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Immunological unresponsiveness in leprosy and its relevance to immunoregulation in man

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Abstract. The varied forms of leprosy form a clinical and immunological spectrum which offers extraordinary possibilities for insight into immunoregulatory mechanisms in man. At one pole, tuberculoid leprosy, patients develop high levels of cell-mediated immunity which ultimately results in killing of bacilli in the tissues, albeit often with damage to nerves. At the lepromatous pole, patients exhibit selective immunological unresponsiveness to antigens of *Mycobacterium leprae.* Even though all currently known protein species of *Mycobacterium leprae* and BCG are cross-reactive, lepromatous patients unreactive to *Mycobacterium leprae* antigens frequently respond strongly to tuberculin. *In vitro* experiments suggest the existence of lepromin-induced suppressor activity, mediated by both monocytes and *T* cells. The *T* suppressor cells have the T_8 phenotype of which 50% express the activation markers, *Ia* and FcR. The one unique species of antigen of the leprosy bacillus is a phenolic glycolipid, and it appears that the T_s cells largely recognize the terminal trisaccharide of this unique antigen. Depletion of T_s cells restores *in vitro* reactivity of lymphocytes to lepromin in a portion of lepromatous patients, and addition of IL-2 containing supernatants partially restores responsiveness to *Mycobacterium leprae* antigens. Vaccination of lepromatous patients with a mixture of *Mycobacterium leprae* and live BCG restores cell-mediated immunity in the majority of lepromatous patients, and concomitantly reduces the *in vitro* suppressor activity and number of activated T_8 cells.

These experiments suggest the existence of stage-of-disease related suppressor cells in leprosy which appear to block the responsiveness of T_H capable of responding to either specific or cross-reactive mycobacterial antigens. The mode of action of these T_S appears to be the inhibition of production of IL-2 and other lymphokines. Successful immunotherapeutic vaccination appears to overcome this block in the majority of patients.

Keywords. Leprosy-immunology-unresponsiveness; lepromin-induced *T* suppressor cells; phenolic glycolipid; interleukin-2; gamma-interferon; immunoprophylaxis.

Introduction

From ancient times, and in virtually every culture, leprosy has evoked singular images of horror and fascination. From the immunological point of view, the disease presents a unique system for probing and intervening to control immunoregulatory mechanisms in man. As a clinical entity, leprosy presents many intriguing challenges. The etiologic agent, *Mycobacterium leprae* remains one of the very few pathogens of man that has not yet been grown in culture. There is a long latency, perhaps five years, between presumed infection and manifestation of the disease, and as a consequence the mode of

Abbreviations used: AFB, Acid fast bacilli; ConA, concanavalin A; IL-2, Interleukin-2.

transmission remains unknown. While thirteen million people are estimated to have leprosy around the world, the disease has a relatively low prevalence, seldom exceeding 1-5/1000 in endemic areas. There is a unique fear and stigma associated with this disease, partially deriving from the deformities which occur in approximately 30 % of its victims. The deformities are most likely not a direct result of infection of nerves, but rather the consequence of immunological reactions to bacillary antigens in and around the nerves, leading to anesthesia followed by mutilation. It remains unclear why leprosy disappeared from Europe at the end of the last century, yet is currently increasing in some developing countries. Clearly, one contributory factor is the recent emergence of primary and secondary drug-resistant organisms. Although it is now known that *M. leprae* can grow in the mouse footpad, in the armadillo and most recently in the mangabey monkey, epidemiological evidence suggests that there is little or no trnasmission from animal to man. This offers the prospect that an effective vaccine against leprosy could, as in the case of smallpox, lead to the eradication of the disease from the face of the earth.

The spectrum of leprosy

In order to describe what is known about the nature and significance of the immunological unresponsiveness in leprosy, it may be helpful to adumbrate some background information on the disease and its immunological features. Leprosy is a spectral disease that presents a diversity of clinical manifestations (Bloom and Godal, 1983; Sansonetti and Lagrange, 1981) (figure 1). A useful histopathological classify-cation system has been developed to stage patients objectively (Ridley and Jopling, 1966) and the histopathological classification correlates extremely well with the

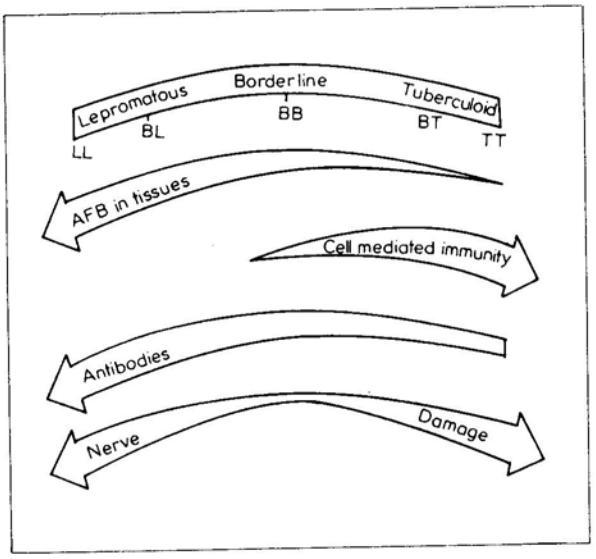

Figure 1. The spectrum of leprosy.

immunological picture. At one pole of the spectrum, tuberculoid leprosy, there are few acid fast bacilli (AFB) in the tissues, and the patients develop high levels of cell-mediated immunity, which ultimately kills and clears the bacilli in the tissues, although often with concomitant immunological damage to the nerves. At the lepromatous pole, the patients exhibit a selective unresponsiveness to antigens of *M. leprae,* and the organisms ineluctably multiply in the skin, often to extraordinary numbers, e.g. 10^{10}/g tissue. Lepromatous patients have few lymphocytes in their lesions, are negative to skin testing with antigens of *M. leprae* or intact bacilli and show little or no lymphocyte transformation to *M. leprae* antigens *in vitro.* The majority of patients exist in the borderline categories between these poles, and, if untreated, gravitate towards one or the other pole of the spectrum.

Antibodies to *M. leprae* occur in $_{all}$ forms of the disease, but attain highest levels in lepromatous disease, suggesting that they have little to do with protection (Abe *et al*., 1976; Young and Buchanan, 1983). Indeed, one of the major clinical problems in this form of the disease is erythema nodosum leprosum, presumed to be caused by immune complexes in the tissues (Bjorvatn *et al*., 1976). Nerve damage occurs with increasing severity at both ends of the spectrum, contributing to the deformities found in 30 % of the cases.

