IgG Antibodies in Leprosy and Their Relation to Thl/Th2 Responses

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Summary

The study of the IgG subclasses of antibodies across the clinical spectrum of leprosy id not reveal differences that could be related to the Th1/Th2 dichotomy; the predominance of antibodies of the IgG1, 2 and 3 subclasses suggest a possible Th1 response across the spectrum in Venezuelan patients. In contrast, the anergic form of American cutaneous leishmaniasis was characterized by a strong IgG4 specific antibody response, suggesting a Th2 response, while localized forms of the disease appeared to be associated with responses of the Th1 type. Persisting antibodies to phenolic glyco- lipid-I in a small percentage of clinically inactive multibacillary patients may reflect differences in the catabolism of this unusual antigen, since persistence was not associated with a unique initial IgG subclass response to a complex extract of *Mycobacterium leprae* antigens.

Introduction

Many recent studies suggest that IgG subclasses of antibodies are at least partially related to the activity of different cytokines. The predominance of specific antibodies of IgG4 and IgE immunoglobulin isotypes has been reported in human filarial and allergic diseases (1,2). Interleukin 4, produced during a predominantly Th2 lymphocyte response, appears to play an important role in the synthesis of these two isotypes of antibodies (3). In contrast, mycobacterial and viral infections are in general characterized by the induction of a Th1-like response, accompanied by production of interleukin-2 and gamma interferon, and the synthesis of IgG1, 2 and 3 antibodies in human beings.

The nature of the T helper (Th) response in leprosy is controversial. While Padmini *et al(4)* and Yamamura *of al* (5) have reported a predominantly Th1 response in tuberculoid leprosy and a Th2 response in lepromatous disease, Mutis *et* a/. (6) reported a Th1 response across the entire spectrum of leprosy. Yet another group has reported a Th0 response, with the synthesis of a mixture of Th1 and Th2 lymphokines, in half of the patients studied, independent of the form of leprosy; in the remainder, Th1 and Th2 responses were associated with tuberculoid and lepromatous leprosy, respectively (7).

We have compared the IgG isotypes of specific antibodies across the spectrum in leprosy, using an antibody capture assay. These results have been are compared with the response observed in a previous study across the clinical spectrum of american cutaneous leishmaniasis (8), which shares many clinical, histopathological, and immunological features with the spectrum of leprosy. The results suggest important differences in the immunological mechanisms associated with anergy in these two intracellular infections.

In addition, we studied the initial IgG antibody subclasses in clinically inactive borderline lepromatous (BL) and lepromatous (LL) patients with persisting high levels of antibodies or with essentially negative responses to the *M.* /eprae-specific antigen phenolic glycolipid-I (PGL-I) after many years of treatment, in order to investigate possible differences in the early stages of the immune response which might explain antibody persistence in some patients in the apparent absence of clinical disease.

Materials and Methods

Sera: Serum samples were taken before treatment from 11 patients with tuberculoid (TT) or borderline tuberculoid leprosy (BT), 6 with borderline leprosy and 18 with lepromatous leprosy. In addition, pre-treatment sera were studied from 11 treated, clinically inactive BULL patients with negligible levels of antibodies to PGL-I after treatment and 12 inactive patients who presented persisting high levels of antibodies after similar treatment. In a previous study (8), we evaluated 20

sera from patients with localized cutaneous leishmaniasis (LCL), 12 with the muco-cutaneous form (MCL) and 20 from patients with diffuse cutaneous leish-maniasis (DCL).

Enzyme-linked immunosorbent assay (ELISA): IgG subclass antibodies to a crude soluble extract of *M. leprae,* obtained by rupturing purified bacilli in a French pressure cell and collecting the supemate after centrifugation, were measured in an antibody capture assay as follows: Microtiter plates were sensitized overnight with 50 pl of a solution containing 5 pg of protein/ml. After washing with PBS containing 0.02% Tween 20 (PBST), blocking with PBST- 1% BSA and decanting, the plates were treated successively with 1:100 dilutions of sera in PBST-1% BSA and peroxidase-labelled conjugates as described below for 1 h each at 37°C, followed by washes after each step and final incubation with the H_20_2-0-phenylene- diamine$_2$HCI substrate for 20 min at room temperature. The reactions were detained with 2.5N H_2SO_4 and read at 488 nm.

The details of the ELISA assay in American cutaneous leishmaniasis have been reported previously (9); the only significant difference in the procedure was the sensitization of microtiter plates with promastigotes or an crude soluble extract of *Leishmania mexicana.*

Peroxidase-labelled monoclonal anti-IgG isotype sera, obtained from BIODESIGN International, Kennebunk, ME, were used in a 1:1200 dilution after preliminary titration. The characteristics of these antisera are as follows: Anti-IgG1 (Fc), clone 8c16-39; anti-IgG2 (Fab), clone HP6014; anti- lgG3 SC3 (F[ab]2 epitope), clone HP6050 and anti- IgG4 (pFc' epitope), clone HP6023.

In the Figure, results are expressed as individual optical densities (OD): mean OD of duplicate antigen-containing wells minus the antigen-free serum control OD. In the Tables, the results are expressed as the mean OD ± the standard error of the mean (SEM).

Results

Sera from TT, BT and BB were characterized by relatively weak antibody responses, predominantly of the subclasses IgG1 and IgG2 (data not shown). The antibody capture assay with LL sera showed a predominance of IgG1 in 8 sera, IgG2 in 6, IgG3 in 2 and of IgG 4 in 1.

