests that nonleprous infection may, in fact, be the cause of many of the humoral changes seen when individuals from different socioeconomic groups are used as controls ([20, 25]). Thus, concurrent nonleprous infection in the Aboriginal population studied was probably responsible for the increase in the "normal" (in the sense of modal) level of IgG found in both patients and their family contacts. Raised globulin levels have been reported in other indigenous populations ([4, 37]), but on the basis of the current findings, may have been an artifact due to the use of controls such as army personnel ([11]) whose general health level would have been significantly higher than that of the leprosy patients ([23]).

Increased levels of IgA in contrast to IgG were restricted to the borderline lepromatous group (Table 2). This has been observed previously ([5, 24]) and may represent another nonspecific change secondary to nonleprous gut or respiratory mucosal infection.

High levels of the acute phase reactant CRP occur in lepromatous leprosy patients during active leprosy and type 2 lepra reactions ([37]). The inactive nonreactional borderline and polar lepromatous leprosy patients studied here showed a significantly greater incidence of raised CRP levels (greater than 0.6 mg/dl) when compared to both noninfected sporadic and family contacts (Table 2). Thus, measurement of CRP cannot be regarded as a reliable way of detecting lepra reactions ([42]) because of the high sensitivity and lack of specificity of the test.

The appearance of many types of autoantibodies in the serum of both lepromatous and tuberculoid leprosy patients is well documented ([6, 20, 26, 30, 32, 41, 44]). The proportion of lepromatous leprosy patients studied here with a positive rheumatoid factor test was similar to that reported by some other authors ([23, 37, 39], and D. Kenny, personal communication, 1980) although lower than that of earlier studies ([2, 7, 27]). As was the case with immunoglobulins and CRP, the results suggest that it was not infection with the *M. leprae* bacillus itself but rather concurrent nonleprous infection which caused the increased incidence of autoantibodies in these groups of subjects.

The current study of inactive, treated, nonreactional leprosy patients did not result in identification of abnormalities of the humoral response which are unique to leprosy. It does, however, point to abnormalities in humoral immunity presumed to be secondary to nonleprous infection in the population from which the leprosy contacts, as well as the patients themselves, were drawn.

On the other hand, the possibility that active leprosy infection may itself be associated with altered control of the humoral immune function ([12, 23, 28]) must also be considered and, in the future, it will be necessary to use purified antigens derived by monoclonal antibody or recombinant DNA techniques to distinguish between primary and secondary changes in the immune system. In addition, further work aimed at examining multiple parameters of the acute phase reaction (such as CRP, alpha-1 globulin and alpha-1 antitrypsin) on a serial basis in individuals with reactional leprosy will be needed to determine if the increased levels of these serum proteins will be useful in a given individual as simple predictive tests for development of reactions or response to therapy.

SUMMARY

Changes in the level of acute phase reactants such as C-reactive protein (CRP), serum globulins, and autoantibodies have been reported previously in patients with leprosy, particularly at the lepromatous end of the spectrum. The clinical significance of these findings was investigated by comparing the same parameters of humoral immune function in populations of Australian Aboriginals with stable treated leprosy and relevant contact groups including a) noninfected European sporadic contacts and b) healthy Aboriginal relatives of patients with confirmed leprosy. Raised levels of CRP and immunoglobulins and the higher frequency of

autoantibodies seen in leprosy patients compared with sporadic contacts are probably related to differences in the incidence of nonleprous infection rather than to leprosy per se. Comparable results were obtained in the leprosy patients and their family contacts. The data highlight the need to use antigen-specific assays for determining the significance of changes in acute phase reactants and for distinguishing between the primary and secondary effects of *Mycobacterium leprae* infection.

RESUMEN

Ya se han descrito la presencia y el incremento en los niveles de las llamadas "proteínas de fase aguda" (proteína C-reactíva, PCR, globulinas séricas y autoanticuerpos) en los pacientes con lepra, particularmente en el extremo lepromatoso del espectro. En este trabajo se investigó el significado clínico de estos hallazgos comparando los mismos parámetros de la función inmune humoral en poblaciones de aborígenes australianos con lepra tratada estable y en grupos de contactos relevantes incluyendo (a) casos esporádicos de contactos europeos no infectados y (b) aborígenes sanos familiares de los pacientes con lepra confirmada. Los elevados niveles de PCR y de inmunoglobulinas y la mayor frecuencia de autoanticuerpos observados en los pacientes con lepra comparados con los contactos esporádicos están probablemente más relacionados a la incedencia de infecciones no leprosas que a la lepra per se. Se obtuvieron resultados similares en los pacientes con lepra y en sus contactos familiares. Los datos indican la necesidad de usar ensayos antígenoespecíficos para determinar el significado de los cambios en las proteínas de fase aguda y para distinguir entre los efectos primarios y secundarios de la infección por el *Mycobacterium leprae*.

RÉSUMÉ

Précédemment, on a rapporté que des malades atteints de lèpre, et particulièrement ceux qui souffrent d'une affection se situant au pôle lépromateux du spectre, présentaient des modifications dans les taux de réactifs tels que la protéine C-réactive (CRP), les globulines sériques, et les autoanticorps. La signification clinique de ces observations a été étudiée en comparant les mêmes paramètres d'immunité humorale, chez des aborigènes australiens ayant été traités pour la lèpre et désormais stabilisés, et dans des groupes pertinents de contacts, à savoir a) des européens non infectés ayant eu des contacts sporadiques; b) des aborigènes en bonne santé, parents de malades atteints d'une lèpre confirmée. L'élévation des taux de CRP et d'immunoglobulines, de même que la fréquence plus élevée d'auto-anticorps, constatées chez les malades de la lèpre, par rapport aux personnes ayant eu des contacts sporadiques, étaient probablement en relation avec des différences dans l'incidence de l'infection non lépreuse, plutôt que dues à la lèpre. Des résultats comparables ont été obtenus en comparant les malades et parents. Ces données soulignent la nécessité d'utiliser des épreuves spécifiques pour l'antigène, en vue de déterminer la signification des modifications notées dans les réactions de la phase aiguë, et de distinguer entre les effets primaires et secondaires de l'infection par *Mycobacterium leprae*.

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