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Antibodies to *Mycobacterium tuberculosis*-specific epitopes in lepromatous leprosy

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SUMMARY

Sera from patients with leprosy or tuberculosis and healthy subjects have been analysed for the presence of antibodies to four species-specific mycobacterial epitopes, four different viruses and five autoantigens. Antibodies to the *Mycobacterium leprae*-specific 35-kD protein and phenolic glycolipid I epitopes were not present in patients with active pulmonary tuberculosis. In contrast, antibody levels to species-specific epitopes of the 38-kD and 14-kD antigens *M. tuberculosis* were significantly elevated in patients with lepromatous leprosy. Neither of the two antigens is cross-reactive with *M. leprae* at the B cell level. However, it was considered that cross-reactive helper T cells could recall the response of *M. tuberculosis*-specific memory B cells, which had been primed through prior self-healing tuberculous infection. As an alternative explanation, the possible role of polyclonal B cell stimulation was considered. This seemed unlikely, however, since: (i) antibody levels to autoantigens, except anti-smooth muscle, were not elevated, and (ii) antibody levels to four distinct viruses, unlike those to all mycobacterial epitopes, showed no correlation with titres, to *M. tuberculosis*-specific epitopes.

Keywords tuberculosis leprosy antibodies mycobacteria original antigenic sin

INTRODUCTION

The specificity of antibody response following successive immunization with structurally related antigens is determined by the cross-reactivity between constituent B cell and T cell stimulatory epitopes. The functional cell interactions are governed by complex, yet poorly understood regulatory mechanisms considering that sharing of antigenic stimulatory epitopes can: (i) enhance the secondary antibody response; (ii) suppress certain anti-hapten response (Herzenberg, Tokuhisa & Herzenberg, 1980); and (iii) recall antibody production with specificity for the primary antigen. This last outcome, termed 'original antigenic sin' (OAS), was discovered by analysis of anti-influenza virus haemagglutinins (Davenport, Hennessy & Francis, 1953; Fazekas de St. Groth & Webster, 1966) and was subsequently observed for viral antigens (Virelizier, Allison & Schild, 1974; Halstead, Rojanasuphot & Sangkawibha, 1983), streptococcal polysaccharide (Cramer & Braun, 1973), HLA (Dorf & Eguro, 1973), protein (Ivanyi, 1972; East, Todd & Leach, 1980), and simple hapten (Strausbach *et al.*, 1972; Deutsch, Vinit & Bussard, 1973) type antigens. The OAS antibodies react only with the first but not second immunogen and are therefore distinct from the commonly occurring antibodies which cross-react with both antigens. The mechanism of this paradoxical type of recognition has been attributed either to the stimulation of cross-reactive T helper cells (Ivanyi, 1972) or to 'degeneracy' of the antigen-binding immunoglobulin receptor of memory B cells (Cramer & Braun, 1973; Virelizier *et al.*, 1974).

The OAS phenomenon has important implications for the development of specific tests for infectious diseases. Several antigenic constituents of *Mycobacterium tuberculosis* and *M. leprae* have been identified by monoclonal antibodies (MoAbs) and T cell clones and several of these proteins have been sequenced using recombinant DNA techniques (reviewed by Ivanyi *et al.*, 1988). Using MoAbs as probes for the serologically defined species-specific epitopes, the solid-phase antibody competition test (SACT) has been used to measure the antibody response to these epitopes in both tuberculosis (Hewitt *et al.*, 1982; Hoeppner *et al.*, 1987; Bothamley *et al.*, 1988) and leprosy (Sinha *et al.*, 1985, 1989; Mwatha *et al.*, 1988; Roche *et al.*, 1990).

In this study we report the surprising observation of a non-reciprocal OAS: *M. tuberculosis*-specific antibodies occurred in lepromatous leprosy but *M. leprae*-specific antibodies were not detected in patients with active pulmonary tuberculosis. Both findings have been made with sera from communities in Nepal and India, where there is endemic exposure to both mycobacterial infections.

