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Specificity of IgG subclass antibodies in different clinical manifestations of leprosy

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SUMMARY

We analysed specific IgG subclasses levels to *Mycobacterium leprae* sonicate extract (MSE), lipoarabinomannan B (LAM) and phenolic glycolipid I (PGL-I) in the sera of leprosy patients with different clinical manifestations. IgG2 was found to be the predominant antibody to MSE regardless of clinical manifestations, and IgG1 response was mostly seen in lepromatous patients. IgG3 reacted only rarely but IgG4 reacted relatively more in certain clinical groups such as borderline lepromatous and lepromatous with erythema nodosum leprosum (ENL) reaction. Most of the IgG subclass responses to MSE could be accounted for reactivity with LAM, suggesting that LAM is the major immunogen involved in the pathogenesis of leprosy. In contrast to LAM, PGL-I antigen showed considerably lower reactivities for IgG subclasses. An association between IgG subclass responses and clinical manifestations of leprosy was also seen. Whereas borderline lepromatous patients were found to have significantly higher levels of IgG2 and IgG4 to MSE, lepromatous patients had elevated levels of IgG1 and lower levels of IgG2. An interesting observation, however, was the significantly higher levels of IgG2 to LAM in the pure neuritic leprosy patients.

Keywords IgG subclasses leprosy clinical manifestations

INTRODUCTION

Leprosy is a chronic infectious disease caused by the non-cultivable bacterial species *Mycobacterium leprae*. It is characterized by a spectrum of clinical manifestations and the differences are due to variability in host immune response to *M. leprae* infection [1]. Patients at the tuberculoid (TT) end of the clinical spectrum show high cell-mediated immune response and low humoral response to *M. leprae* antigens; the opposite is true for lepromatous (LL) patients [2]. The humoral immune response in leprosy has been studied by several investigators by measuring circulating immunoglobulin levels with radial immunodiffusion technique [3–9]. Although the results of these studies were often contradictory as to the levels of immunoglobulin isotypes, markedly raised IgG levels in the sera of leprosy patients compared with healthy subjects have been noticed by some investigators [3,7,9]. Our unpublished observations, and others [10,11] have also revealed that IgG is the predominant circulating antibodies against *M. leprae* antigens.

Human IgG is composed of four subclasses which differ in structure and biological activities such as binding capacity to mononuclear phagocytic cells, reacting with staphylococcal proteins and activating complement fixation [12]. The quantitative response of each IgG subclass varies with the nature of antigens; IgG1, IgG3 and IgG4 respond mostly to protein antigens [13], and IgG2 is primarily induced by carbohydrate antigens [14]. In recent years, increasing importance has been given to the IgG subclass levels as they are found to be of pathogenetic significance in viral [15–17], bacterial [14] and parasitic [18–22] infections. In particular, an association between specific IgG subclasses levels and different type of clinical manifestations has been noticed in AIDS [17], lymphatic filariasis [19] and onchocerciasis [20]. However, little is known regarding *M. leprae*-specific IgG subclass levels and different clinical manifestations of leprosy. Here we have examined *M. leprae*-specific IgG subclass levels in the sera of leprosy patients.

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MATERIALS AND METHODS

Sera

Serum samples were obtained from patients with active leprosy attending clinics of Central Leprosy Teaching and Research Institute, Chengalpattu, Tamil Nadu, India. In total, 138 patients were analysed. These sera from patients classified according to Ridley & Jopling [23] were of 18 tuberculoid (TT), 23 borderline tuberculoid (BT), eight mid-borderline (BB), 27 borderline lepromatous (BL), 38 lepromatous (LL), 15 LL with erythema nodosum leprosum (ENL) reaction and nine pure neuritic (PN). Sera from 49 healthy subjects were also analysed as controls.

Antigens

M. leprae antigens were obtained through the IMMLEP section of the World Health Organization. *M. leprae* sonicate extract and native phenolic glycolipid-I (PGL-I) were provided by Dr R. J. W. Rees, National Institute of Medical Research, London, UK. Lipoarabinomannan B (LAM) from *M. tuberculosis* H37Ra was a kind gift from Dr P. J. Brennan, Colorado State University, Fort Collins, CO.