While leprosy is commonly described in textbooks as the least infectious disease, in fact, studies in Ethiopia (Godal, 1974) revealed that most lepromin skin test negative individuals working with active leprosy patients converted to skin test positivity in a period of several months, although they showed no evidence of disease. This implies that infection is much more common than generally assumed, and that most infections remain subclinical and are eliminated by an appropriate cellular immune response.

Antigens of M. leprae

The fundamental advance that made it possible to characterize the antigens of the leprosy bacillus and to contemplate vaccines was the observation that *M. leprae* derived from lepromatous patients would grow in enormously high levels in the spleens and livers of armadillos (Kirchheimer and Storrs, 1971). It is possible to obtain 10^{12} AFB from a single armadillo. Elegant purification methods were developed using percoll gradients and two-phase separation to obtain purified bacilli virtually free of contaminating host tissues (Draper, 1976). The fundamental serological finding of relevance is that when hyperimmune sera were developed against *M. leprae* and BCG and compared on crossed immunoelectrophoresis, it was observed that essentially all the protein and glycoprotein antigens detected in *M. leprae* were also recognized by antisera prepared against BCG (Harboe *et al*., 1978). No evidence has yet emerged for a completely unique species of protein or glycoprotein in *M. leprae.* However, a number of laboratories have developed monoclonal antibodies specific for antigens of *M. leprae* (Gillis and Buchanan, 1982; Ivanyi *et al*., 1983). The majority of monoclonal antibodies do not react with proteins on the surface of the intact organism, but rather recognize a set of *M. leprae* specific epitopes on internal polypeptides of 68 kd, 34 kd and 14 kd. It thus appears that there are unique epitopes specific for *M. leprae* found on discrete protein species which contain other determinants cross-reactive with a variety

of mycobacteria. Whether any of these epitopes is necessary or sufficient for protective immunity remains an important question.

The one unique antigen of *M. leprae* thus far identified is a complex phenolic glycolipid (Hunter *et al*., 1982). It consists of a phthiocerol C_{29} backbone with two $C_{30\text{-}34}$ mycocerosic acid sidechains linked by a phenolic linkage to a simple trisaccharides 3,6-di-*O*-MeGlu(l-4)-2,3-di-*O*-MeRha(l–2)-3-*O*-MeRha (*cf.* figure 9). It is this trisaccharide which is unique for *M. leprae* and contains the antigenic specificity. Monoclonal antibodies which react with the intact bacillus recognize this unique trisaccharide, as do IgM antibodies in the sera of most leprosy patients. This phenolic glycolipid-I is produced in abundance in the tissues, and only 10% of the tissue glycolipid is associated with the isolated bacilli.

Specific unresponsiveness in lepromatous leprosy

There have been a number of hypotheses to explain the unresponsiveness of lepromatous leprosy patients to skin tests or *in vitro* lymphocyte transformation to antigens of *M. leprae.* The older view was that lepromatous leprosy patients had generalized anergy for cell-mediated immune responses. While it may be true that in untreated polar lepromatous leprosy there is depletion of the paracortical areas in lymphoid tissues and occasionally a generalized anergy, in the vast majority of patients with lepromatous leprosy there is good responsiveness to a variety of recall antigens, including reactivity to PPD. This is illustrated in figure 2, which shows the *in*

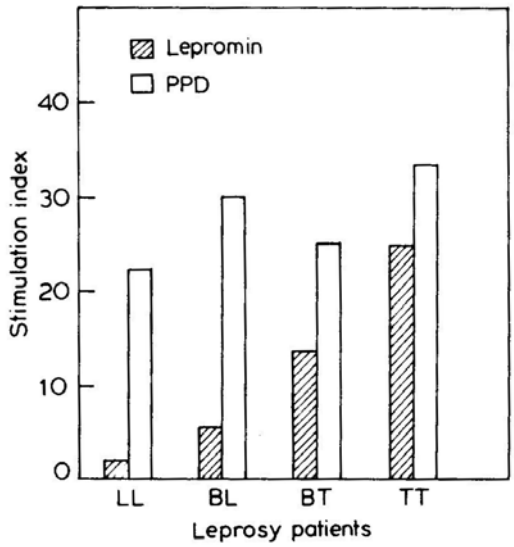

Figure 2. Stimulation of peripheral blood lymphocytes from leprosy patients by lepromin and PPD.

Peripheral blood lymphocytes were cultivated in microtiter plates at a density of 2×10^5 cells/well in RPMI 1640 containing 10% heat-inactivated, pooled human AB serum with; (i) Dharmendra lepromin (1:10), (ii) PPD (10 μg/ml), and [^{3}H]-thymidine incorporation was measured at day 6. The ratio of the mean incorporation of [^{3}H]-thymidine in the presence (E) or absence (C) of antigen was taken as measure of lymphocyte stimulation (stimulation index = E/C).

vitro lymphocyte transformation of 222 leprosy patients across the spectrum to lepromin and to PPD. It can be seen that patients throughout the spectrum show high lymphocyte transformation to tuberculin PPD, while lepromatous and borderline lepromatous patients show no or very poor lymphocyte transformation to lepromin. This selective unresponsiveness constitutes a fascinating immunological paradox. How is it possible, if most of the protein antigens of *M. leprae* are either identical or cross-reactive to those in BCG, that lymphocytes react with the antigenic determinants when they are present on the tubercle bacillus, but not when they are present on the leprosy bacillus? Currently, the most widely held view is that the unresponsiveness in lepromatous leprosy is determined by a poor constellation of immune response genes. Yet, there are several studies of segregation within families which have failed to find a significant association between HLA and lepromatous leprosy, but do reveal a weak association in segregants for tuberculoid leprosy and HLA, suggesting the possible existence of genetic 'resistance factors' linked to HLA (Fine, 1982). However, a recent study has documented an HLA association in segregation of lepromatous leprosy in 18 large families from Venezuela, indicating that there may well be some genetic factors associated with lepromatous leprosy (Van Eden *et al*., 1983). There exists, however, a study of identical twins of which four twin pairs had discordant forms of leprosy, one with tuberculoid, the other with lepromatous, indicating that genetic factors alone are unlikely to be the principal determinant of the unresponsive state (Chakravartti and Vogel, 1973).