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Since the rise in antibody level to a variety of autoantigens and infectious agents is a feature of lepromatous leprosy (Kano *et al.*, 1981), we have also measured antibody levels to viral and autoantigens and examined their relationship to anti-mycobacterial antibody titres.

PATIENTS AND METHODS

Patients and sera

Patients attending leprosy clinics in either Anandaban, Nepal or in Agra, India were classified by attending physicians on the basis of standard clinical and bacteriological criteria (Ridley & Jopling, 1966). Sera were collected from 33 patients with polar lepromatous (LL), 31 patients with borderline lepromatous (BL) and 27 patients with borderline or polar tuberculoid leprosy (BT). None of these patients presented with symptoms or clinical signs of tuberculosis; in lepromatous patients a sputum smear was negative for acid-fast bacilli in 45 patients tested and in 26 patients a chest radiograph was obtained and showed no evidence of active pulmonary tuberculosis. Sera were also obtained from 19 patients with radiologically active pulmonary tuberculosis and from 20 clinically healthy subjects. All sera were kept frozen and thawed not more than twice before serological analysis.

Solid phase antibody competition test (SACT)

This method estimates antibody levels of specificities which overlap with the binding of selected MoAbs (Hewitt *et al.*, 1982). *M. tuberculosis*-specific MoAbs TB72 directed to a 38-kD protein (Young *et al.*, 1986; Andersen & Hansen, 1989) and TB68 directed to a 14-kD protein (Ivanyi, Morris & Keen, 1985) were radioiodinated and competed with test sera for binding to microtitre plates (M24; Dynatech, Billingshurst, UK) coated with the soluble extract from *M. tuberculosis* (MTSE) following the procedures described in detail elsewhere (Bothamley *et al.*, 1988). The antibody ML04 directed to the 35-kD protein of *M. leprae* (Ivanyi *et al.*, 1985) was labelled with peroxidase and used on plates (Immulon M129B; Dynatech) coated with *M. leprae* soluble extract (MLSE) (Sinha *et al.*, 1985; Mwatha *et al.*, 1988). Sera were tested at serial dilutions of 1/5–1/625 and antibody titres (ID_{50}) were expressed as reciprocals of the dilution of serum which reduced the binding of the labelled MoAb to 50% of binding to wells with no competing serum. Antibody titres were calculated by interpolation between the two serum dilutions either side of the 50% inhibition values.

Western blots

SDS–PAGE (12% w/v final acrylamide concentration) was run under reducing conditions at 17 mA per gel for 60 min, using the SE250 Minigel system (Hoeffer Scientific Instruments, San Francisco, CA). Proteins after separation were transferred to nitrocellulose membrane by blotting for 1 h at 60 V. The membrane was washed once with phosphate-buffered saline (PBS) containing 0·05% w/v Tween 20 (PBST) and non-specific binding was blocked by incubation with 1% w/v dried milk, in PBST for 20 min. The membranes were cut into 0·4 cm width strips longitudinally and incubated for 2 h with 1/100 diluted either human sera or pooled serum from *M. leprae* infected armadillos. After washing three times with PBST, peroxidase-labelled affinity purified goat anti-human IgG or anti-armadillo IgG was added for 1 h and developed with diamino-benzidine dihydrochloride (0·1 mg/ml, Sigma Chemical Co., Poole, UK) and 0·01% H_2O_2 in 0·1 M citrate buffer (pH 5).

ELISA

IgM anti-phenolic glycolipid (PGL-I) antibodies were determined using as antigen the glycolipid disaccharide-bovine serum albumin (dBSA, provided by IMMLEP, WHO, Geneva, Switzerland) (Roche *et al.*, 1990). Briefly, microtitre trays (Dynatech, Alexandria, VA) were coated with dBSA at 250 ng/ml, blocked with 1% BSA and reacted with patient sera at a dilution of 1/300 in duplicate. The second antibody was goat anti-human IgM-peroxidase (Cappel, West Chester, PA) at a dilution of 1/4000 and the enzyme substrate o-phenylenediamine (Sigma, St. Louis, MO). Absorbance in control wells coated with BSA alone was subtracted from test wells and samples with an absorbance at 492 nm greater than 0·199 (mean absorbance + 3 × s.d. of 91 healthy Nepali control subjects) were considered positive.