MoAbs

Mouse MoAbs to human IgG1 (clone SG-16), IgG2 (clone HP-6014), IgG3 (clone HP-6050), IgG4 (clone Sk-44) were purchased from Sigma Chemical Co. (St Louis, MO).

ELISA for IgG subclasses

IgG subclass levels to specific antigens were measured semi-quantitatively by ELISA. Three antigens were used and each had a different reactivity with the serum samples. It became necessary, therefore, to optimize the ELISA condition for each antigen separately. For this purpose, 10-fold dilutions of antigens and serum samples were made and assayed for the reactivity in the presence of optimal concentrations of different anti-human IgG MoAbs. The lowest concentrations of antigens and the highest dilution of serum samples that gave maximal absorbances in combination with the lowest background were chosen. The concentrations of antigens selected were 10 μg/ml MSE, 1 μg/ml LAM and 50 μg/ml PGL-I. Carbonate buffer, pH 9·6, was used for coating the antigens, except for native PGL-I, for which hexane was used. Sera dilutions of 1:300, 1:300 and 1:20 were selected to quantify MSE, LAM and PGL-I specific IgG subclasses, respectively.

Fifty microlitres of antigen solution were added to each well of 96-well flat-bottomed polystyrene microtitre plates (Dynatech, Plochingen, Germany) and incubated at 37°C overnight. After washing the plates five times with PBS, 100 μl of 1% bovine serum albumin (BSA) in PBS were added to each well and incubated for 1 h to block the antigen-free surface of the wells. Then serum samples diluted appropriately were added to the wells (50 μl/well) and incubated at 37°C for 90 min. Each serum sample was tested in duplicate antigen-coated and non-coated control wells for each IgG subclass. After washing, 50 μl of MoAbs directed against human IgG subclasses were added and incubated at 37°C for 90 min. The dilutions of mouse MoAbs used were: IgG1, 1:5000; IgG2, 1:5000; IgG3, 1:5000; and IgG4, 1:7500. The plates were then washed and 50 μl/well of peroxidase-conjugated rabbit anti-mouse IgG (1:2000) (Dakopatts, Glostrup, Denmark) were added for 60 min at 37°C. Subsequently, 50 μl of citrate buffer, pH 5·0, containing 0·4 mg/ml of the chromogenic substrate OPD and 0·006% H_2O_2 were added to each well. The reaction was arrested after 30 min by adding 50 μl/well 5 M H_2SO_4 and absorbance was measured at 492 nm in a Titertek Multiscan (Flow Labs).

The mean optical density (OD) + 3 s.d. of healthy subjects for each IgG subclass with respect to each antigen was used as cut-off value to determine positivity.

Statistical analysis

The data were subjected to one-way classification of analysis of variance (ANOVA) using Hewlett Packard statistical software. The mean IgG subclass levels between different groups were compared with Student–Newman–Keuls (SNK) multiple range test and $P < 0.05$ was considered significant.

RESULTS

Since the sonicate extract of *M. leprae* (MSE) has been reported [24] to contain all major antigens including the carbohydrate

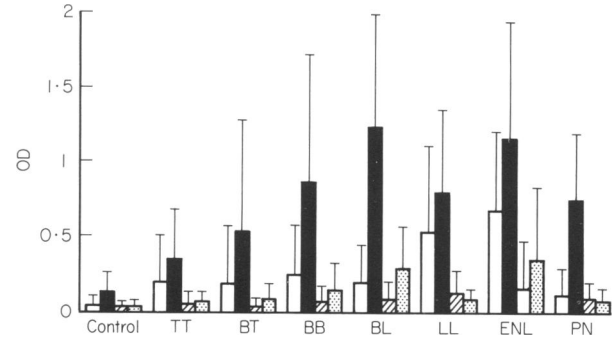

Fig. 1. Distribution of *M. leprae* sonicate extract (MSE) specific IgG subclasses in different groups of sera. □, IgG1; ■, IgG2; ▩, IgG3; ▦, IgG4. TT, tuberculoid; BT, borderline tuberculoid; BB, mid borderline; BL, borderline lepromatous; LL, lepromatous; ENL, lepromatous with ENL reaction; PN, pure neuritic.

