Immunology, Epidemiology and Social Aspects of Leprosy
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THE ANTIBODY RESPONSE IN LEPROSY

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Overwhelming evidence that cell-mediated immune reactions provide the basis for an effective protective response to infection by *Mycobacterium leprae*, together with rapid technological advances in the evaluation of T-lymphocyte reactivity, have led to important advances in our understanding of cell-mediated activity in leprosy. Circulating anti-mycobacterial antibodies, in contrast, do not play a direct role in protection, and their measurement has not been useful for diagnostic or prognostic purposes. The role of antibody-mediated hypersensitivity phenomena, such as erythema nodosum leprosum appears to be well established, but these phenomena are normally managed at the clinical level. Antibody- or immune complex-mediated modulation of cell-mediated responses has not been clearly established *in vivo*. With relatively few exceptions, leprologists and investigators have not concerned themselves with antibody responses in leprosy during the last decade.

Perhaps four factors have been fundamental in stimulating a renewed interest in the evaluation of anti-mycobacterial antibody activity in leprosy:

1. *The isolation and characterization of phenolic glycolipid I and the demonstration of its highly specific serological reactivity* by Patrick Brennan and his colleagues [1]. This fundamental contribution has provided the basis for the development of specific tests which are not handicapped by the extensive cross reactivity with environmental mycobacteria which has characterized anti-mycobacterial serology.

2. *The widespread application of enzyme-linked immunosorbent (ELISA) techniques for antibody measurement.* These techniques are

highly sensitive, employ stable reagents and a minimum of sophisticated equipment, and are readily adapted to the study of large numbers of small serum samples. ELISA procedures are particularly suited for use in those areas of the world with endemic leprosy, where resources are often severely limited.

3. *The development of hybridoma technology and its application to leprosy.* The production of antibodies specific for simple antigenic molecules and individual epitopes on complex molecules offers the possibility of detecting a specific antigen-antibody reaction within a complex system by suitable inhibition tests, isolating single antigens or detecting their presence in tissues, and elucidating possible mechanisms of antibody-mediated immunomodulation, among others. These types of study are still in quite early stages in leprosy, but several *M. leprae*-specific epitopes and antigenic molecules can be identified by the monoclonal antibodies produced by Buchanan [2], Ivanyi [3] and Bloom. Monoclonal antibodies have of course been used with great success in characterizing the cell-mediated response in leprosy, both *in vitro* and at the level of organization and functional activity in the granuloma.

4. *Renewed interest and new approaches to leprosy control*, stimulated by the activities of the Special Program for Research and Training in Tropical Diseases of the UNDP/World Bank/WHO as well as independent centers. Leprosy control still depends on early detection of clinical disease and effective treatment. Field trials in prophylactic vaccination are being initiated in several areas which may introduce fundamental modifications in the strategy of leprosy control, and the determination of anti-mycobacterial antibodies may play an important role in this strategy.

The availability of suitable immunological tools for serological study allows the definition of very clear areas of interest for the application of these studies. These areas can be divided into studies in clinical leprosy and in subclinical infection, immuno-epidemiology and related fields.

Studies in clinical leprosy with more traditional approaches as well as newer applications permit several fundamental generalizations. Anti-mycobacterial levels appear to depend rather closely upon the bacterial load; specific as well as cross-reacting antibodies are present in relatively low titers at the tuberculoid end of the clinical spectrum of leprosy and increase across the spectrum, reaching maximum levels in

lepromatous leprosy. Within each clinical category there is, nevertheless, considerable individual variation. No clear qualitative differences have as yet been demonstrated in different clinical forms of leprosy; it has not been possible to ascribe possible *in vivo* antibody-mediated immunoregulatory functions to antibodies of a particular specificity. Interestingly enough, Mehra has demonstrated that monoclonal antibodies to phenolic glycolipid I (PGL-I) block the suppressor activity induced by this antigen *in vitro* [4]; to my knowledge, the relevance of this mechanism *in vivo* has not been evaluated, though the information to do so would be readily available.

We have determined levels of IgG and IgM antibody to PGL-I in serum samples from lepromatous and borderline lepromatous patients who showed significant histopathological changes, including reversal reactions and shifts toward more resistant forms of leprosy by the Ridley-Jopling criteria, and patients who did not show such changes. As shown in Tables 1 and 2, we could not demonstrate any relation between the initial levels of antibody of either class and the subsequent response to immunotherapy. Antibody levels did not show dramatic changes in a period of two years after the initiation of immunotherapy, though many decreased.