The influenza virus core antigen (IVC) prepared by the method of Brand & Skehel (1972) was used for the coating of microtitre wells at a concentration of 30 μg/ml. Antibody binding to IVC was measured by ELISA using sera diluted from 1/1000 to 1/100 000. Antibody titres were expressed as the dilution giving 30% binding (ABT_{30}) of the plateau represented by a rabbit hyperimmune serum (OD 0·9). Antibodies to herpes simplex virus (HSV), measles virus (MEV) and cytomegalovirus (CMV) were sought with Enzygnost kits OUWP-11, OSOK-03 and OSDL-03 (Behring Diagnostics, Hounslow, UK) according to the manufacturer's instructions: assays were recorded in optical density units.

Autoantibodies

Rheumatoid factor (IgM) was detected with the Human RF latex kit (Biostat, Stockport, UK). Anti-nuclear, anti-mitochondrial and anti-smooth muscle autoantibodies were sought with indirect immunofluorescence tests on cryostat sections of rat liver and kidney and were demonstrated using anti-human immunoglobulin FITC conjugate (MF01, Wellcome Reagents, Beckenham, UK). Autoantibodies to gastric parietal cells were assayed by ELISA using microtitre plates coated with an extract of porcine parietal cells.

Statistical analysis

Antibody levels between different groups were compared using the non-parametric Mann–Whitney *U*-test. Correlation between antibody levels to different epitopes or antigens was performed using Spearman's rank correlation test. The probability was corrected for the number of tests (n) according to the formula $P_{corr} = 1-(1-P)^n$. The frequencies of positive autoantibodies were compared using the χ^2-test.

RESULTS

Antibody levels to M. leprae*-specific antigenic determinants*

The mean ML04 (35 kD) specific ID_{50} titres and anti-PGL-I OD binding values in the examined groups of patients and controls are shown in Fig. 1. The stringent *M. leprae* specificity of both these epitopes is evident from the absence of any positive values in healthy controls and in patients with active pulmonary tuberculosis. Antibody titres to PGL-I-disaccharide and to the ML04 epitope were significantly higher in patients with LL compared to those with BL leprosy (Mann–Whitney *U*-test:

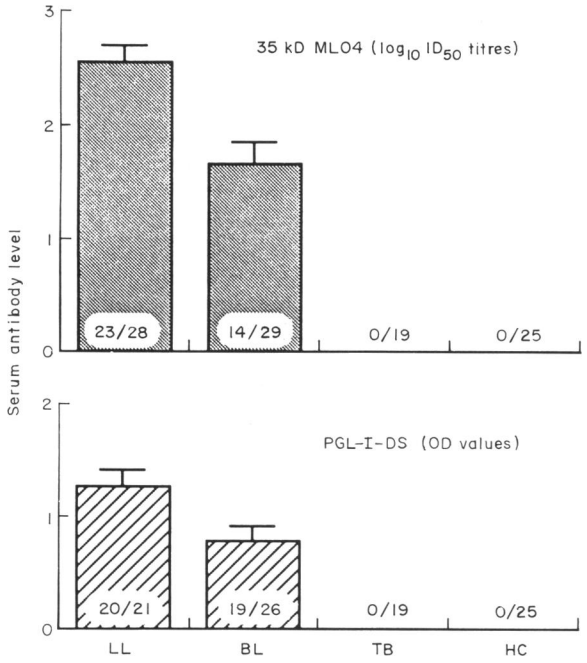

Fig. 1. Serum antibody levels to *M. leprae*-specific epitopes. Columns corresponding to groups of patients (see footnote to Table 1 for abbreviations) represent mean values and vertical bars indicate the standard error. The numbers indicate antibody-positive/total examined.

