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LEARNING FROM LEPROSY: A PERSPECTIVE ON IMMUNOLOGY AND THE THIRD WORLD

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"If we take the widest and wisest view of a Cause, there is no such thing as a Lost Cause, because there is no such thing as a Gained Cause. We fight for Lost Causes because we know that our defeat and dismay may be the preface to our successors' victory, although that victory itself will be temporary; we fight rather to keep something alive than in the expectation that anything will triumph."

—T.S. Eliot

"A Map of the World Without Utopia on It Is not Worth Glancing At."

—Oscar Wilde

Let me begin with a case history, not of an individual, but rather of a country, any of the forty poorest nations on earth. Let me ask you to try to imagine our quality of life, if life expectancy at birth in this country were 42 yr, if infant mortality at birth were 140 per thousand, if 40% of our children suffered from malnutrition, and if only 10% of children were immunized against diphtheria, pertussis, tetanus, tuberculosis, polio, and measles, and consequently 0.5% of them became lame from polio, 1% died from neonatal tetanus, 2% succumbed to whooping cough, and 3% died from measles. We would be living in a country whose average gross national product per capita would be $310/yr; in which 37% of males, but only 14% of females, would be literate. Access to water would

be enjoyed by 50% of the urban population, but only 15% of the rural population. Government expenditures on health and education would represent only 3.9% of the central budget, and there would be 15,932 people per physician. This is the quality of life, differing only in detail, endured by 200 to 300 million people in Africa, Asia, and Latin America (1). This is the Third World, in which 75% of the planet's population lives, where 86% of all children are born and 98% of all infant and child deaths occur, and where 10 kids die of vaccine-preventable illness every minute.

This was not my world when I embarked on a career in immunology. That world was the Rockefeller University where I was trained in cellular immunology by Dr. Merrill Chase. Dr. Chase was one of the founders of cellular immunology, who first established that delayed-type hypersensitivity (DTH) could be passively transferred by cells, not serum, and whose example impressed upon me the importance of discipline and commitment in science. That too was not the world of St. Mary's Medical School in London where, as a postdoc, I learned something of the structure of antibodies from Rodney Porter. I came, in addition, to learn three important things from my experience in Rod Porter's lab: *i*) the active site of antibodies was not on isolated heavy chains, *ii*) I was not destined to be a great chemist, and *iii*) most importantly, it was ultimately possible to do the highest quality of science with in no way compromising one's dignity, generosity, humor, or humanity. I cannot imagine being more privileged than to have had these two wonderful and inspiring teachers.

To convey a sense of time in that "world," the least understood phenomenon in immunology was "delayed-type hypersensitivity," not then even dignified by the term "cell-mediated immunity." Pioneering studies in vitro on lymphocytes and macrophages by Hirshhorn, Bach, George, and Vaughan and John David were just breaking new ground. The most burning question, incredible as it may sound, that Boyce Bennett and I first tried to address at Albert Einstein, was whether sensitized lymphocytes or macrophages, or both, carried the immunological specificity for DTH. We were able to show in the macrophage migration inhibition reaction *i*) that only immune lymphocytes had the ability to recognize specific antigen and inhibit macrophage migration; indeed, as few as 0.6% of sensitized cells were sufficient; and *ii*) that the in vitro reaction was mediated by a nonantibody product of the antigen-activated lymphocytes (2). John David made the same observation essentially simultaneously (3). Migration inhibitory factor, MIF, thus became the first of the lymphokines. Bennett and I really first began to believe in the lymphokines when we found (Fig. 1) that partially purified MIF injected into normal guinea pig skin produced reactions as early as 4 hr consisting of almost pure mononuclear cell infiltrates resembling DTH reactions (4). Although the chemical nature of MIF has been somewhat elusive and its biological effects on macrophages may well be mediated by more than one molecular entity, it was gratifying to learn only last year from the work of Thurman et al. (5) that the in vitro activities of MIF were produced on human monocytes by cloned human IFN-γ, and conversely, that MIF activity induced by concanavalin A (Con A) activation of human lymphocytes was neutralized by monoclonal anti-interferon (IFN)-γ antibodies. Recently, Nathan et al. (6) have observed skin reactions, similar to those induced by MIF in guinea pigs, produced interestingly in the skin of lepromatous leprosy patients after inoculation of recombinant IFN-γ.

As the in vitro study of cellular immune reactions became accepted as a legitimate immunological endeavor, I was invited by the World Health Organization to help evaluate whether these emerging in vitro techniques might have some conceptual or practical relevance to understanding immunity to tropical diseases. One such meeting, held in India in 1971, introduced me to the Third World and the problem of leprosy, and was one from which I have never quite recovered. In the course of my wanderings in immunology and around the globe, I have been privileged to have been able to collaborate with a great many wonderful scientists and colleagues, both in this country and abroad, and would like to express my sincere appreciation for all they have contributed. I am deeply grateful for the dedicated efforts and valuable advice provided me by many members of the Secretariat of the World Health Organization.

Leprosy—the disease. From ancient times and in every culture, leprosy has evoked singular images of horror and fascination. Many aspects of the disease remain mysterious to this day. Although the leprosy bacillus was the first identified bacterial pathogen of man (7), it remains one of the very few pathogens of man that cannot yet be grown in culture. There is generally a long latency, perhaps 5 yr between presumed infection and manifestation of disease, and as a consequence the mode of transmission remains largely unknown. Although 13 million people are estimated to have leprosy around the world, the disease has a relatively low prevalence, seldom exceeding 1 to 5 per 1000 in endemic areas. There is a unique fear and stigma associated with this disease, partially deriving from the deformities that occur in approximately 30% of people afflicted by it. There is no other disease whose victims were burned at the stake by Henry II of England and Philip V of Spain, or buried alive by Henry's grandson, Edward I, or given the last rites of the dead by the Church in medieval Europe and turned outside the city walls (8).