Suppressor cell hypothesis

We have suggested a hypothesis that could resolve the immunological paradox in lepromatous leprosy, namely, that there must be one or a small number of unique antigenic determinants of *M. leprae* capable of engendering suppressor cells (T_s) which have the ability to block the responsiveness of helper *T* cells (T_H) to other specific or cross-reactive determinants (Bloom and Mehra, 1981). In order to try to examine such a hypothesis, and lacking HLA-matched individuals with leprosy, we developed a simple *in vitro* model for measuring the ability of *M. leprae* to induce suppression of *T* cell responses to the mitogen concanavalin A (ConA) (Mehra *et al*, 1979). Lymphocytes from leprosy patients across the spectrum are stimulated with ConA alone, or ConA in the presence of lepromin, [^{3}H] -thymidine incorporation is measured at 72 h, and the diminution in thymidine incorporation in the lepromin-containing culture is taken as the degree of suppression. We have found a consistent suppression of ConA stimulation in lepromatous and borderline leprosy patients, but not in tuberculoid patients, lepromin-positive contact's or normal donors (figure 3). There thus appears to be a suppressor activity specifically associated with the unresponsive form of leprosy.

Subsets of suppressor cells

Separation of peripheral mononuclear cell population into adherent and non-adherent subsets indicated that approximately 84% of the patients had a non-adherent cell capable of suppressing the ConA responses of normal donors' lymphocytes, and about 64% had an adherent suppressor cell, presumably a macrophage, as well

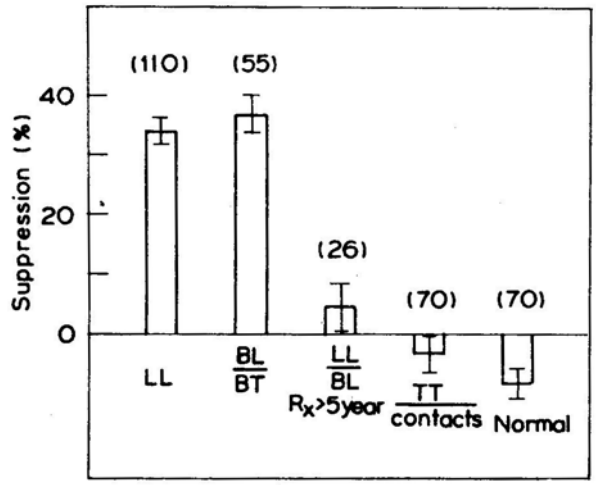

Figure 3. Lepromin-induced suppression of ConA responses of lymphocytes from leprosy patients across the spectrum.

Lymphocytes were cultured with: (i) ConA (2.5 μg/ml), (ii) ConA and Dharmendra lepromin (1:10), and (iii) no additions, as previously described (Mehra *et al*., 1979). The cultures were labelled at 2 days with 1.0 μCi [^{3}H]-thymidine/well and harvested 18 h later.

(table 1). Most lepromatous patients have adherent and non-adherent suppressor cells (i) but some patients have only non-adherent (ii) or only adherent (iii) *in vitro* suppressor activity. The adherent suppressor activity in lepromatous patients, presumably due to monocytes, has been confirmed and extended by studies reporting suppressive activity in extracts of lepromatous monocytes (Salgame *et al*., 1983) and findings of a suppressor factor secreted in culture by macrophages, from lepromatous

Table 1. Lepromin-induced suppression of ConA responses of normal mononuclear cells by adherent celts (monocytes) and non-adherent cells of leprosy patients.

Adherent cells	Non-adherent cells	Suppression in experiment (%) I	II	III
Patient	Patient	70·6	61·2	25·0
Normal	Normal	−35·1	−24·4	2·0
—	Patient	54·1	32·9	0
Patient	Normal	41·0	−3·3	26·6
Normal	Patient	58·8	33·9	2·4

Adherent cells were separated from non-adherent cells by incubating mononuclear cell suspensions in medium containing 12·0% pooled *AB* serum at 37° C for 1 h, in the microtiter culture plates (2×10^5 cells/well). Subsequently, nonadherent cells were removed and the wells were washed twice with warm medium containing 5% FCS. 2×10^5 nonadherent cells from normal individuals or leprosy patients were then added to each well containing autologous or allogeneic adherent cells. The cells were cultured in medium alone, ConA alone or lepromin + ConA, labelled at 2d with 1·0μCi of [^{3}H]-thymidine and harvested 18 h later (from Mehra *et al*., 1979).

patients (Sathish *et al*., 1983). A similar macrophage-like suppressor cell has been found in spleens of mice 5–10 weeks after inoculation with *Mycobacterium lepraemurium* (Bullock *et al*., 1978).

We have pursued the characterization of the non-adherent suppressor cell in the blood of lepromatous leprosy patients (Mehra *et al*., 1980). The non-adherent suppressor activity is totally associated with *T* cells prepared by *E*-rosetting or by cell sorting (figure 4). Using the fluorescence-activated cell sorter and monoclonal and polyclonal antibodies to lymphocyte surface markers we have shown that all of the suppressor activity in lepromatous patients is in the 20 % subset recognized by OKT5 or a horse anti-human *T* cell serum (anti- TH_2) or in the 30% subset recognized by OKT8 antibodies (figure 5), and since most studies were carried out with the latter reagent, these cells will be referred to as T_8 cells. Admixture of the T_8^+ subset to allogeneic normal mononuclear cells results in suppression of ConA responses of normal mononuclear cells in the presence of lepromin, indicating that the suppressor activity is not HLA restricted.

When the T_8^+ subset was examined for expression of *T* cell activation markers, namely *Fc* receptors and *Ia* antigens, we found that approximately 50 % of the T_8^+ subset expressed both *Fc* receptors and monomorphic *Ia* antigens (Mehra *et al.,* 1982) (table 2). As will be documented below, monitoring of the activation markers on the. T_8^+ subset may provide an *in vitro* means for estimating the degree of activation of these cells for suppression *in vivo.*

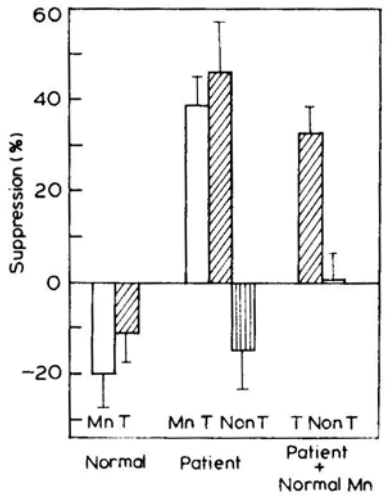

Figure 4. Suppression of ConA responses of normal mononuclear cells by *T* cells from lepromatous patients.