Antibody titers decrease after chemotherapy; studies by Melsom and collaborators [5] report the most rapid decrease in the IgG class of antibodies, while our studies [6] as well as those of Brennan's group [7] show a more rapid decrease in antibodies of the IgM class to glycolipid as well as to a complex soluble extract which contains PGL-I (Figs 1 and 2). Yoder *et al.* [8] have reported an increase in antibody titers prior to relapse in borderline tuberculoid leprosy. All of these studies taken together indicate that serological studies may provide a useful approach to the evaluation of chemo- and immunotherapy in clinical leprosy, to the early detection of relapse or drug resistance, and to the possible detection of persisting viable organisms. All of these studies are of relatively long duration and require close collaboration between clinical and laboratory personnel. It would appear to be of great interest to include the storage of an initial serum sample from new cases of leprosy as part of the routine procedure in different areas of the world, since regional differences may be important and the necessary technology should be quite readily available.

The use of serological tests may be of even greater interest and potential importance in aspects of leprosy control related to early detection of disease, including subclinical infection, immuno-

TABLE 1

*Titer of IgM and IgG antibodies to phenolic glycolipid I in the sera of patients who showed histopathological changes after M. leprae-*BCG immunotherapy.

Patient	IgM		IgG	
	Initial	Two years	Initial	Two years
AMM	16000[a]	8000	1000	2000
SG	8000	8000	4000	500
JPV	8000	2000	2000	500
UM	8000	2000	1000	1000
EP	8000	2000	1000	500
AH	8000	2000	<250	<250
DC	4000	4000	1000	500
RE	4000	4000	1000	500
AJO	4000	1000	250	500
JJG	2000	2000	1000	500
SD	1000	4000	500	500
JC	1000	1000	250	250
HG	1000	1000	<250	<250
LP	500	500	1000	250
JHM	<250	<250	500	500
LR	<250	<250	500	250

[a] Reciprocal of the highest dilution giving an OD >.02 at 405 nm.

TABLE 2

Titer of IgM and IgG antibodies to phenolic glycolipid I in the sera of patients who did not show histopathological changes after immunotherapy.

Patient	IgM		IgG	
	Initial	Two years	Initial	Two years
JY	16000	16000	8000	2000
TC	16000	4000	500	250
GN	8000	1000	500	500
RV	2000	1000	1000	250
JLR	1000	1000	<250	<250
JR	500	250	<250	<250

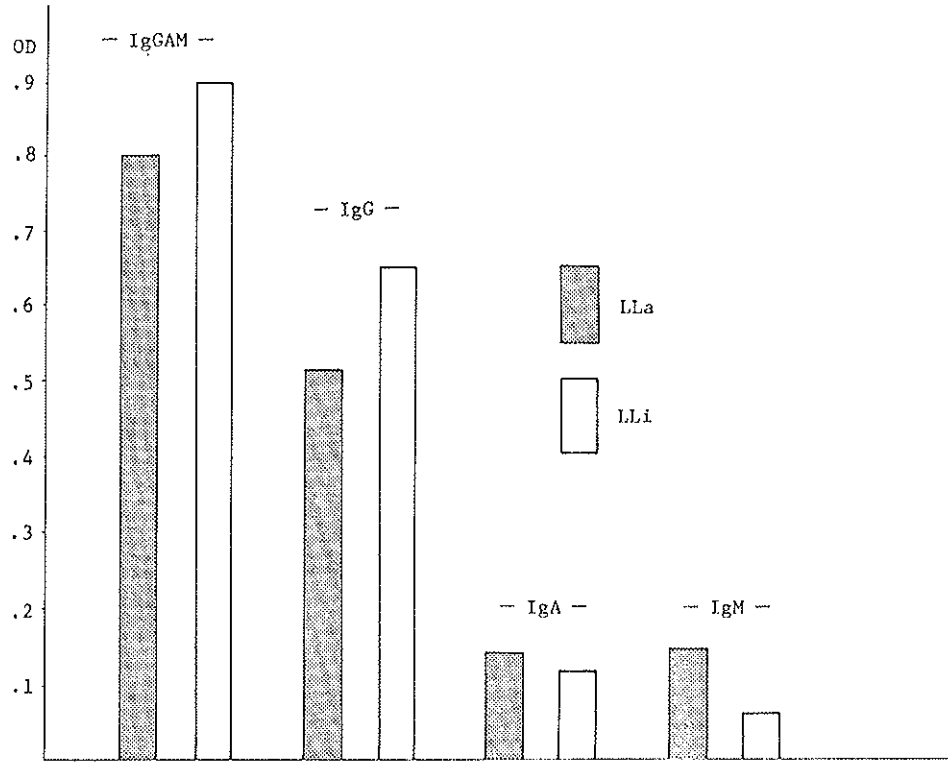

FIG. 1. Antibody levels to soluble extract of *M. Leprae* in active and inactive lepromatous leprosy.