The spectrum of leprosy. There has been a renaissance of scientific interest in leprosy, one reason being the extraordinary possibilities it offers for gaining insight into immunoregulatory mechanisms in man (9, 10). For leprosy is not a single clinical entity, but rather a spectral disease that presents a diversity of clinical manifestations (Fig. 2). At one pole of the spectrum, tuberculoid leprosy, patients develop high levels of specific cell-mediated immunity that ultimately kills and clears the bacilli in the tissues, although often with concomitant immunological damage to the nerves. At the lepromatous pole, patients exhibit a selective unresponsiveness to antigens of *Mycobacterium leprae* and the organisms ineluctably multiply in the skin, often to extraordinary numbers—as many as 10^{10} per gram of tissue. It remains unclear why the vast majority of people exposed to infection with *M. leprae* develop no clinical disease, and why only a minority who do develop clinical disease become lepromatous and remain immunologically unresponsive to the antigens of the organism.

Mechanism of nerve damage in leprosy. To a visitor

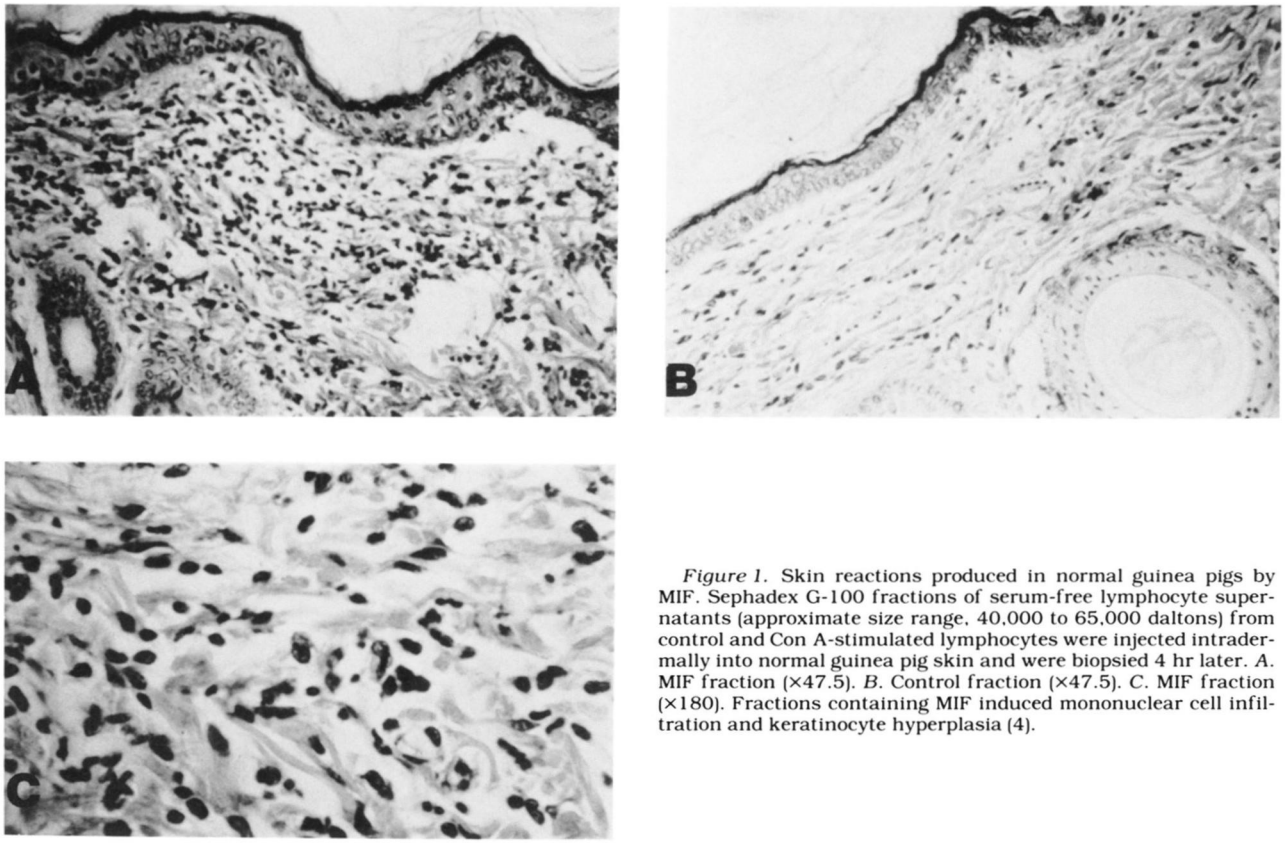


Figure 1. Skin reactions produced in normal guinea pigs by MIF. Sephadex G-100 fractions of serum-free lymphocyte supernatants (approximate size range, 40,000 to 65,000 daltons) from control and Con A-stimulated lymphocytes were injected intradermally into normal guinea pig skin and were biopsied 4 hr later. *A.* MIF fraction (×47.5). *B.* Control fraction (×47.5). *C.* MIF fraction (×180). Fractions containing MIF induced mononuclear cell infiltration and keratinocyte hyperplasia (4).

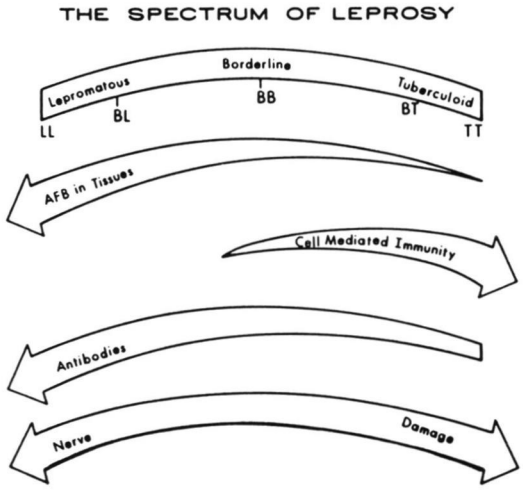


Figure 2. The spectrum of leprosy.