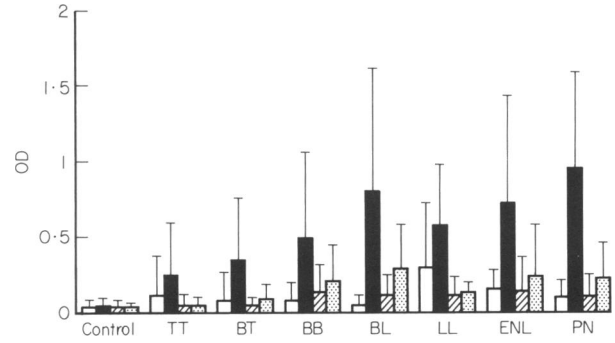

Fig. 2. Distribution of lipoarabinomannan-B- specific IgG subclasses in different groups of sera. □, IgG1; ■, IgG2; ▩, IgG3; ▦, IgG4. TT, tuberculoid; BT, borderline tuberculoid; BB, mid borderline; BL, borderline lepromatous; LL, lepromatous; ENL, lepromatous with ENL reaction; PN, pure neuritic.

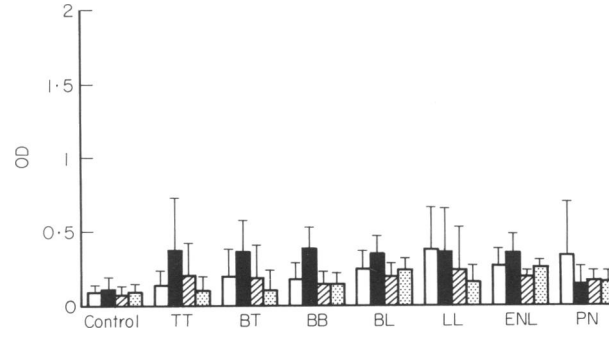

Fig. 3. Distribution of phenolic glycolipid-I-specific IgG subclasses i different groups of sera. □, IgG1; ■, IgG2; ■, IgG3; ▦, IgG4. TT tuberculoid; BT, borderline tuberculoid; BB, mid borderline; BL borderline lepromatous; LL, lepromatous; ENL, lepromatous wit ENL reaction; PN, pure neuritic.

Table 1. Positive individuals to IgG subclasses in different groups of subjects

Serum no.	Antigen	IgG class	Cut-off value*	Groups							
				HS (46)	TT (18)	BT (23)	BB (8)	BL (27)	LL (38)	ENL (15)	PN (9)
1	MSE	IgG1	0·190	2 (4)	3 (17)	3 (13)	3 (37)	8 (27)	21 (55)	11 (73)	1 (11)
		IgG2	0·460	0	6 (33)	8 (35)	4 (50)	19 (70)	24 (63)	11 (73)	5 (55)
		IgG3	0·060	1 (2)	2 (11)	1 (04)	3 (37)	9 (33)	15 (39)	4 (27)	4 (44)
		IgG4	0·120	1 (2)	2 (11)	4 (17)	2 (25)	18 (67)	8 (21)	11 (73)	2 (22)
2	LAM	IgG1	0·090	1 (2)	3 (17)	4 (17)	1 (12)	4 (15)	16 (42)	7 (47)	3 (33)
		IgG2	0·160	1 (2)	6 (33)	10 (43)	5 (62)	17 (63)	28 (74)	11 (73)	8 (89)
		IgG3	0·150	1 (2)	1 (5)	0	1 (12)	7 (26)	10 (26)	1 (07)	1 (11)
		IgG4	0·060	2 (4)	3 (17)	9 (39)	3 (37)	18 (67)	28 (74)	10 (67)	7 (77)
3	PGL-I	IgG1	0·210	0	2 (11)	6 (26)	3 (37)	15 (55)	27 (71)	10 (67)	4 (44)
		IgG2	0·290	0	8 (44)	11 (48)	7 (87)	17 (63)	15 (39)	9 (60)	2 (22)
		IgG3	0·220	0	3 (16)	3 (13)	1 (12)	9 (33)	9 (23)	6 (40)	2 (22)
		IgG4	0·180	0	1 (5)	3 (13)	2 (25)	21 (78)	11 (29)	13 (86)	2 (22)

* Cut-off values represent mean OD + 3 s.d. of the healthy subjects.
Numbers in parentheses are percentages.