T cells were isolated from total mononuclear cells or non-adherent cells by rosetting with AET-treated sheep erythrocytes and separating the rosetted from non-rosetting cells by flotation on Ficoll-Hypaque. Subsequently, erythrocytes were lysed by 0·83% ammonium chloride. Normal mononuclear cells and patients' *T* cells were combined in 1:1 ratio. The results represent the means of six experiments (from Mehra *et al.,* 1979).

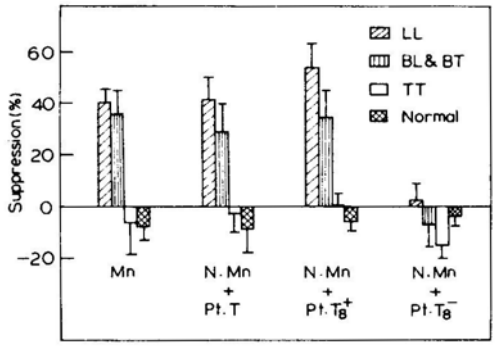

Figure 5. Lepromin-induced suppression of ConA responses of normal mononuclear cells by the T_8^+ and T_8^- cell subsets from leprosy patients.

The T_8^+ and T_8^- subsets were admixed with normal mononuclear cells at a density of 25,000 sorted cells/1 × 10^5 mononuclear cells (from Mehra *et al.,* 1980).

Table 2. Expression of activation markers on *T* cell subsets from lepromatous patients by FACS analysis.

Subjects	Percentage Ia^+ cells among TH_2^+/T_8^+	TH_2^-/T_8^-	Percentage of FcR^+ cells among TH_2^+/T_8^+	TH_2^-/T_8^-
Lepromatous patients	47·1 ± 3·6	20·9 ± 2·3	50·8 ± 6·8	16·9 ± 3·8
Normals	7·1 ± 2·3	8·7 ± 2·5	25·6 ± 5·6	24·6 ± 3·8

Adapted from Mehra *et al.* (1982).

If the T_8^+ subset, responsible for the lepromin-induced suppression of mitogenic responses, is related to the unresponsiveness to the antigens of *M. leprae* in these patients, then depletion of the T_8^+ subset should restore responsiveness to specific antigens in a six day lymphocyte transformation in some unresponsive patients. As shown in figure 6, removal of the T_8^+ cells from lepromatous lymphocytes resulted in high levels of responsiveness to lepromin at six days in about a third of the patients, exclusively patients with borderline, and not polar lepromatous leprosy. This result indicates that the unresponsiveness in at least some lepromatous patients cannot be due to the total absence of antigen responsive cells, and that some of the unresponsiveness must be attributed, particularly in BL patients, to the presence of effector suppressor cells.

Antigen specificity of suppressor T cells in lepromatous leprosy

Initial studies to ascertain whether the *in vitro* suppressor activity of lepromatous lymphocytes was specific only for antigens of *M. leprae* were confounded by relatively high levels of suppression of ConA responses, even in normal individuals, induced by including any species of mycobacteria in the cultures. After some effort, we learned that all the species of mycobacteria tested induced IFN-α secretion in lymphocytes from

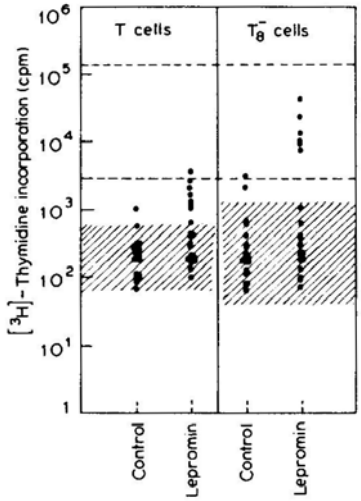

Figure 6. Response of peripheral blood *T* cells and OKT8-depleted *T* cells of lepromatous leprosy patients to lepromin.

Shaded area represents 95 % confidence limits for the control group. Area enclosed within the broken lines indicates the 95% confidence limits for [^{3}H]-thymidine incorporation by peripheral blood lymphocytes from tuberculoid leprosy patients (20) stimulated with Lepromin (from Mehra *et al.,* 1982).

normal, PPD-negative donors, and this correlated with the degree of non-specific suppression. Consequently, the specificity of suppression in lepromatous patients could be critically examined in the presence of a mixture of monoclonal antibodies to three species of IFN-α (figure 7). The results indicate that the *in vitro* suppression induced by killed mycobacteria in normal lymphocyte responses to ConA was completely eliminated by the antibodies to IFN-α, and that suppression seen with cultivable

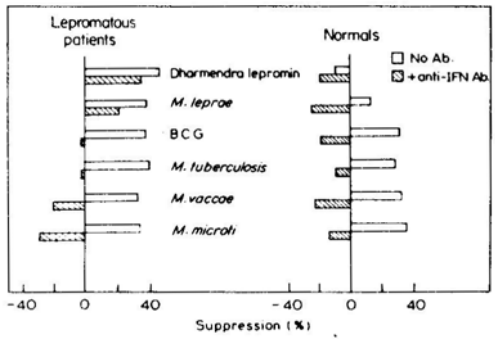

Figure 7. Effect of monoclonal antibodies to human IFN-α on the suppression of ConA response induced by various mycobacteria.

Peripheral blood lymphocytes from leprosy patients and normal donors were cultured with: (i) ConA, (ii) mycobacteria (10 μg/ml) + ConA, and (iii) mycobacteria, ConA and 200 units of mixture of monoclonal antibodies to three species of human IFN-α.

mycobacteria was similarly eliminated by the anti-IFN antibodies in lepromatous patients. In contrast, the suppression induced by Dharmendra lepromin and by purified killed *M. leprae* remained only in the lepromatous group, indicating that the T_s cells recognized only *M. leprae*-specific antigens.