to the Third World for the first time, the most devastating aspect of leprosy was the horrible deformities seen in people suffering from the disease. A particular paradox was the nerve damage in patients with tuberculoid leprosy in which cellular immunity had virtually eliminated the acid-fast bacilli (AFB) from the tissues. Because *M. leprae* has selectivity for growth essentially only in macrophages and Schwann cells, Henry Wisniewski and I undertook to develop a model to analyze this type of nerve damage (11). Guinea pigs were sensitized to Freund's adjuvant and were challenged with purified protein derivative (PPD) near myelinated fibers of the sciatic nerve or ventricles. The question asked was, "What would happen to nerves as a result of a cellular immune reaction to foreign antigens in the vicinity of myelinated fibers?" The results clearly demonstrated primary focal demyelination of these myelinated fibers as innocent "bystanders" of a specific cellular immune reaction to the foreign antigen (Fig. 3). Together with Celia Brosnan, Wendy Cammer, Gerry Stoner, Siamon Gordon, and Bill Norton, we were able to demonstrate that activated macrophages secreted neutral proteinases that had the ability to degrade purified myelin proteins in vitro (12). One of these was inhibitable by EDTA and was probably a collagenase; the other was plasminogen activator, dependent on plasminogen and blocked by inhibitors of plasminogen activator and plasmin. This suggested that activated macrophages could secrete small amounts of neutral proteases, one of which could act upon the very large reservoir of plasminogen (~1 mg/ml) found in serum and extravascular fluids, releasing plasmin that would have the ability locally to degrade myelin proteins. If that were true, then specific protease inhibitors should be capable of blocking demyelination in vivo. Celia Brosnan and I set up the model of experimental allergic encephalomyelitis (EAE) in rats, and 7 days after the animals were sensitized and the spleen cells were capable of transferring EAE to normal recipients, the animals were treated with a variety of protease inhibitors (13). Two of these inhibitors, *p*-nitrophenyl guanidinobenzoate and transaminomethylcyclohexane carboxylic acid, quite effectively blocked demyelination histologically and reduced paralysis in the vast majority of animals. These studies introduced us to two new mechanisms of immunological damage to myelin, bystander demyelination and an antibody-dependent, cell-mediated demyelination (14) that could have relevance to inflammatory demyelination, perhaps in multiple sclerosis, and to general mechanisms of tissue damage.

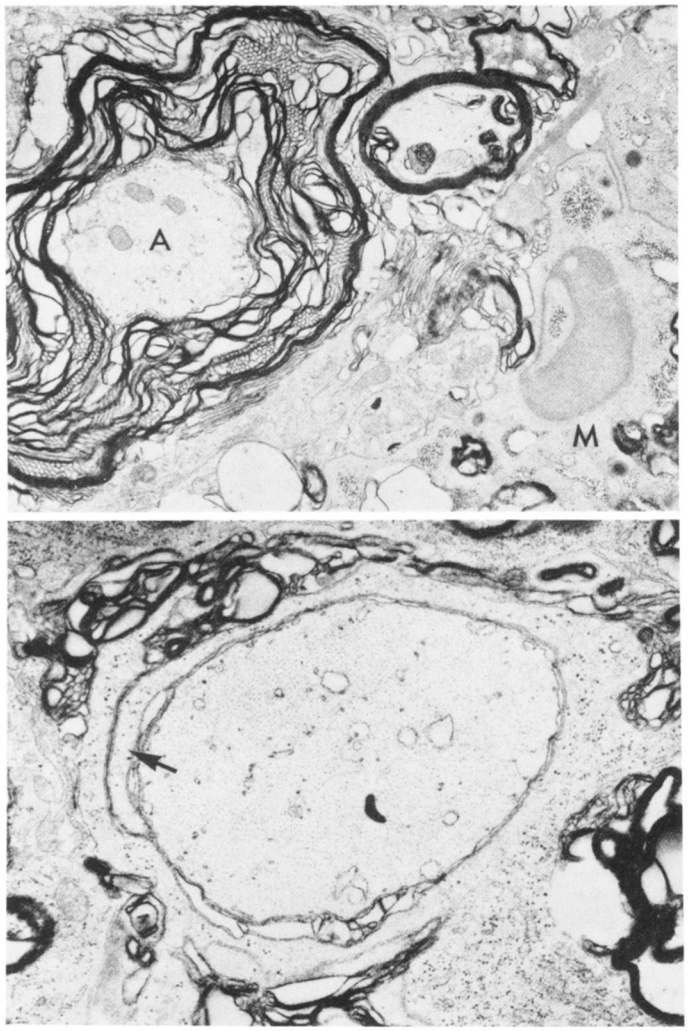


Figure 3. Bystander demyelination. Guinea pigs were sensitized to Freund's complete adjuvant and were challenged with PPD inoculated above the sciatic nerve. Focal demyelination was produced. *Top.* Disruption of the myelin lamellae with normal appearing axoplasm (*A*) of nerve adjacent to a mononuclear phagocyte (*M*). *Bottom.* Pseudopod of mononuclear phagocyte insinuating itself between lamellae and stripping the myelin sheath. Note ingested myelin figures are seen in phagocytic cell (11).

There is far less information on the mechanism of nerve damage in lepromatous leprosy, in which the lesions indicate fibrosis and death of sensory nerves in the absence of a cellular infiltrate. Perhaps the major clue to pathogenesis derives from studies of Bjorvatn et al. (15) that showed that in lepromatous patients, particularly in reactional episodes, there was a large excess of C3d found in the circulation, suggesting that antibody-antigen complexes are occurring in the tissues with fixation of complement, probably producing edema and compression neuropathy.

Specific unresponsiveness in lepromatous leprosy. At a time in immunology when the genes and their rearrangements that confer specificity on B and T cells are being revealed, when a variety of lymphokines and immunoregulatory molecules and their receptors have been cloned and are available for study and clinical trials, and when the major histocompatibility complex (MHC) restrictions on helper and cytotoxic T cell recognition have been elaborated in molecular terms, in my judgment the most important and fundamental immunologic issue that remains unresolved is that of tolerance and unresponsiveness. The special aspect of this unresponsiveness in lepromatous leprosy is that it appears to be acquired, occurs over a long period of time, and in general is not life threatening. Consequently, leprosy provides unique opportunities for study of unresponsiveness in man and for immunologic intervention.