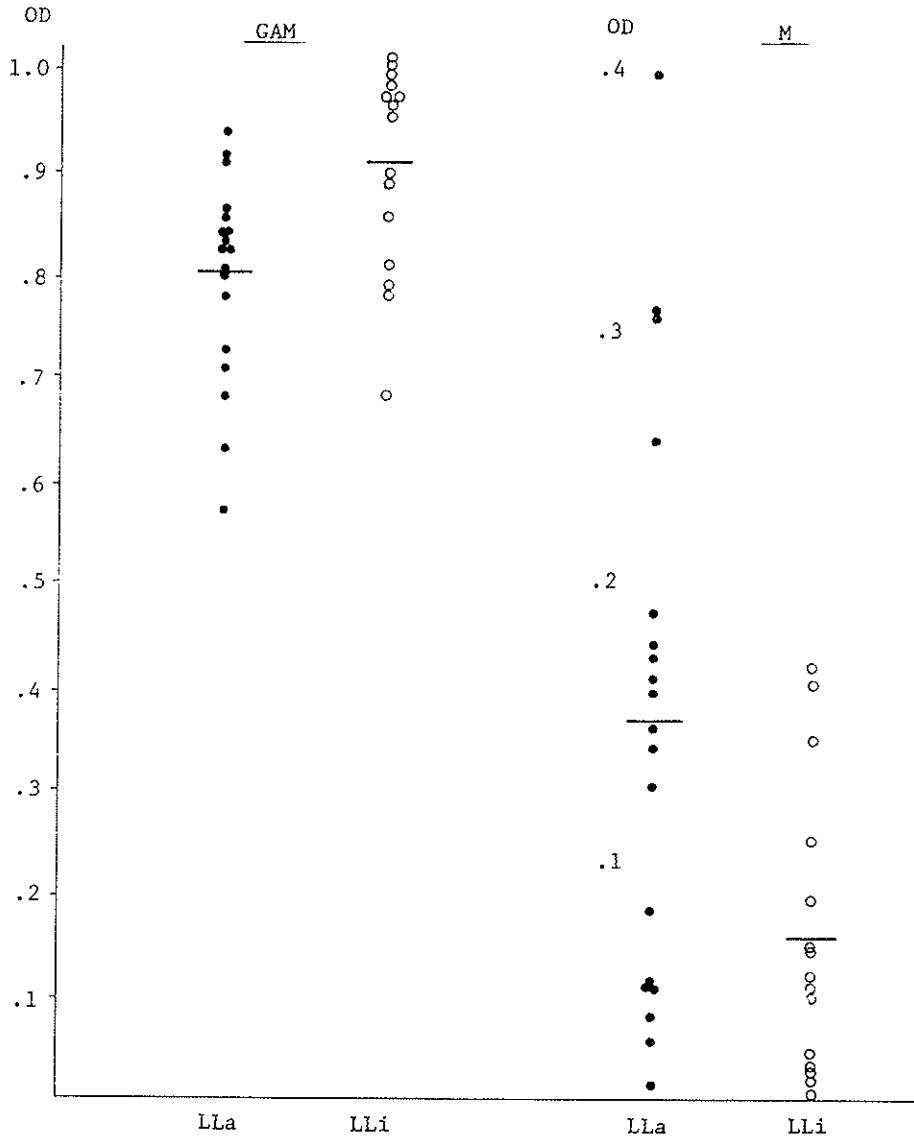

FIG. 2. Individual antibody responses to *M. leprae* soluble extract in active and inactive LL.

epidemiological studies and immunoprophylaxis. Progressive forms of leprosy with an average incubation period of several years may be the source of new infections in the community during a period when there is no clinical evidence of disease. The availability of a specific serological test for detecting infection by *M. leprae* may offer the only possibility for detection of potentially progressive disease during the incubation period, since cell-mediated reactivity to *M. leprae* is absent. Buchanan [9] has recently reported four cases of clinical leprosy (2 TT, 1 BT and 1 LL) in the follow-up of a group of 1096 household contacts from Sri Lanka; the contact who subsequently developed LL had exceptionally high levels of antibody to PGL-I. We have detected strong serological reactivity to PGL-I and to a complex antigenic extract of *M. leprae* in partially overlapping groups of about 3% of a group of 302 household and non-household contacts who gave negative skin tests to a soluble antigen of *M. leprae* (Figs. 3 and 4) [10]. Since these individuals are incorporated into an immunoprophylaxis trial and have been vaccinated, there is some question whether clinical disease will occur even if they now have subclinical infection. About 18% of the skin-test negative contacts with ELISA indices greater than 4 did not respond to vaccination, while the overall percentage of non-response was less than 3%. These results indicate that a small group of contacts possess immunological peculiarities similar to those observed in lepromatous leprosy—high anti-mycobacterial antibody titers together with negligible cell-mediated reactivity to *M. leprae*. The study of T-cell suppressor activity of the type described by Mehra and colleagues [4] will be of particular interest in this group.