LAM, we first analysed the IgG subclasses against this so that an overall picture could be obtained. As can be seen in Fig. 1, IgG2 subclass was the predominant antibody to MSE regardless of clinical status. The highest level of IgG2 (mean OD 1·230) was seen in the serum of BL leprosy patients. Significantly lower levels of IgG2 (mean OD $\leqslant 0{\cdot}790$; $P < 0{\cdot}05$) were noticed in all other groups, except ENL patients who showed IgG2 levels (mean OD 1·150) comparable to that of BL patients. After IgG2, IgG1 was found to be the second largest antibody that reacted with MSE. Although few sera from BT and TT groups showed strong reactivity for IgG1, LL sera either with or without ENL reactions reacted more frequently to IgG1 and thus the levels of IgG1 were significantly ($P < 0{\cdot}05$) higher in these groups (mean OD $\geqslant 0{\cdot}530$) than other patient groups (mean OD $\leqslant 0{\cdot}240$). IgG3 and IgG4 reactivities to MSE were mainly seen in BL, LL and ENL groups with a relatively higher titre to IgG4 antibody.

The quantification of IgG subclass antibodies to the mycobacterial cell wall polysaccharide LAM in different groups of leprosy sera showed (Fig. 2) a pattern almost similar to that noticed for MSE, but with slightly decreased levels of reactivity. The remarkable differences between the profiles of MSE and LAM specific reactivity were the decreased IgG1 levels in LL patients with ENL reaction and increased IgG2 levels in PN patients.

Figure 3 shows IgG subclasses reactivity with *M. leprae*-specific cell wall PGL-I. Unlike MSE and LAM, PGL-I showed a different pattern of reactivity. Sera from all clinical groups other than PN showed more or less similar IgG2 levels to PGL-I (range OD 0·340–0·370). In PN, IgG2 levels (mean OD 0·130; $P < 0{\cdot}05$) was significantly lower, but IgG1 (mean OD 0·330; $P < 0{\cdot}05$) levels were higher than in other groups. No significant differences in IgG3 and IgG4 levels to PGL-I antigen were noticed between the different groups of sera tested.

The numbers of positive responders to each IgG subclass in different groups of patients with respect to different antigens are summarized in Table 1.

DISCUSSION

Little progress has been made in understanding the role of antigen-specific humoral immune responses in the pathogenesis of leprosy. Although this may be attributed to the lack of well-defined antigens from *M. leprae* and from related mycobacterial species for many years, application of modern biological techniques in leprosy research [25] has now changed the situation dramatically. Several carbohydrate and some protein antigens of *M. leprae* have become available in large quantities, enabling more detailed study. In the present study, two purified carbohydrate antigens (LAM and PGL-I) were used, in addition to whole MSE, to quantify specific IgG subclass levels in different clinical manifestations of leprosy. We used mouse MoAbs directed against human IgG subclasses which ensured the specificity of the IgG subclass measured in addition to reducing background due to non-specific binding in ELISA.