The textbook description of the "anergy" of lepromatous leprosy is that such patients are totally unresponsive to new and recall antigens. Although nonspecific anergy may in fact be seen in small numbers of late-stage untreated patients, clearly the vast majority of patients exhibit normal cell-mediated immunity to a variety of recall antigens including PPD, although they are totally unresponsive to antigens of *M. leprae* (Fig. 4). Because antibodies to all the known protein and glycoprotein antigens of *M. leprae* appear to be cross-reactive with homologus antigens in a variety of other mycobacteria, the immunological paradox becomes, "How is it possible to respond to the same or cross-reactive antigens when they are associated with *M. tuberculosis*, bacillus Calmette-Guérin (BCG), or other mycobacteria, yet be totally unresponsive when these same or related antigens are associated with *M. leprae*?" We suggested the hypothesis that there might be one or a small number of unique epitopes associated with *M. leprae* capable of inducing T suppressor cells that had the ability to suppress responses of potentially cross-reactive helper clones.

Dr. Vijay Mehra and I developed a simple in vitro model for examining the ability of *M. leprae* antigens (Dharmendra lepromin) to induce suppressor activity (16). Lacking HLA-matched individuals with leprosy, we used *M. leprae* antigens to induce suppression of Con A responses measured at 72 hr. In over 200 patients studied, suppression was found in 84% of lepromatous and borderline patients, but not in tuberculoid patients, lepromin-positive contacts, or normal donors. Separation of the peripheral mononuclear cell populations into adherent and nonadherent subsets indicated that lepromatous and borderline patients had nonadherent cells capable of suppressing Con A responses, and a lesser proportion had adherent suppressor cells, presumably macrophages. In collaborative experiments with Ellis Reinherz and Stuart Schlossman, we were able to show that the nonadherent suppressor activity was totally associated with the 20 to 30% T cell subset recognized by OKT5, a horse anti-human T cell serum (anti-TH2), and the OKT8 antibody (17). When the T8 subset from lepromatous patients was examined for expression of T cell activation markers, namely Fc receptors and Ia antigens, we found that approximately 50% expressed both monomorphic HLA-DR antigens and Fc receptors, and we believe these markers may represent useful indices of their functional suppressor activity in vivo (18). In our hands, in vitro suppression was not overcome by interleukin 2 (IL 2), but removal of the $T8^+$ cells from lymphocytes of lepromatous patients resulted in very high levels of specific antigen responsiveness to *M. leprae* at 6 days in about one-third of the patients, exclusively in the borderline lepromatous category (18). This result encourages us to believe that the immunologic unresponsiveness in at least a proportion of lepromatous patients cannot be due to the total absence of antigen-responsive T cells, and that it may be possible to overcome the suppression at least in some patients with leprosy.

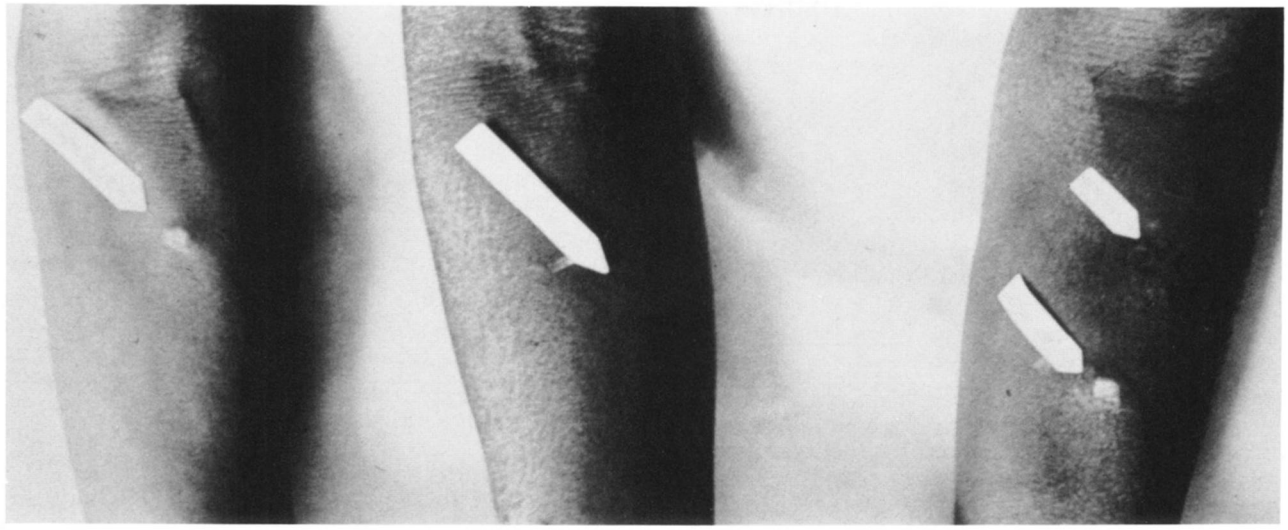
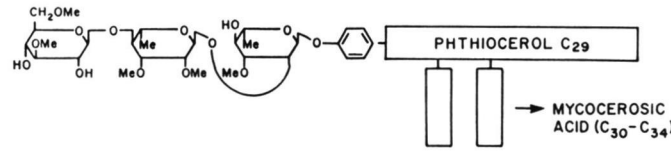


Figure 4. Selective unresponsiveness in leprosy. *Left.* Lepromin test in tuberculoid leprosy patient. Note positive indurated reaction at 28 days. *Center.* Lepromin test in patient with lepromatous leprosy. Note total lack of reaction. *Right, lower arrow.* Positive skin test with killed *M. tuberculosis* in same lepromatous patient. The author is indebted to Dr. Tore Godal for kindly providing this figure.

The specificity of the suppressor T cells in lepromatous leprosy. Initial studies to ascertain whether the in vitro suppressor activity was specific only for antigens of *M. leprae* were confounded by relatively frequent suppression of Con A responses, even in normal individuals, induced by a variety of species of cultivable mycobacteria added to the lymphocyte cultures. After much effort, we learned that most species of mycobacteria tested induced IFN-α secretion by mononuclear cells from normal, PPD-negative donors, which correlated with the degree of nonspecific in vitro suppression. Consequently, it became possible to examine the specificity of suppression in lepromatous patients by testing induction of in vitro suppression by a variety of species of mycobacteria in the presence of a mixture of monoclonal antibodies to three subsets of human IFN-α (11). The results clearly indicated that the nonspecific in vitro suppression induced by killed mycobacteria in normal lymphocyte responses was completely eliminated by the antibodies to IFN-α. Suppression was still induced in the lepromatous patients only by lepromin and by killed *M. leprae*, demonstrating that the T suppressor cells studied were specific for antigens of *M. leprae*.