Because the phenolic glycolipid-I is currently the only unique species of antigen demonstrated in *M. leprae,* in collaboration with Drs. P. Brennan and J. Convit, we examined the possibility that this phenolic glycolipid might activate the suppressor cells from lepromatous patients (Mehra *et al*., 1984). As shown in figure 8, when glycolipid-I, which is highly insoluble in aqueous media, was incorporated in liposomes and added to lymphocytes of lepromatous patients, it was as effective as lepromin in inducing suppression. Glycolipid-I did not induce suppression in tuberculoid patients, contacts or normal donors. The optimal dose of glycolipid in liposomes inducing *in vitro* suppression was 0.5 μg/ml. As previously, depletion of the T_8^+ *T* cell subset eliminated *in vitro* suppression observed both by lepromin and the phenolic glycolipid-I.

We were fortunate to have available a series of chemically modified *M. leprae* glycolipids and structurally related glycolipids prepared from other mycobacteria to probe the specificity of recognition of this unusual antigen by the T_s cells. As illustrated in figure 9, the results indicate that: (i) removal of the mycocerosic acid side chains (de-acylated glycolipid-I) had no effect on *in vitro* suppression; (ii) removal of the terminal 3'-methyl group abolished the suppression; and (iii) removal of the terminal sugar markedly reduced the suppression. Of particular interest is the observation that no suppression was induced by the liposomes alone, or by analogous glycolipids from *M.*

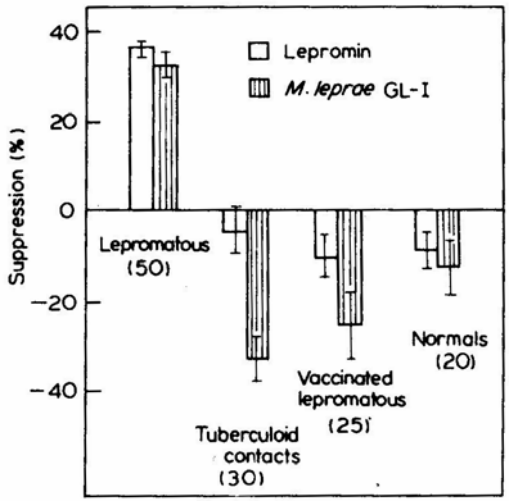

Figure 8. Suppression of mitogenic responses of peripheral blood lymphocytes of leprosy patients and normals to ConA in the presence of *M. leprae*-specific phenolic glycolipid-I and Dharmendra lepromin.

Glycolipid-I is highly insoluble in aqueous medium, and was incorporated into liposomes and then added to the lymphocytes at a concentration of 0.5 μg/ml. Liposomes contained 2.0 mg sphingomyelin, 0.73 mg cholesterol, 0·065 mg dicetylphosphate and 0·23 mg *M. leprae* glycolipid-I in 125 μl of Tris-NaCl buffer (pH 8·0) (from Mehra *et al*., 1984).

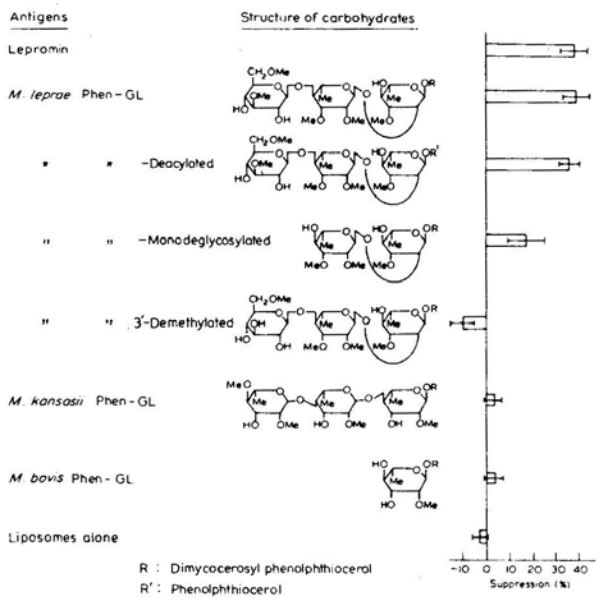

Figure 9. Suppression of ConA responses of peripheral blood lymphocytes of lepromatous leprosy patients by modified *M. leprae* glycolipid and phenolic glycolipids from other mycobacteria.

All the preparations were added in liposomes to the cultures at 0·5 μg/ml, except deacylated GL-I, which was suspended in PBS and added at the same concentration (from Mehra *et al*., 1984).

The chemical nature of the lipids tested are as follows:

M. leprae phenolic glycolipid-I:

3,6-di-*O*-Me-*ß* -*D*-Glcp(l–4)2,3-di-*O*-Me-α-L-Rha*p*(l-2)3-*O*-Me-α-*L*-Rha*p*-R

M. leprae phenolic glycolipid-I (monodeglycosylated):

2,3-di-*O*-Me- α -*L*-Rha*p*(l-2)3-*O*-Me-α-*L*-Rha*p*-R

M. leprae phenolic glycolipid-III (3'-demethylated):

6-*O*-Me -*ß*-*D*-Glcp(l-4)2,3-di-*O*-Me-α-*L*-Rha*p*(l-3)3-*O*-Me- α -*L*-Rha*p*-R

M. kansasii phenolic glycolipid (mycoside *A*)*:*

2,4-di-*O*-Me-*L*-Rha*p* (l-4)2-*O*-Me-*L*-Fuc*p*(l-4)2-*O*-Me-*L*-Rha*p*-R (tentative structure)

M. bovis phenolic glycolipid (mycoside B):

2-*O*-Me- α -*L*-Rha*p*-R

Glcp, glucopyranose; Rhap, rhamnopyranose, Fucp, fucopyranose; R, Dimycocerosylphenolphthiocerol

kansasii and *M. bovis*, which differ from *M. leprae* glycolipid-I only in the terminal saccharide moiety. These data indicate that the suppressor cell has rather exquisite specificity for the terminal trisaccharide of the phenolic glycolipid of *M. leprae.* This conclusion was confirmed by testing a series of monoclonal anti-*M. leprae* antibodies for their ability to inhibit suppression induced by lepromin or the phenolic glycolipid-I (table 3). We found that only monoclonal antibodies specific for the terminal disaccharide of the *M. leprae* glycolipid-I completely inhibited the suppression induced by glycolipid-I in liposomes and markedly reduced that by intact lepromin.