There is some doubt whether the PGL-I system is ideal for detecting early infection by *M. leprae*. The concept of "original mycobacterial sin" [11] would suggest that the earliest antibody response to infection by *M. leprae* might be to common antigens shared with environmental mycobacteria; a specific antibody response may develop much more slowly. Additionally, glycolipids are not particularly strong antigens. The development of an appropriate inhibition test with monoclonal antibodies to a *M. leprae*-specific proteic epitope might offer a more sensitive tool for detecting early infection. Until the nature of the early response has been more clearly defined, there would appear to be justification for measuring both specific and non-specific anti-mycobacterial antibody, in conjunction with skin testing and, in selected individuals, *in vitro* studies of cell-mediated responses in contacts.

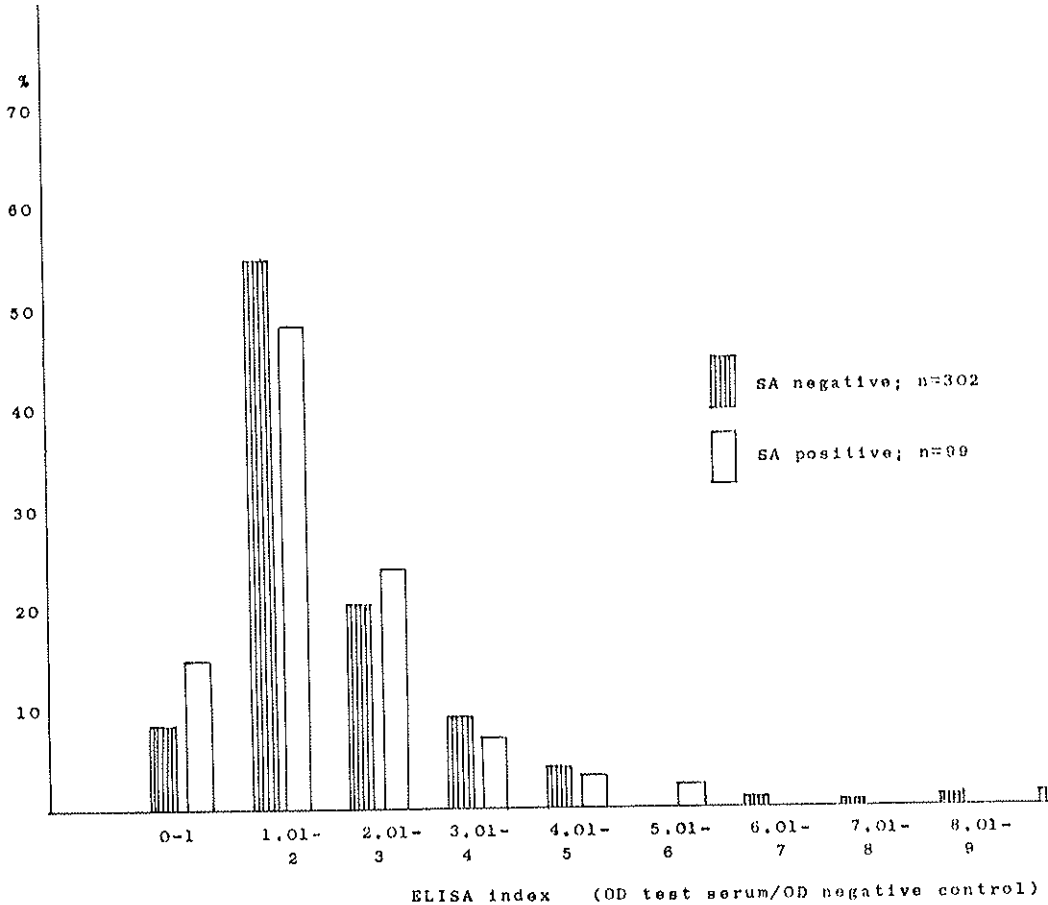

FIG. 3. Antibody levels in SA-negative and SA-positive contacts, measured by microELISA with soluble antigen from *M. leprae*.