There has thus far been demonstrated only one unique species of antigen associated with *M. leprae*, a phenolic glycolipid-1 (PGL-1) elucidated by Hunter and colleagues (19). It consists of a complex carbon backbone structure phenolically linked to a unique trisaccharide found only in *M. leprae*. In collaboration with Drs. Brennan and Convit, we were able to examine the possibility that this phenolic glycolipid might stimulate suppressor cells from lepromatous patients (20). When PGL-1, which is highly insoluble in aqueous solution, was incorporated in liposomes and added to lymphocytes of lepromatous patients, it was almost as effective as lepromin in inducing suppression (Fig. 5). It did not induce suppression in tuberculoid patients, contacts, or normal donors. Depletion of the T8 cell subset eliminated in vitro suppression produced both by lepromin and phenolic PGL-1. We were fortunate to have available a series of chemically modified *M. leprae* glycolipids and structurally related glycolipids derived from other mycobacteria to probe the specificity of recognition of this unusual antigen by the T suppressor cells. The results indicated that *i*) the removal of the mycocerosic acid side chains (deacylated glycolipid-1) had no effect on the in vitro suppression, *ii*) removal of the terminal 3′ methyl group abolished the suppres-



M. leprae phenolic glycolipid - I

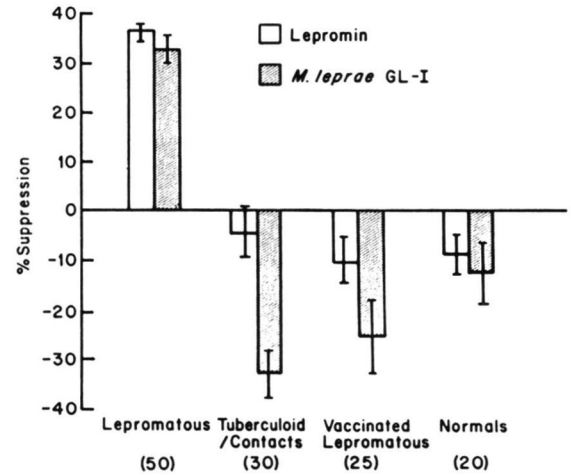


Figure 5. Top. Structure of *M. leprae* phenolic glycolipid-1. It is composed of 3,6-di-O-methylglucose,2,3-di-O-methylrhamnose, 3-O-methylrhamnose linked to phenol dimycocerosyl phthiocerol. *Bottom.* Suppression of Con A responses of peripheral blood lymphocytes of leprosy patients and normals by Dharmendra lepromin and by *M. leprae* phenolic glycolipid-1 incorporated into liposomes (17).

sion, and *iii*) removal of the terminal sugar markedly reduced the suppression. No suppression was induced by liposomes alone or analogous glycolipids from other mycobacteria differing in the terminal saccharide moiety. Monoclonal antibody to the terminal disaccharide completely abolished the suppression induced by the phenolic glycolipid and partially inhibited that induced by *M. leprae*. These data indicate that the T8 suppressor cells in lepromatous leprosy have rather remarkable specificity for the terminal trisaccharide of the phenolic glycolipid of *M. leprae* and provides strong structural evidence that T cells are capable of recognizing carbohydrate epitopes or possibly idiotypes reacting with them.

Relationship of in vitro suppression to the disease state in leprosy. Leprosy is basically a localized disease in which the battle between the immune system and the pathogen is fought out in the tissues, primarily skin and nerves. If the T suppressor cells studied in vitro in lepromatous patients were involved in suppressing DTH reactions in the tissues, one might expect to find macrophages laden with (AFB) and a predominance of T8 cells over T4 cells in lesions of lepromatous leprosy. Indeed this is found to be the case (21, 22). Robert Modlin and Thomas Rea have been able to isolate small numbers of T cells from biopsies of leprosy lesions, sort them into T4 and T8 subsets, and establish cell lines in culture.[1] In collaborative studies with our laboratory, we were then able to establish clones that provided a unique opportunity to test the function of the cells found at the sites of lesions in patients. Two of these T8 clones, when added either to peripheral blood mononuclear cells or to T4 clones, were able, in the presence of lepromin, to suppress Con A responses, but only when there was matching at HLA-D (23). Through the use of cloned cell lines, it became possible directly to ask whether T8 suppressor clones were capable of blocking antigen-specific responses of T4 clones and whether that suppression was MHC restricted. Our two T8 suppressor clones were able to suppress proliferation of the *M. leprae*-specific T4 clones only when the cells shared MHC class II antigens. These results provide the first evidence for the presence of suppressor cells at lesions in any human disease and provide evidence that their activity can be restricted by class II MHC antigens. We suspect that, as in the mouse, other levels of suppressor activity will be identified, perhaps including idiotype and anti-idiotype recognition and possibly other MHC restrictions. Overall, the results support the view that the spectrum of leprosy represents a subtle regulatory balance of T helper and T suppressor cells within lesions that determines the ultimate course of the disease.

Nevertheless, these findings raise more questions than they answer. The basic question becomes what factors determine the predominant development of suppressor cells in a very small percentage of people infected with *M. leprae*. Is it a matter of genetic or environmental predisposition of the individual, variations in the genetics of the organism, the route of infection, escape from cytocidal mechanisms of host macrophages, or subversion of antigen-presenting macrophages, dendritic cells, or Langerhans cells leading, by default, to suppressor cell dominance? Do the suppressor cells act by regulating IL 2 or IFN-γ production or receptors for IL 2 or antigen on T helper cells, by blocking chemotaxis of T helper cells to lesions, by blindfolding or killing T helper cells, or by killing or blocking the function of antigen-presenting cells? I find these questions intriguing and curiously central to understanding the process of immunoregulation. Leprosy is one of the few models in which these questions can be fruitfully explored in man.