Table 3. Inhibition of phenolic glycolipid and lepromin induced suppression of ConA responses of peripheral blood lymphocytes of lepromatous leprosy patients by monoclonal antibodies.

Monoclonal antibodies to *M. leprae*	Specific for	Suppression of ConA response (%) Phen-GL-I	Lepromin
—	—	42·9 ± 3·9	38·1 ± 2·6
46·7	Phen-GL-I terminal disaccharide	−18·9 ± 7·2	17·8 ± 6·6
γ_1	Specific epitope on 68 Kd protein	45·4 ± 3·8	27·4 ± 3·5
46·7 + γ_1	—	−23·0 ± 6·7	16·6 ± 12·5
D_{17}	Cross-reactive with surface antigen on *M. leprae* and other mycobacteria	35·3 ± 4·9	41·8 ± 5·4
J_{12}	Dharmendra lepromin	45·5 ± 4·2	47·9 ± 5·3

Monoclonal antibodies were produced by the fusion of spleen cells from Balb/C mice immunized with *M. leprae* to myeloma × 63 Ag 8·653 cells. The reactivity and specificity of monoclonal antibodies, all of which are IgG_1, was determined by enzyme-linked immunosorbent assay analysis of 18 mycobacterial species (manuscript in preparation). In enzyme-linked immunosorbent assay, mAb 46·7 showed an O.D. of 2·23 with gly-I, 0·19 with gly-I lacking the terminal sugar, and 0·01 with gly-I lacking the terminal disaccharide.

Suppression was measured as before in the presence or absence of 20 μl/well culture supernatants from the hybrids making the monoclonal antibodies with the above mentioned reactivities (from Mehra *et al.,* 1984).

Relationship of T_s to the disease state in leprosy

Leprosy is basically a localized disease in which the battle between the immune system and *M. leprae* is fought out in the tissues, primarily skin and nerves. If the T_s cell found in *vitro* in lepromatous patients were involved in suppressing delayed-type hypersensitivity, the expansion of T_H cells and activation of macrophages, one might expect to find a predominance of T_8 cells, a paucity of T_4 cells, and macrophages laden with acid fast bacilli in lesions of polar lepromatous leprosy. Indeed, this is found to be the case. Using immunocytochemistry with monoclonal reagents against lymphocyte subset markers, two groups have shown that T_8 cells predominate in lepromatous lesions and that T_4 cells predominate in tuberculoid lesions (Van Voorhis *et al.,* 1982; Modlin *et al.*, 1983). In lepromatous lesions the macrophages are either laden with AFB or with large lipid vacuoles (the Virchow cell), recently shown to consist of the phenolic glycolipid-I (R. Modlin, personal communication). In tuberculoid lesions, there are small granulomas consisting of T_4 lymphocytes and macrophages with very few AFB.

We would presume that the spectrum represents varying contributions of T_H and T_s cells within the local tissue foci of infection. Indeed, one of the extraordinary forms of the borderline disease, formerly termed dimorphic, is characterized by the presence of lesions with the histopathological characteristics of lepromatous and tuberculoid lesions simultaneously at different skin sites in the same individual. While clearcut examples of this are relatively uncommon, they serve as paradigms for the delicate regulatory balance within the lesions that determines the outcome and ultimate course of the disease.

One of the characteristics of lepromatous patients, even following elimination of most or all detectable acid fast bacilli by means of chemotherapy, is that they remain lepromin negative and unresponsive to antigens of *M. leprae* generally for the lifetime. In studies on a small number of patients with lepromatous leprosy undergoing combined chemotherapy, it was surprising to note how long lepromin-induced suppressor cells were detectable in peripheral blood, often waning only after two years of treatment (figure 10), under conditions in which killing of the vast majority of organisms was completed in 1–3 months. Of interest in this regard are observations on two polar lepromatous patients treated with dapsone, a cytostatic drug, for 10–15 years after all AFB had been cleared from the skin (Pearson, 1979). Upon cessation of chemotherapy, lesions emerged which initially took the form of borderline tuberculoid reactions and over a two-year period downgraded until they had the characteristics of florid polar lepromatous leprosy. These clinical observations indicate that long after chemotherapy some viable organisms persisted, and suggested that when they emerged there were competent *T* cells capable of forming granulomatous responses in the skin characteristic of tuberculoid lesions, which may eventually have been suppressed by the re-emergence of T_s cells.

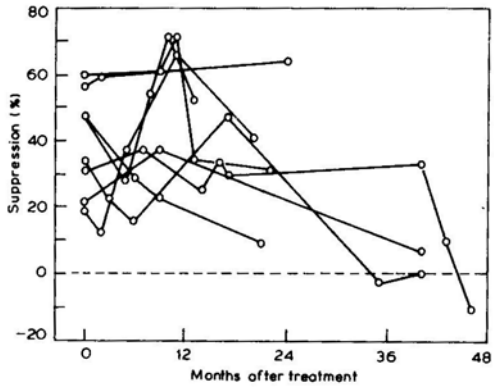

Figure 10. Lepromin-induced suppression of ConA responses of lepromatous leprosy patients undergoing combined chemotherapy for varying lengths of time, ranging from 1 month to 4 years. Patients were tested on several occasions during this period.

Vaccines and immunotherapy of leprosy

It was noted many years ago that killed *M. leprae* inoculated into the skin of lepromatous patients persisted in acid fast staining form for considerable periods of time. However, when live BCG was inoculated together with killed *M. leprae,* a granulomatous response to the BCG was produced and all the acid fast bacilli were degraded, including the *M. leprae* (Convit *et al.,* 1974). This suggested the possibility that immunization with a mixture of live BCG plus *M. leprae* might bring about a state of both specific and non-specific reactivity to *M. leprae* antigens and might serve as a useful vaccine. Pursuing this rationale, Convit and his colleagues have vaccinated

several hundred lepromatous leprosy patients with a mixture of live BCG and killed purified *M. leprae* (Convit *et al*., 1979; 1982). The results indicate that immunologic conversion, clearance of bacilli from the skin, histopathological upgrading towards the tuberculoid end of the spectrum and clinical improvement were found in approximately 85% of the borderline lepromatous patients, and in 65% of the polar lepromatous patients. These are rather dramatic results in patients who have been immunologically unresponsive often for long periods of time, and suggest a potentially general means for overcoming specific immunological unresponsiveness, at least to certain antigens, in man.