The results indicate that IgG antibodies to *M. leprae* antigens are predominantly of IgG2 subclass as in other bacterial infections [14]. The IgG2 levels for MSE show an increasing trend from paucibacillary leprosy (TT, BT) to multibacillary leprosy (BB, BL, LL), possibly indicating a correlation with bacillary load. Most of these IgG2 responses to sonicate extract, and in fact for all IgG subclass responses, could be accounted for reactivity with LAM. This suggests that LAM is the major immunogen involved in the induction of *M. leprae*-specific humoral immune responses and consequently in the immunopathogenesis of leprosy. This is consistent with the earlier observations that LAM has many B cell epitopes [25, 26]. *In vitro* studies have also indicated that LAM may play a suppressive role on cell-mediated immunity against *M. leprae*. Whereas Kaplan *et al.* [27] and Moreno *et al.* [28], respectively, showed that LAM inhibits the antigen responsiveness of the human peripheral blood leucocytes and antigen-induced proliferation of $CD4^+$ T cell clones, Sibley *et al* [29] reported inhibition of interferon-gamma-mediated activation of macro-

phages by LAM. Even though we used LAM from *M. tuberculosis* in this study, it may not have altered the results because LAM from *M. leprae* as well as *M. tuberculosis* are largely similar in structure and have many cross-reacting epitopes [25].

Compared with LAM, PGL-I in general showed considerably low reactivity with IgG subclasses in this study. Similar to this, Levis *et al.* [30] observed low anti-PGL IgG antibodies but high anti-PGL-I IgM antibodies in the sera of leprosy patients. Although both PGL-I and LAM are cell wall carbohydrates, the reason for the differential induction of immunoglobulin isotypes in the host is not clear at present. However, the fact that a few patients sera do have high titre of IgG subclasses to PGL-I tends to suggest that PGL-I might play some role in IgG-mediated pathogenesis in leprosy.

There seems to be a striking association between MSE-specific IgG subclasses levels and clinical manifestations of leprosy. LL patients are found to have significantly elevated IgG1 levels but reduced IgG2 levels when compared with other multi-bacillary patients, particularly BL patients. By contrast, BL patients have remarkably higher IgG2 levels accompanied by a slight increase in IgG4 levels. Only during ENL reaction did LL patients have some IgG4 antibodies but with a corresponding increase in IgG2. Although these discrepancies appear to exist only at subclass level without affecting much the total MSE-specific IgG between BL and LL patients, it appears to increase the total MSE-specific IgG in LL patients with ENL reaction. IgG subclass binding patterns further reveal that IgG2 and IgG4 levels in BL as well as in LL patients are mainly directed to LAM whereas IgG1 levels in LL patients are in response to both LAM and PGL-I. It is not clear why the levels of certain IgG subclasses increase and others decrease in some clinical conditions of leprosy. Clinically BL leprosy resembles lepromatous (LL) disease in many respects, except for the relatively widespread peripheral nerve trunk and skin lesions with predominant macrophages and lymphocytes [2]. ENL reaction, however, is an inflammatory episode mediated by circulating immune complex which occurs mostly in LL leprosy and very rarely in BL [2]. A previous study [31] found no increase in *M. leprae*-specific immunoglobulin isotypes, including IgG, during ENL reaction, although total increase in immunoglobulin levels during ENL has been noticed by some investigators [8]. However, an interesting observation is the significantly higher levels of anti-LAM IgG2 in PN leprosy patients. Although PN is often considered a pauci-bacillary disease between BT and BB [2] in the classification of leprosy, a recent histopathological study [32] in neuritic patients demonstrates features ranging from indeterminate to LL. Our study is the first one to measure antibody levels in these patients as a separate group; whether the observed antibody levels have any specific role in the nerve damage remains to be investigated.

Specific IgG subclasses differ with the nature of infection. Unlike bacterial infections which predominantly elicit IgG2, viral infections and parasite infections induce IgG1 and IgG4 as predominant specific antibodies, respectively [15–22]. Virus-specific IgG1 has been demonstrated to play a key role in antibody-dependent cellular cytotoxicity [16] and the parasite-specific IgG4 antibodies are reported to block the killing mediated by IgG effector antibodies [33]. However, the precise role of IgG2 antibodies in bacterial infection is not clear. Circumstantial evidences suggest that IgG2 mediates complement-activated killing of bacteria but experimental evidence is lacking (see [12] for review).

To date, no attempt has been made on these lines in leprosy. Future studies directed towards the functional role of humoral antibodies would lead to a better understanding of humoral responses in leprosy. The association of antigen-specific IgG subclass levels with certain clinical manifestations could also be better explained once the functional role of these antibodies is known.

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