Vaccines against leprosy. The key finding that initially permitted the possibility of producing a vaccine against leprosy to be contemplated was that *M. leprae* can be grown in vary large amounts in the armadillo, e.g., 10^9 AFB/gram of tissue (24). Initially, the armadillo was chosen because of its low body temperature, comparable with that of human skin, but it is likely that the undeveloped immune system in this host contributes as well. The first vaccine strategy was developed by one of the truly great experts in leprosy, Dr. Jacinto Convit in Caracas, Venezuela, on the basis of observations he made earlier that killed *M. leprae* inoculated into the skin of lepromatous patients persisted as AFB for long periods of time (25). However, when live BCG was inoculated together with killed *M. leprae*, a granulomatous response was produced to the BCG, and all AFB were rapidly degraded including the *M. leprae*. This suggested the possibility that immunization with a mixture of live BCG plus killed *M. leprae* might bring about a state of both cross-reactive and specific reactivity to *M. leprae* and thus serve as a therapeutic vaccine.

Convit and his colleagues (26) have now vaccinated several hundred lepromatous leprosy patients with a mixture of live BCG and killed purified *M. leprae*. Their results have shown immunologic conversion to positive skin test reactivity, clearance of bacilli from the skin, histopathological upgrading towards the tuberculoid end of the spectrum, and clinical improvement in approximately 85% of borderline lepromatous leprosy 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. We have had the opportunity, in collaboration with Dr. Convit, to examine the in vitro suppressor activity and activation markers on peripheral T cells from 10 such patients in a completely blind fashion, prior and subsequent to immunotherapy (18). The results indicate that in vitro suppressor activity in all successfully vaccinated patients disappeared after immunotherapy, and expression of HLA-D was reduced to normal levels in seven of eight patients examined. These results provide objective evidence for immunological changes brought about by this vaccine, and establish a correlation between the in vitro T cell suppressor activity and the degree of immunologic unresponsiveness in vivo. Thus, leprosy remains the only example of specific immunological unresponsiveness in man that can, with reasonable success, be overcome by immunologic intervention. It is important to try to develop an understanding of the mechanism by which this is accomplished. The ability of BCG-responsive T cells to produce IL 2 and IFN-γ locally is likely to be important.

Although it would have been inconceivable even 5 yr ago, there are currently three major controlled preventive

[1] **Modlin, R. L., V. Mehra, L. Wong, Y. Fujimiya, W-C. Chang, D. A. Horwitz, B. R. Bloom, T. H. Rea, and P. K. Pattengale.** 1986. Suppressor T lymphocytes from lepromatous leprosy skin lesions. *Submitted for publication.*

vaccine trials against leprosy in progress or about to get underway involving 450,000 people in three parts of the world, largely as a result of the work of the Committee on the Immunology of Leprosy (IMMLEP) of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. On the assumption that the combined vaccine or a cross-reactive cultivable mycobacteria will be as effective as *M. leprae* in providing immunity to noninfected individuals and likely to be therapeutic in those in the population who have subclinical infection, all three trials are testing the efficacy of killed *M. leprae* + BCG. However, even if the armadillo-derived vaccine should be effective, there is real question whether its cost and the inevitable shortage of the bacilli would permit it to be widely used. For this reason, it is important to search for potentially protective antigens in *M. leprae*. Because *M. leprae* has not been cultivated in vitro, biochemical studies of *M. leprae* antigens present a unique challenge. To meet this challenge, we were fortunate enough to be able to collaborate with Drs. Richard Young and Ron Davis who had just developed the λ gt11 expression system. In collaboration with Josephine and Roy Curtiss, who had just worked out methods for preparing high m.w. DNA from *M. leprae*, and Clemens Grosskinsky in my lab, Rick Young produced recombinant λ gt11 genomic libraries to *M. tuberculosis* (27) and *M. leprae* (28) that are fully representative of the entire genome. Although *M. leprae* cannot be grown, it was very exciting to know that its genes could be propagated infinitely and expressed in a vicarious host, *E. coli*.

Although I indicated earlier that all the known protein and glycoprotein antigens of *M. leprae* appear to cross-react with antibodies to homologous proteins in other species of mycobacteria (29), the development of monoclonal antibodies has permitted the identification on each of the major proteins of unique epitopes possessed only by *M. leprae* or shared with only a few other species (30, 31). Screening of the *M. leprae* library with 38 monoclonals permitted the identification of λ-phage expressing the five major protein antigens of *M. leprae* of m.w. 55,000 to 65,000, 36,000, 28,000, 18,000, and 12,000. Because multiple monoclonal antibodies exist to the largest of the polypeptides, it seemed possible to attempt to identify epitopes recognized by specific and cross-reacting monoclonals and by T cells by using recombinant DNA technology. To do this, a mini-library was constructed by Vijay Mehra and Rick Young of fragments of approximately 250 to 1000 base pairs from the gene encoding the 65,000 dalton protein. These were screened with six monoclonals antibodies, and from the pattern of clones reacting with a given antibody it was possible to predict the sites of individual epitopes (32). Because they obtained the DNA sequence of the entire gene, an amino acid sequence for each of the fragments of the "epitope library" was predicted.

Peptides are now being synthesized, and two have already been found to bind the appropriate monoclonal antibodies. The identification of these polypeptide species containing unique epitopes permitted, in collaboration with Salim Mustafa and Tore Godal in Norway, the development of a simple method for testing whether these antigens could be recognized by T helper cells, which are most likely to be critical for providing protection against leprosy (33). Through the use of *M. leprae*-specific T cell clones it was possible to screen crude phage lysates of the lysogens expressing major antigens and to identify four human T cell clones that recognized one of the proteins (18,000 daltons) (Fig. 6). This approach should permit testing for T clone recognition in other systems by using crude bacterial lysates without the need to purify the antigens. Finally, by using the computer algorithm of DeLisi and Berzofsky (34), predictions of amphipathic structures likely to be recognized by T cells on the 65,000 dalton *M. leprae* protein have been made, and one predicted peptide has been synthesized by Peter Kim and found by Johanne Melancon in my lab to be recognized by T cell clones from lepromin-positive donors. From these molecular approaches, we hope to be able to develop more sensitive sero-diagnostic and skin test reagents that could be used on a wide scale for detecting infection by *M. leprae* and possibly predicting the form of disease while infection is still subclinical.