We had the opportunity to examine the *in vitro* suppressor activity and *T* cell phenotype of peripheral *T* cells from 10 such patients, in a blinded fashion, prior and subsequent to immunotherapy. The results, illustrated in figure 11, indicate that *in vitro* suppressor activity in all such vaccinated patients decreased to normal levels following immunotherapy, and expression of *Ia* was reduced to normal levels in 7 of 8 patients examined. These results provide objective evidence for immunological changes brought about by the vaccine, and establish a correlation between the *in vitro T* cell suppressor activity observed and the degree of the immunologic unresponsiveness *in vivo*.

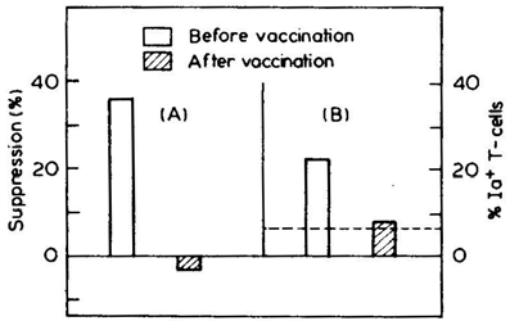

Figure 11. Diminution of *in vitro* lepromin-induced suppression of ConA responses and percentage of Ia^+ *T* cells in lepromatous patients tested both before and 18–24 months subsequent to immunotherapeutic treatment with BCG plus *M. leprae* (adapted from Mehra *et al*., 1982).

Relevance of findings in leprosy to general questions of immunoregulation in man

There are elaborate and sophisticated models of immunoregulatory networks in experimental animal systems (Eardley *et al*., 1980; Greene *et al*., 1982). For the induction of suppression in these systems, it appears that a suppressor-inducer *T* cell acts on an amplifier cell converting it to a functional suppressor cell. In general, the inducer cell has an Lytl, T_H phenotype and expresses *I-J* determinants; in some systems the amplifier cell expresses the Lytl, 2,3 phenotype and the competent suppressor cell has an Lyt2 phenotype. The first interaction is *I-J* restricted, and in some systems the effector Lyt2 T_s cell is *I-J* unrestricted. Of interest is a recently described effector

suppressor murine *T* cell line which is carrier-specific, MHC unrestricted and expresses high levels of both *Ia* and *I-J* antigens (Nakauchi *et al.,* 1984). It would appear that the suppressor cell observed in the experiments described by us most closely corresponds to those of this *T* cell effector suppressor, in that both possess the suppressor phenotype and express high levels of *Ia.* There are several examples in man of helper-inducer cell lines, most notably in influenza in which there is a MHC-restricted suppression by *T* cells of the T_4 phenotype (Fischer *et al*., 1982). No such cell has yet been found in leprosy, although it has been reported that cultivation of lymphocytes from BCG-vaccinated normal donors with BCG results in the development of a suppressor cell *in vitro* which is MHC restricted and has a T_4 phenotype (Mustafa and Godal, 1983).

While it has been possible to measure suppression of mitogen responses at 72 h, induced by lepromin, it has been very difficult to demonstrate suppression of specific responses to *M. leprae* in 6 day cultures. Attempts to suppress lepromin responses of lepromin-positive contacts using HLA–*A,B,* and *D*-matched lymphocytes from lepromatous patients have not been successful (Stoner *et al.,* 1978; Nath *et al.,* 1980). In essence, these experiments may be analogous to attempting to transfer tolerance into an already immune animal, a phenomenon that has been elicited, but only rarely and with great difficulty. This remains a problem, although it could be argued that once cells are sensitized and exposed to antigens they may be capable of producing all the lymphokines and factors required for amplification of the response *in vitro*, even in the presence of T_s, although within lesions this may be more easily regulated.

The role of lymphokines

Despite the dazzling interactions of regulatory *T* cell networks in model systems, the ultimate mechanism by which suppressor cells prevent competent lymphocytes from functioning remains unresolved. Recent findings in experimental models indicate that mitogen-, alloantigen- or hapten-induced suppressor cells inhibit the proliferation of *T* lymphocytes by blocking the production of Interleukin-2 (IL-2) (Gullberg and Larsson, 1982; Kramer and Koszinowski, 1982). A soluble suppressor molecule has been obtained from *T* cells of mice rendered unresponsive to chemical allergens which mediates suppression by blocking IL-2 production by T_H cells. This factor is neither antigen-specific nor MHC-restricted (Malkovsky *et al.,* 1982).

The first evidence that a similar mechanism may pertain to suppressor cell regulation in man derives from the experiments of Haregewoin *et al.* (1983), which demonstrated that lymphocytes from lepromatous patients cultured with *M. leprae* failed to produce IL-2. More importantly, addition of supernatants from mitogen-stimulated lymphocytes known to contain IL-2 restore, at least partially, the ability of lymphocytes from lepromatous patients to respond to *M. leprae.* These results again indicate that some T_H cells reactive to *M. leprae* exist in the blood of lepromatous patients, an observation consistent with our findings that depletion of T_8 cells restores *in vitro* lepromin responsiveness in a portion of these patients. This work represents the first report of some degree of restoration of immunological competence in a disease-associated state of immunological unresponsiveness in man by means of lymphokines. These studies have been extended by other studies indicating that lymphocytes from lepromatous

leprosy patients failed to produce IFN-γ (Nogueira *et al.,* 1983), the lymphokine which appears to be required for activation of macrophages for production of $O\bar{2}$ and H_2O_2 (Nathan *et al*., 1983). Exposure of the cells to IL-2 restored their ability to proliferate in response to *M. leprae* and also to produce IFN-. γ The ability of *T* cells from lepromatous patients to respond to IL-2, and the presence of Tac receptors, the putative receptor for IL-2, on lymphocytes in lesions of leprosy patients (R. L. Modlin, personal communication) indicates that the defect in lepromatous lesions is related to the inability to produce IL-2. If so, these studies in leprosy, taken .together, would provide first insight on the mode of action of T_s cells in man. It remains unclear, at present, whether there is any defect in IL-1 production in lepromatous lesions, or whether the primary defect can be pinpointed as being the failure to produce IL-2.