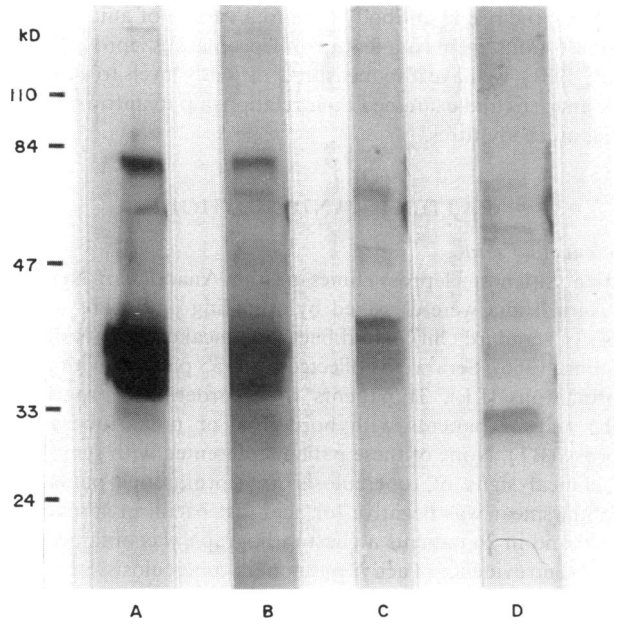

Fig. 3. Western blot analysis of anti-38-kD antibodies. Binding to recombinant semipurified 38-kD protein by: TB72 MoAb (A), sera from patients with active tuberculosis (B), LL (C) or pooled serum from heavily *M. leprae*-infected armadillos (D).

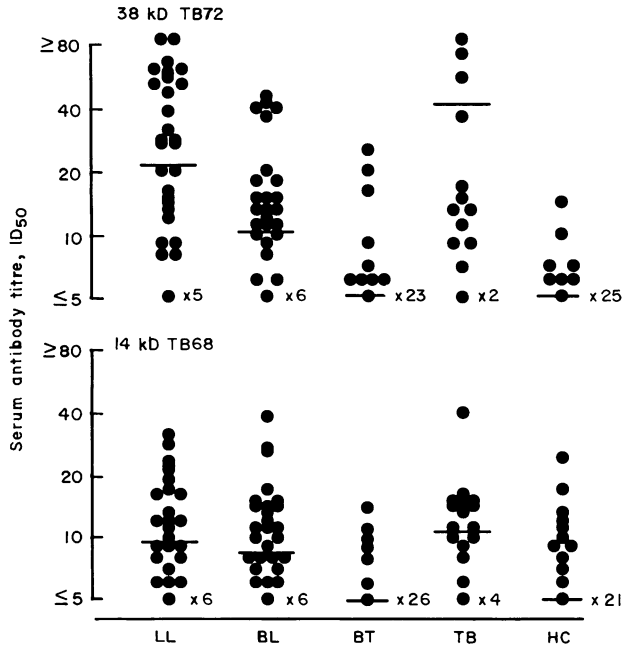

Fig. 2. Serum antibody levels to *M. tuberculosis*-specific epitopes. Individual values (●) and geometric means (horizontal bars) in groups of patients and controls. Abbreviations, see footnote to Table 1; BT, tuberculoid leprosy.

$P=0{\cdot}05$ and $P=0{\cdot}004$ respectively). Defining the cut-off points for positivity as an ID_{50} of 10 for ML04 and an OD of 0·2 for IgM anti-PGL-I, the sensitivity of leprosy detection was somewhat greater for the ML04 specificity, since eight sera were $ML04^+/PGL\text{-}I^-$ and only two sera were $ML04^-/PGL\text{-}I^+$. These results confirm the previous comparative serological studies (Mwatha *et al.*, 1988; Sinha *et al.*, 1989; Roche *et al.*, 1990). Only 8/31 patients with BT had positive antibody titres to the ML04 epitope and their mean titre (geometric mean $ID_{50}=12$) was significantly lower than in those with lepromatous leprosy ($P<0{\cdot}0001$).