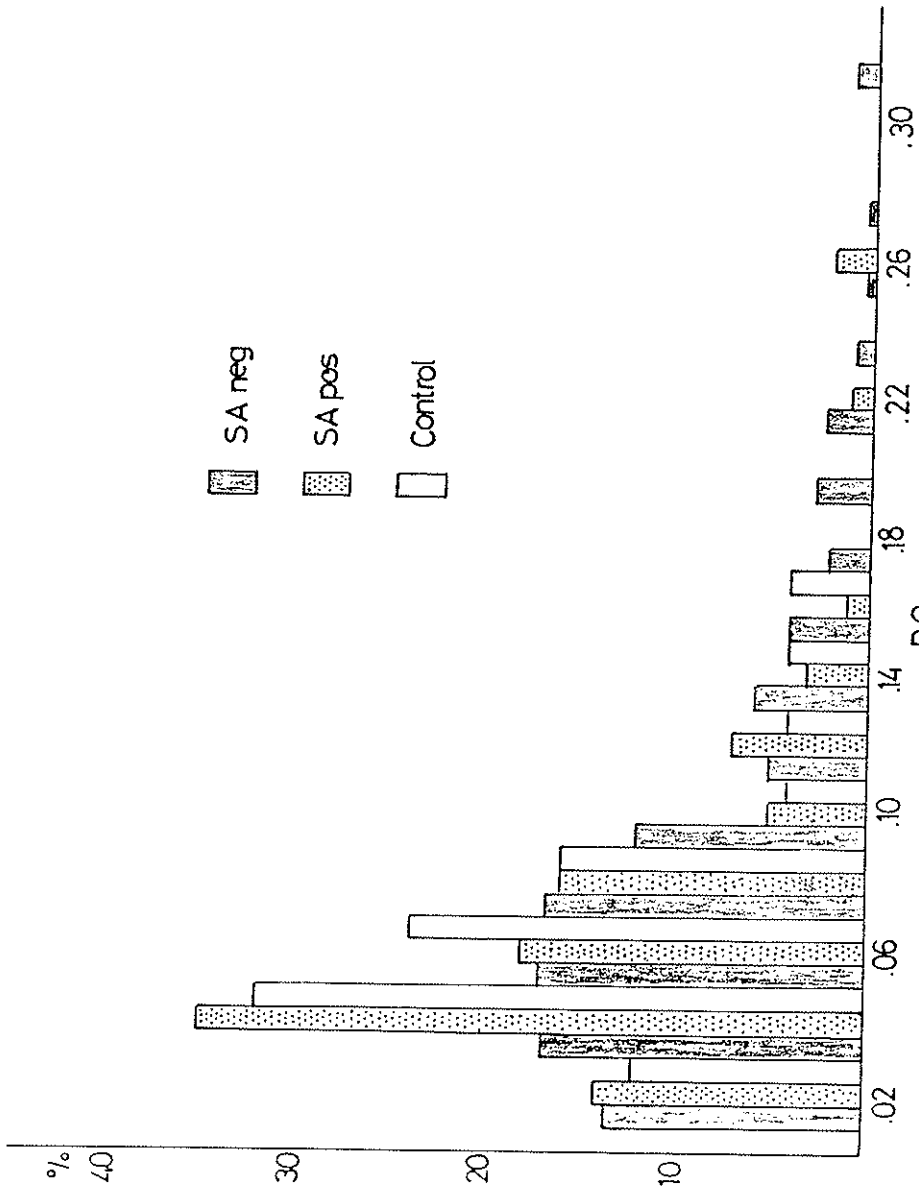

FIG. 4. Antibody levels to PGL-I in healthy contacts.

Bloom has suggested that the measurement of anti-PGL-I may offer useful information during the course of vaccine evaluation, since the *M. leprae* preparation being used (as well as BCG) would not be expected to stimulate the formation of these antibodies. If they were to appear during the course of follow-up, that would suggest the presence of active infection by *M. leprae* (and vaccine failure). The obvious corollary is that diminution or disappearance of anti-PGL-I antibodies in the vaccinated group but not in the controls would clearly suggest that vaccination possesses a therapeutic effect in subclinical infection.

In the relatively near future, serological tests could be used in leprosy to: (1) monitor the course of clinical infection and evaluate the response to chemo- and immunotherapy; (2) detect multibacillary infection during its subclinical stage. Combined studies of cell-mediated reactivity and serological activity may permit the definition of a susceptible population among apparently healthy individuals and contribute to the evaluation of immunoprophylactic procedures. All of these possibilities could contribute in one way or another to more successful programs of leprosy control.

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