Factors predisposing for development of T_s cells in leprosy

While there is an enormous body of literature on the requirements for *Ir* gene products and antigen presentation to T_H cells, there is very little lnformation on the requirements for development and amplification of T_S in any system. There are very few experiments in which suppressor cells have been produced *de novo in vitro.* Interestingly, this has been reported in a mycobacterial system in mice, after cultivating *Ia*-depleted spleen cells with BCG (Nakamura *et al*., 1982). This resulted in the development of BCG-specific T_S cells. Upon transfer of these cells in normal mice, the ability of recipients to develop delayed hypersensitivity foot pad reactions to PPD was markedly suppressed. The phenotype of the suppressor cell was *I-J* positive, and the development of suppressor cells *in vitro* could be blocked by anti-*I-J* serum.

It remains very important to identify the factors in man which determine the part of the spectrum to which any individual infected with *M. leprae* will gravitate. Clearly, the vast majority of people infected by *M. leprae* develop appropriate cell-mediated immunity and no evidence of clinical disease. It is unclear what determines whether infection will be contained or will develop towards the lepromatous or tuberculoid poles of the spectrum. One view would hold that the *Ir* genes may be determinative, although the association is sufficiently weak that, in our view, they are likely only to be contributory. Another possible explanation is the route of infection; if *M. leprae* is introduced into the skin, taken up by macrophages or presented by *Ia*-positive Langerhans cells, dendritic cells or macrophages, it is likely that some degree of cell mediated immunity will develop. If *M. leprae* is contracted by a route of infection which tends to bypass appropriate antigen presentation, possibly by the oral or respiratory routes, the possibility exists that T_H cell development will be retarded and suppressor cells will be developed by default. If this rather simplistic model of antigen presenting capability as being the determinative factor for development of different forms of disease were true, one might have expected that fewer macrophages in lepromatous lesions would express *Ia* antigens than those in tuberculoid reactions. Both studies of the histopathology of lesions have clearly found that almost all macrophages of both lepromatous and tubeculoid lesions are positive for HLA-Dr monomorphic antigens. One wonders if these determinants are sufficient for adequate presentation of complex antigens such as bacteria to T_H cells. Since the classical experiments of Macher and Chase (1969), demonstrating that the introduction of antigens simultaneously leads to the development of sensitization and unresponsiveness, it remains quite unclear, in

leprosy or any other clinical state of unresponsiveness, precisely what factors determine which form of response will develop.

Mechanisms of vaccine action

Leprosy remains the only example in man of specific immunological unresponsiveness which can, with reasonable frequency, be overcome by immunization or immunotherapy. While several potential vaccines derived from cultivable mycobacteria are being developed (Deo *et al*., 1981; Chaudhuri *et al*., 1983), the most extensively detailed studies on histopathology and clinical improvement are those of Convit (Convit *et al*., 1979; 1982), vaccinating lepromatous patients with live BCG and killed *M. leprae.* It is important to try to develop an understanding in immunological terms of the mechanism by which this breaking of unresponsiveness is accomplished. Among the possibilities to be considered are: (i) BCG represents an immunologically cross-reactive antigen which can break tolerance and cause the emergence of cross-reactive clones that are protective against *M. leprae*; (ii) BCG provides an amplification of BCG-reactive clones which, in local lesions, could produce enough IL-2 to permit the expansion of that small number of *M. leprae* specific T_H remaining at a level sufficient to provide activation of macrophages and protection; (iii) BCG causes elevated expression of *Ir* gene products, perhaps as a result of local IFN production, required for antigen presentation and thereby permitting the amplification of *M. leprae*-specific T_H cells. Two approaches to the dynamics and mechanisms of immunoconversion during vaccination would be to follow the changes in lymphocyte subpopulations and production of IL-2, IL-1 and IFN-γ in the lesions of lepromatous patients, or to treat patients with cloned IL-2 or IFN- γ either locally or systemically.

Do suppressor T cells recognize polysaccharide and lipid antigens?

Evidence presented here suggests that the T_s cells of lepromatous patients have remarkable specificity for the terminal di- or trisaccharide of the *M. leprae*-specific phenolic glycolipid. Yet it has long been problematic whether *T* cells are capable of recognizing polysaccharide antigens or sugars. Earlier studies on antibody responses to pneumococcal polysaccharide III indicated a role for *T* suppressor cells *in vivo*, although there remains no convincing evidence for recognition of SSS-III by *T* cells (Baker, 1975; Braley-Mullen, 1980). Recent evidence indicates that the determinant recognized by the suppressor cell in this system is the idiotype on the IgM antibody initially formed (Taylor *et al*., 1983). Clearly, antibody responses to hapten-coupled ficoll (Letvin *et al*.,1981) and streptococcal A carbohydrate (Eichmann, 1975) have been found to be *T* cell dependent, but again there is little direct evidence that *T* cells recognize the carbohydrate. Delayed type hypersensitivity to tuberculocarbohydrates has been reported (Baer and Chaparas, 1964). Recently, cell-mediated immunity to *Bacillus fragilis* has been shown to be induced by the capsular polysaccharide, and curiously, is mediated by an MHC-unrestricted, Lyt2, *T* cell in the mouse (Shapiro *et al*., 1982). These findings indicate that some carbohydrate moieties can be recognized by *T* cells, perhaps preferentially by T_S cells.

It is not without interest that the tumor-associated antigens recognized by monoclonal antibodies in a variety of human tumor systems, including melanomas (Dippold *et al*., 1980; Pukel *et al*., 1982; Yeh *et al*., 1982), Burkitt's lymphoma (Nudelman *et al*., 1983), and colonic tumors (Koprowski *et al*., 1981; Magnani *et al*.,

1981) have been found to be glycolipid antigens. In the case of *M. leprae,* we have been unable to demonstrate any specific *in vitro* lymphocyte transformation induced by the glycolipid-I and it may well be that this type of antigen is not readily recognized by T_H or CTL in man. What remains to be explored, however, is whether in the tumor systems these immature surface glycolipids may be recognized by T_s and contribute to the immunological unresponsiveness. If T_s recognition of carbohydrate or lipid moieties is found to be a general phenomenon, since it is relatively easy to produce monoclonal antibodies to these lipid determinants, it is conceivable that anti-idiotypic antibodies could be produced which would react with the idiotypic receptor on the specific T_s blocking the specific suppressor activity *in vivo.* While speculative, this could provide a novel general approach for overcoming unresponsiveness and restoring specific cell-mediated immunity to bacterial, parasitic and viral infections and tumor cells.

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