Antibody levels to M. tuberculosis*-specific epitopes*

Antibody titres to the TB72 (38-kD) and TB68 (14-kD) protein antigens (Fig. 2) were significantly greater in patients with active pulmonary tuberculosis compared to healthy controls (Mann–Whitney *U*-test: all $P<0{\cdot}001$). Although neither of these two MoAbs bind to *M. leprae*, paradoxically raised antibody levels were found in lepromatous leprosy (LL and BL) patients compared with healthy controls (Fig. 2: TB72: $P=0{\cdot}0004$; TB68: $P<0{\cdot}0001$). Antibody levels to the TB72 and TB68 epitopes in LL patients were indistinguishable from values obtained in patients with pulmonary tuberculosis ($P=0{\cdot}46$ and 0·94 respectively). Antibody levels to the TB72 epitope were lower in BL patients compared with both LL and tuberculosis patients (both $P=0{\cdot}04$), although antibody levels to the TB68 epitope in BL patients were indistinguishable from those in patients with tuberculosis ($P=0{\cdot}58$). Separate analysis of the data from 26 lepromatous patients with a sputum-smear without acid-fast bacilli on microscopy and with a normal chest radiograph, confirmed that antibodies to *M. tuberculosis*-specific epitopes in patients with lepromatous leprosy appeared in the absence of concurrent tuberculosis (Mann–Whitney *U*-

Table 1. Anti-viral antibody levels

Antigen specificity*	End-point†	Antibody level (mean ± s.d.) LL	BL	TB	HC
IFV	ABT30	3·50 ± 0·70	3·86 ± 0·66	3·53 ± 0·61	4·00 ± 0·80
MEV	OD	1·95 ± 0·11	1·97 ± 0·05	1·95 ± 0·06	1·95 ± 0·09
CMV	OD	1·87 ± 0·11	1·82 ± 0·17	1·79 ± 0·19	1·87 ± 0·13
HSV	OD	1·90 ± 0·19	1·90 ± 0·24	1·80 ± 0·27	1·75 ± 0·41

* IFV, influenza virus; MEV, measles virus; CMV, cytomegalovirus; HSV, herpes simplex virus.
† ABT30, $\log_{10}$ serum dilution giving 30% of the plateau OD binding; OD, optical density. LL, lepromatous leprosy ($n=33$); BL, borderline lepromatous ($n=31$); TB, tuberculosis ($n=19$); HC, healthy controls ($n=33$).

Table 2. Correlation between antibody titres in lepromatous leprosy

Epitope/ antigen*	Spearman's rank correlation (r) value† TB72	TB68	ML04	PGL-I
TB68	0·808			
ML04	0·632	0·439		
PGL-I	0·487	0·515	0·397	
IFV	0·122	0·343	0·089	0·081
MEV	0·171	0·143	0·168	0·173
CMV	0·126	−0·001	0·166	0·057
HSV	−0·130	−0·150	−0·025	−0·055

* See footnote to Table 1.
† Underlined values are significant ($P<0·05$) when corrected for the number of tests (n) according to the formula: $P_{corr}=1-(1-P)^n$. Values combined from LL and BL groups (54 sera).

Table 3. Incidence of autoantibodies in leprosy and tuberculosis patients

Autoantibody specificity	Number (%) of positive sera LL/BL ($n=57$)	TB ($n=19$)	Healthy ($n=19$)
Rheumatoid factor (IgM)	10 (18)	2 (10)	5 (21)
Anti-nuclear	3 (5)	1 (5)	0 (0)
Anti-mitochondrial	1 (2)	1 (5)	2 (8)
Anti-smooth muscle	20 (35)	5 (26)	1 (4)
Anti-gastric parietal cell	5 (9)	2 (10)	4 (17)

test, comparing antibody titres in leprosy patients with control values: both $P<0·02$). Antibody titres in patients with BT were significantly lower than in patients with pulmonary tuberculosis ($P<0·01$) and indistinguishable from control sera ($P>0·30$).