Vaccines and the Third World. Leprosy is but one of many diseases that selectively exact major morbidity and mortality in the Third World. Five million children die each year of the simple vaccine-preventable illnesses diphtheria, whooping cough, tetanus, polio, measles, and tuberculosis (1) (Table I). Seven and one-half million die annually from diarrheal diseases, and 4.5 million die from respiratory diseases (35). Five hundred million people suffer from malnutrition. With the development of monoclonal antibodies and recombinant DNA technology, it is possible to identify and produce protective antigens and to modify the genetics of organisms to produce effective recombinant vaccines.

Yet even with the simple childhood vaccines there remain the major problems of timing and delivery. In the case of DPT, in many countries 60% of the population receives one DPT shot but only 20% receive the full course of three immunizations (1). The cost of vaccines from development to production represents only 14% of the cost of global immunization, the remaining 86% being the costs associated with the health infrastructure, transport, and cold chain required for effective delivery, which must generally be borne by the developing country (36) (Fig. 7). Yet vaccines are probably the most cost-effective public health measure, other than hand washing, available to the Third World. In the case of smallpox, the expenditure of $46 million over a period of 12 yr resulted in a vaccine that saves the United States an estimated $500 million each year in not having to vaccinate all overseas travellers. The Institute of Medicine has recently developed criteria for evaluating the cost-benefits of vaccines, and has developed a sophisticated analytical framework for setting vaccine priorities relevant to the United States and the Third World (37). They developed a means of comparing acute diseases and chronic diseases by normalizing to a value termed the "infant mortality equivalent" (IME), i.e., values that represent the number of acute morbidity days or chronic cases considered to be equal in undesirability to the death of an infant. A simplified example of the kind of priority decisions that could be made on the basis of how much one is prepared to spend per IME is given in Table II (38).

One of the major conceptual breakthroughs for immunizing vast numbers of children in the Third World was the development of multi-vaccine vehicles by Moss and Paoletti in vaccinia (39, 40). It has been possible to im-

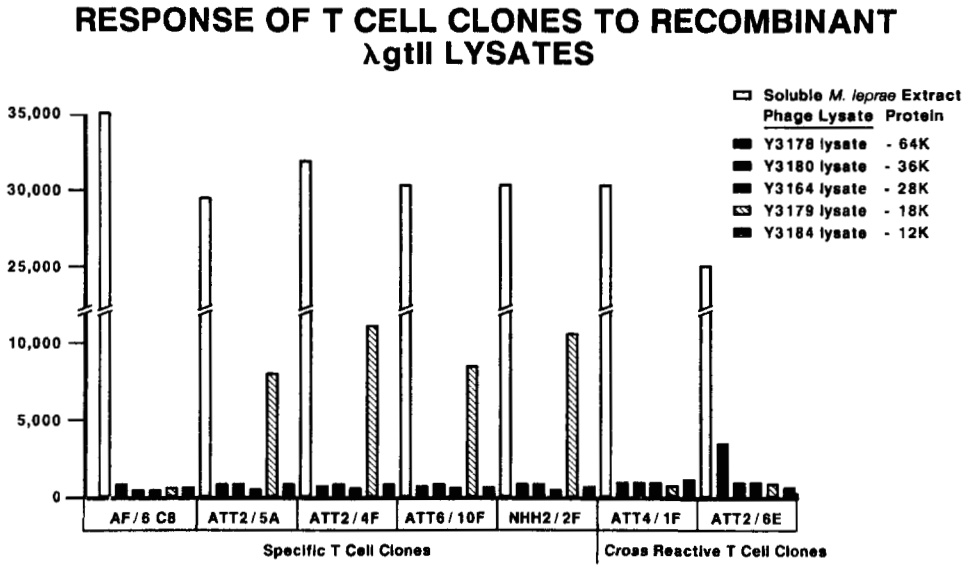


Figure 6. T cell clones derived from volunteers vaccinated with killed *M. leprae* screened against crude phage lysates of λ gt11 lysogens expressing major antigens of *M. leprae*. Four *M. leprae*-specific T cell clones responded to the lysogen Y3179 expressing the 18,000 dalton protein (34).

TABLE I

Vaccine-preventable mortality

	Deaths Millions/Year
I. Currently existing vaccines	
Neonatal tetanus	1.14
Measles	2.60
Pertussis	0.84
	4.75
II. Vaccines under development	
Diarrheal diseases	7.50
Acute respiratory illness	6.50
	14.00

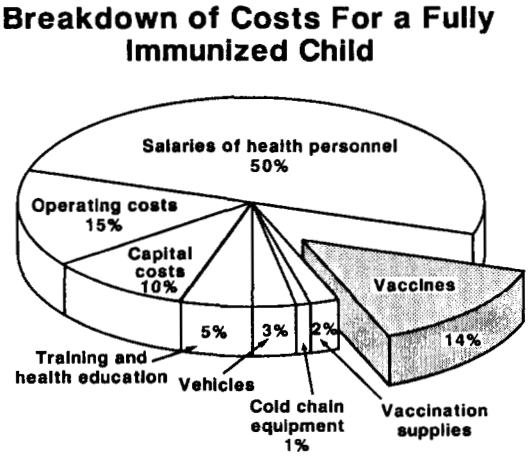


Figure 7. Breakdown of costs for a fully immunized child in the WHO Expanded Program for Immunization (39).

TABLE II

Total disease burden values and vaccine priorities[a]

Disease	Total Disease Burden Value (IME units)	Priority[b] (cost per IME averted) Unrestricted	$10,000
Streptococcal pneumonia	6,612,261	1	1
Hepatitis B	2,394,256	6	—
Malaria	2,111,795	3	4
Typhoid	1,308,121	4	3
Escherichia coli (ETEC)	978,248	13	7
Rotavirus	925,042	2	2
Shigellosis	828,068	5	5
Streptococcus (Group A) infection	811,477	8	9
Leprosy	657,349	11	11
Hemophilus influenzae type B	471,336	7	7
Cholera	229,217	10	8

[a] Infant mortality equivalent (IME) values represent the number of acute morbidity days or chronic cases considered to be equal in undesirability to the death of an infant (39).