An earlier study (8) showed a clear predominance of IgG1, IgG2 and IgG3 antibodies in LCL and MCL forms of the localized cutaneous and muco-cutaneous forms of American cutaneous leishmaniasis; IgG4 antibodies predominated in this assay in 19 of 20 sera from the anergic form of the disease, DCL.

Table I shows the optical densities of antibody subclasses in sera from LL and DCL, the anergic forms of leprosy and american cutaneous leishmaniasis, respectively.

As shown in Table 2, we could not detect differences in the initial IgG subclass response to *M. leprae* in clinically inactive multibacillary patients with persisting high or sharply decreased levels of antibodies to PGL-I after treatment.

Discussion

The nature of the T helper lymphocyte response in leprosy has not been resolved. As cited in the Introduction, lepromatous leprosy has been associated with Th1, Th2 and Th0 responses in different studies. We hoped that the study of IgG subclasses of antibodies might provide additional data to clarify this situation. While switching factors for each IgG subclass have not been fully clarified in human beings, specific antibodies of the IgG4 subclass have been clearly associated with a Th2 response in several clinical situations, including allergy and helminth infestations.

Sera from patients with TT, BT and BB gave predominantly IgG1 and IgG2 antibody responses, as might be expected in a Th1 response. Our results showed a single LL patient with predominant IgG4; the 17 other sera from this group gave stronger reactions than those observed in the paucibacillary group, but with a similar subclass distribution.

These results were in marked contrast to the subclass response across the spectrum in American cutaneous leishmaniasis, where the response of LCL and MCL sera appeared to correspond to a Th1 response, but 19 of 20 patients with DCL gave a predominant IgG4 response.

The relationships between Th responses, cytokines and clinical characteristics in human

Table 1. Mean ELISA reactivity of each IgG isotype in LL and DCL

		IgG isotype			
Group	*n*	IgG1	IgG2	IgG3	IgG4
LL	18	0.3218* ± 0.06	0.4192 ± 0.16	0.3043 ± 0.09	0.0899 ± 0.06
DCL	20	0.2327 ± 0.03	0.1141 ± 0.03	0.0172 ± 0.01	0.6450 ± 0.09

* Expressed as mean OD ± SEM

Table 2. Initial antibody responses to soluble extract of *M. leprae* in patients with persistence or disminution of anti-PGL-I after treatment.

	High final	Low final
	anti-PGL-1	anti-PGL-I
Antiserum:		
a-IgG1	0.9191*	1.2623
a-IgG2	0.2721	0.2885
a-IgG3	0.7278	0.7385
a-IgG4	0.0607	0.1344

*Mean optical density

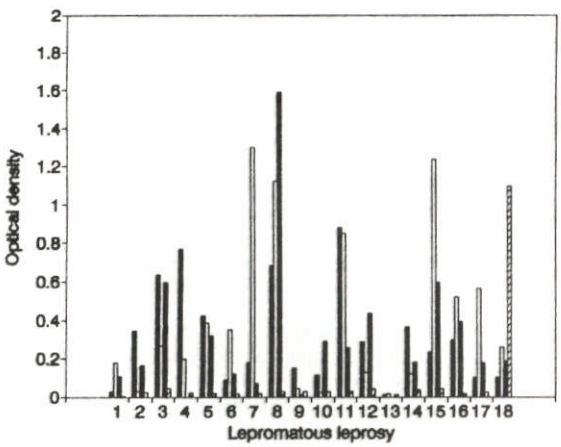

Figure - Concentration of antibodies of the four IgG subclasses on sera from untreated lepromatous patients. Key: IgG1 vertical lines, IgG2 open bar, IgG3 solid bar, IgG4 slash.

cutaneous leishmaniasis (10) appear to be very similar to the characteristics reported in infections with *Leishmania major* in inbred mouse strains (11), which clearly facilitates the extrapolation of many observations in the murine model to human disease.

The study of leprosy is handicapped by the absence of an experimental model similar to the murine model for leishmaniasis; all normal mice present a tuberculoid leprosy-like disease, while athymic mice and armadillos share characteristics of human lepromatous disease. We are unaware of any antibody isotype studies in these lepromatous-like infections.

Our results with lepromatous patients from Venezuela suggest that the T helper responses may be predominantly of the Th1 type. It should be noted that we are unaware of Th0-IgG subclass associations. We have not studied the subclass of antibodies *in situ* in lesions, but the results in DCL

would seem to suggest that there is no marked sequestering of antibodies at the tissue level.

We might have expected the sera from anergic forms of two intracellular infections with clinical and pathological similarities to give similar responses. Since this was not the case, it is clear that anergy may reflect varying mechanisms, unrelated to or over-riding the Th1/Th2 dichotomy.

Measurement of circulating antibodies to PGL-1 is used as one of the criteria for patient release and subsequent post-treatment surveillance in the Venezuelan leprosy control program. While these antibodies decrease significantly in two or three years in most patients, about 5% show persisting high levels for many years in spite of any evidence of clinical activity or presence of bacilli. Therefore we were interested in studying the early IgG antibody subclass response to determine possible differences between the two groups.

We could not detect differences in the early IgG subclass response to multiple *M. leprae* antigens, which might be associated with the subsequent persistence of anti-PGL-I antibodies in a small percentage of multibacillary patients after apparent clinical cure. Additional observations (unpublished data) appear to suggest that differences in the catabolism of PGL-I in some patients might be a more important factor in antibody persistence to this unusual antigen than persistence of bacilli or other antigens. Further studies using more than one antigenic preparation may be useful in refining serological tests for detection of relapse and other aspects of post-treatment surveillance.

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