*Failure of 38-kD protein binding by anti-*M. leprae *sera*

Although the MoAb TB72 does not cross-react with *M. leprae* (Ivanyi *et al.*, 1985), we further investigated whether the TB72 competition by LL sera could have resulted from steric hindrance by antibodies against putative cross-reactive epitopes of the 38-kD molecule. Since human anti-*M. leprae* sera, with definitely no exposure to tuberculosis, could not be obtained, we examined a pooled serum from heavily *M. leprae*-infected armadillos (obtained from J. Colston, NIMR, Mill Hill, UK) which contained high antibody levels against phenolic glycolipid I. Western blot analysis of this serum using the partially purified recombinant 38-kD protein showed no binding against the 38-kD band whilst other weak bands were apparently directed towards *Escherichia coli*-derived contaminants (Fig. 3, lane D). However, the 38-kD band was detected by human sera from tuberculosis as well as LL patients and by the TB72 MoAb used as the positive control (Fig. 3, lanes A, B and C). The lack of anti-38-kD binding was also confirmed by competition assay and using antisera from rabbits (obtained from Prof. M. Harboe, Oslo, Norway) and BALB/c mice which had been hyperimmunized with either whole cells or extracts from *M. leprae* (data not shown).

Antibody levels to viral antigens

To explore further whether the *M. tuberculosis*-specific antibodies could have been raised as a result of polyclonal B cell stimulation, sera from LL, BL, tuberculosis and control groups were tested for antibody binding to four different viruses to which subjects were naturally exposed. Antibody activity with individual variation was observed but antibody levels to influenza core antigen, measles virus, cytomegalovirus and herpes simplex virus showed no significant difference between the four groups (Table 1).

Concordance between anti-mycobacterial but not with anti-viral antibody levels

The Spearman's rank correlation analysis was performed for the anti-*M. leprae*, anti-*M. tuberculosis* and anti-virus antibody levels in 54 sera from the lepromatous (LL and BL) leprosy patients (Table 2). This analysis showed significant ($P_{corr}<0·05$) correlation between paired TB72, TB68, ML04 and PGL-I levels with the single exception of the ML04/PGL-I combination which marginally failed to obtain statistical significance when corrected for the number of tests made ($r=0·397$; $P=0·006$, $P_{corr}=0·13$). However, no correlation between antibody titres to the viral antigens and the mycobacterial epitopes could be demonstrated, even if the analysis were restricted to LL patients or to those in whom antibody titres to viral antigens

were such as to suggest exposure. Similarly, no correlation in antibody titres between any of the four viral antigens was observed.

Autoantibody levels

The presence of autoantibodies was examined in sera from LL/BL, tuberculosis and healthy control groups (Table 3). The incidence of anti-nuclear, anti-mitochondrial, anti-gastric parietal cell antibodies and rheumatoid factor was uniformly low and no differences were found between patients and controls (Table 3). However, anti-smooth muscle antibodies were found more frequently in lepromatous leprosy (35%) and in tuberculosis (26%) than in controls ($\chi^2=4{\cdot}9$, $P=0{\cdot}03$). LL patients with a positive anti-smooth muscle antibody titre had higher antibody levels to the TB68 epitope when compared with LL patients without such autoantibodies (Mann–Whitney *U*-test: $P=0{\cdot}025$).

DISCUSSION

The novelty of this study rests with the finding of elevated levels of antibodies to *M. tuberculosis*-specific epitopes in patients with lepromatous leprosy in whom concurrent tuberculosis has been formally excluded by clinical examination, sputum microscopy and chest radiographs. *M. tuberculosis*-specific antibodies in patients with pulmonary tuberculosis were directed to the TB72 (38-kD) and TB68 (14-kD) epitopes (Hewitt *et al.*, 1982; Ivanyi, Krambovitis & Keen, 1983; Hoeppner *et al.*, 1987; Bothamley *et al.*, 1988). Thus, the serological diagnosis of tuberculosis, in common with the false positive serology in respect of other infections (Kano *et al.*, 1981), is clearly not feasible in patients with lepromatous leprosy. In contrast, antibody levels to the *M. leprae*-specific ML04 (35-kD) epitope and to PGL-I which are consistently elevated in leprosy patients with a lepromatous polarity (Mwatha *et al.*, 1988; Sinha *et al.*, 1989; Roche *et al.*, 1990) were not demonstrable in patients with active pulmonary tuberculosis. Patients were from areas where both leprosy and tuberculosis are endemic. Sensitization by natural exposure to cases of infectious tuberculosis is likely to be more frequent than to leprosy and might therefore account for the non-reciprocal occurrence of the antibody recall.

The *M. tuberculosis*-specific antibodies occurred selectively in patients with multibacillary (LL and BL) lepromatous leprosy but not in those with tuberculoid disease. Similarly, antibodies to the *M. leprae*-specific antigenic determinants were found predominantly in lepromatous leprosy patients. This fact together with the markedly higher antibody levels to the 38-kD antigen in patients with smear-positive than in smear-negative pulmonary tuberculosis (Bothamley *et al.*, 1988; Jackett *et al.*, 1988) suggests that multibacillary stimulation is an essential requirement for anti-mycobacterial antibody formation. However, it is not clear why the antibodies to the TB68 (14-kD) epitope, which is also immunogenic in healthy subjects who have been exposed to tuberculosis by occupational or household contact (Hoeppner *et al.*, 1987; Jackett *et al.*, 1988), were not raised in tuberculoid leprosy. Although the peripheral blood of tuberculoid leprosy patients contains an abundance of T cells responding to mycobacteria by proliferation, they appear to be lacking either the helper subset of T cells or primed B cells required for the recall of TB68 (14-kD) specific antibodies.

We failed to detect any cross-reactive antibodies to the 38-kD antigen of *M. tuberculosis* following infection or immunization with *M. leprae* alone, using armadillo, rabbit and mouse antisera. Moreover, antisera to the recombinant 38-kD protein of *M. tuberculosis* did not bind to a corresponding antigen within the *M. leprae* soluble extract (unpublished data). These results argue strongly against the presence of protein, serologically cross-reactive with the 38-kD protein of *M. tuberculosis*, in the *M. leprae*-soluble extract but do not preclude its secretion *in vivo*. Furthermore, the results allow for the cross-recognition by T cells of a homologous protein in both species of mycobacteria. A selective cross-reactivity between antigens for T but not for B cells was proposed to be the basis for the OAS (Ivanyi, 1972). This phenomenon represents the recall of antibody synthesis, specific for the priming but not for the eliciting antigen, following successive immunization with structurally related molecules. The most compelling evidence for the role of cross-reactive T cell epitopes for the OAS was presented in adoptive transfer experiments using mixtures of T and B cells each primed to different polypeptides which demonstrated that the recall of antibody was dependent on stimulation with polypeptides of the T cell specificity (Parhami-Seren *et al.*, 1982).

Cross-reactive T cell stimulatory epitopes have been demonstrated for the 14-kD antigen which carries the TB68-defined antibody specificity. Mice and guinea pigs immunized with an extract of *M. leprae* gave a significant delayed-type hypersensitivity (DTH) and lymphocyte proliferative response to the recombinant 14-kD protein of *M. tuberculosis* and conversely, immunization with the recombinant 14-kD protein significantly increased the responses to *M. leprae* (Kingston *et al.*, 1987). Corresponding analysis of the 38-kD antigen showed that guinea pigs sensitized with *Mycobacterium intracellulare*, *Mycobacterium scrofulaceum* or *Mycobacterium kansasii* (*M. leprae* was not tested) showed lymphocyte proliferation or skin DTH responses to the 38-kD antigen purified from *M. tuberculosis* (Worsaae *et al.*, 1987; Kadival, Chaparas & Hussong, 1987). Broad bacterial cross-reactivity of T cells would also be expected in view of the structural homology between the 38-kD protein of *M. tuberculosis* with the phoS gene product of *E. coli* (Andersen & Hansen, 1989). Conversely, the failure to detect antibody to the *M. leprae*-specific ML04 epitope in patients with tuberculosis might then indicate the absence in *M. tuberculosis* of a homologue of the *M. leprae* 35-kD antigen. This observation is in accord with the previous report that the 35-kD antigen represented by line 2 in crossed immunoelectrophoresis was not recognized by potent antisera against *M. bovis*-bacille Calmette–Guerin (BCG) or *M. tuberculosis* (Harboe & Ivanyi, 1987).

Polyclonal stimulation of B cells might be an alternative explanation for the presence of antibodies to *M. tuberculosis*-specific epitopes in lepromatous leprosy. Antibody to ABO blood groups or *Candida albicans* (Buck & Hasenclaver, 1963), typhoid antigens (de Almeida *et al.*, 1964) and enhanced development of immunoglobulin-secreting cells following *in vitro* stimulation of lymphocytes with pokeweed mitogen (Bullock *et al.*, 1982) have been observed in patients with lepromatous leprosy. However, the present analysis showed that antibody levels to four common viruses did not differ when comparing lepromatous leprosy, tuberculosis and control groups. Furthermore, no correlation between viral and anti-mycobacterial antibody titres was observed. Thus, unrestricted

polyclonal B cell stimulation cannot explain the presence of anti-*M. tuberculosis*-specific antibody in lepromatous leprosy patients.

Autoantibodies have previously been reported in patients with leprosy (Mathews & Trautman, 1965; Masala *et al.*, 1979; Kano *et al.*, 1981) and tuberculosis (Lindquist, Coleman & Osterland, 1970; Sela *et al.*, 1987). In this study, only autoantibodies directed against smooth muscle were of greater frequency in both lepromatous leprosy and tuberculosis patients and their occurrence was positively associated also with higher titres to the TB68 epitope. Since the latter has been reported to express the 16/6 public idiotype (Shoenfeld *et al.*, 1986) and to compete with the 1/17 ssDNA binding MoAb it is possible that the TB68-specific recall might involve the stimulation of germ-line B cell precursors involving the common idiotypes which have been associated with anti-DNA autoantibodies in mycobacterial disease (Mackworth-Young, Sabbaga & Schwarty, 1987; Sela *et al.*, 1987; Locniskar *et al.*, 1988).

In conclusion, prior sensitization of T and B cells as a result of clinically inapparent infection with *M. tuberculosis* might explain the presence of antibodies to the 14-kD and 38-kD antigens in patients with lepromatous leprosy. Sensitization to T cell epitopes shared between *M. leprae* and *M. tuberculosis* has generally been indicated by the strong correlation of proliferative responses to the respective crude antigen extracts in healthy subjects (Rawlinson & Basten, 1989) and by the similar degree of depression in response to both extracts in lepromatous leprosy (Closs *et al.*, 1982). However, it should be pointed out that T cell proliferative assays may be poorly representative of the specificity of those T cells which act as helpers for antibody responses. The relationship between T and B cells in the production of specific antibody in the context of natural infection needs to be further explored by structural identification of the cross-reactive T cell epitopes of these proteins. Finally, the question whether the cross-stimulation in mycobacterial infections is protective (e.g. against plague as suggested by Girard (1975)) or harmful (e.g. high death rate of Norwegian leprosy patients from tuberculosis (Fine, 1984)) remains open for future analysis.

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