[b] Priorities can be assigned to specific vaccines by considering the following factors: *i*) vaccine practicability (number of strains, antigenic drift/shift), *ii*) target population selection (cost effectiveness), *iii*) proportion of illness falling in target populations (including opportunity to deliver at optimal age), *iv*) efficacy (including duration of immunity), *v*) capacity to deliver vaccine (health system functionality), *vi*) characteristics of vaccine (stability), *vii*) utilization (including provider and lay attitudes, and number of doses needed), *viii*) amount of funds one is prepared to allocate to avert one IME relative to the cost of each vaccine (39).

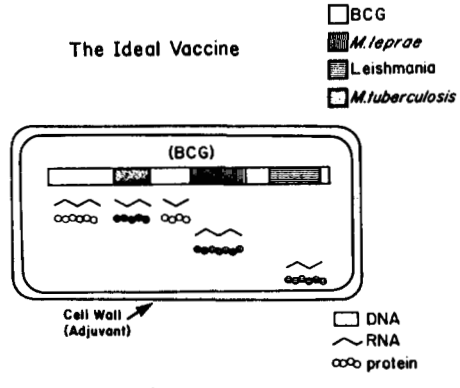


Figure 8. Conception of a recombinant mycobacterial vaccine expressing specific protective antigens for other infectious agents.

munize animals with recombinant vaccinia to produce antibodies against four independent antigens simultaneously. Recombinant vaccines capable of providing simultaneous protection against *Salmonella* and *Shigella* are being developed (41). It is our hope to develop a recombinant mycobacterial vaccine that is capable of engendering cell-mediated immunity and antibodies to multiple antigens (Fig. 8). A recombinant mycobacterial vaccine would have a number of unique advantages: *i*) it is a superb adjuvant, perhaps the most effective known for induction of cell-mediated immunity, *ii*) BCG has been used in more than 4 billion people around the world with minimal toxicity (42) (reported fatal complications occur at a frequency of about 60/billion), which is about 100-fold less than that of vaccinia, *iii*) it is the only vaccine that can be given at birth, *iv*) it requires only a single

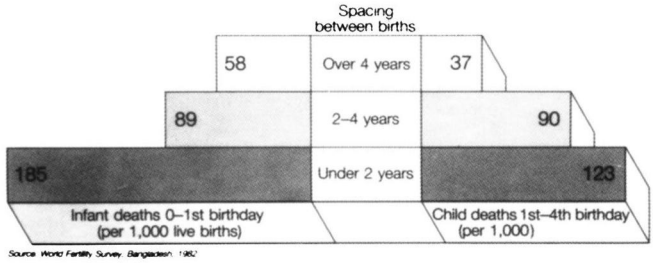


Figure 9. Spacing between births in relation to infant and childhood deaths in Bangladesh (1).

TABLE III

A perspective on priorities[a]

Defense appropriation	$210 billion
Alcoholic beverages	$22 billion
Tobacco	$12 billion
Toys	$4 billion
F-18 fighter plane	$32 million
Biomedical research	$4.5 billion
Tropical diseases research	$100 million
WHO tropical diseases programme	$25 million
WHO/UNICEF expanded programme for immunization	$300 million (proposed 1986)

[a] Figures obtained from fiscal years 1982 and 1983.

dose, *v*) it can engender DTH that persists for 5 to 50 yr, and *vi*) it costs $0.55/dose. One of the major difficulties one might have anticipated in the development of such a vaccine would be introducing DNA through the waxy coat, glycolipids, and peptidoglycan cell wall structure of mycobacteria. Bill Jacobs in my laboratory (43) has recently developed methods for spheroplasting and transfecting mycobacteriophage DNA into a cultivable mycobacterium, *M. smegmatis*. These experiments give hope to the possibility of introducing appropriate vectors with selectable markers expressing foreign genes into some species of cultivable mycobacteria, preferably BCG. An ideal vaccine would probably require, in addition to the adjuvant effect of the mycobacterium, both specific neutralizing or protective determinants of the foreign pathogen and a T helper cell-recognized epitope of the pathogen to confer memory that will be stimulated by infection.

If highly effective and low cost vaccines were developed, there are two major impediments to their use. One is the view in the developed countries that immunization of children will only lead to more babies and simply exacerbate the shortages of food and resources in the Third World, and hence should not be supported. There are a number of objections to this line of argument, of which I shall mention only two. The first is a matter of historical fact: birth rates in no country of the world have decreased prior to a decrease in death rates. It is simply not possible effectively to persuade people not to have large families unless one can demonstrate that children will survive with sufficient frequency to care for them in old age. This persuasion may not take generations. There are data from Bangladesh (Fig. 9) indicating that reducing infant and child mortality has a profound effect in increasing the spacing between births (1). Finally, it may well be that immunology has an even more crucial role to play. Although not widely known by immunologists, there are two vaccines containing antigen determinants from the β-chain of human chorionic gonadotrophin (44, 45), a hormone required for implantation, which are about to undergo clinical trials to learn if one can safely and effectively immunize to produce long-term but reversible reduction in fertility. Perhaps it will be possible some day simply to immunize against babies.

The second impediment is a matter of will and commitment. When this country spends (Table III) vast amounts of funds on national security (this year's appropriation request is $317 billion and undoubtedly comparable amounts are spent by the Soviet Union), and our people spend almost $40 billion annually merely on alcoholic beverages, tobacco, and toys, one wonders why it is so difficult to raise the $300 million that would permit every child in the world to be vaccinated now against the major childhood diseases. Of the $5 billion spent on biomedical research, less than one-fiftieth is spent on diseases of greatest concern to the developing countries by all agencies of the U.S. government.[2] It is a telling indictment of the priorities of the developed nations that the entire WHO Special Programme for Research and Training in Tropical Diseases still receives less funding for research in immunology, chemotherapy, and epidemiology of six major tropical diseases than the cost of a single jet fighter plane (46). One wonders how much of the preoccupation of world leaders with "national security" actually makes a significant positive impact on the "personal security" and daily survival of the people in the poorest nations. One wonders also to what extent the political tensions in the Third World arise from despair at the disparity in the quality of life there relative to life in developed countries.

The preamble to the charter of the World Health Organization states that "Access to the highest attainable standard of health is a basic human right". We as immunologists and as Americans have important contributions, and commitments, to make.

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[2] A report of the Office of Science and Technology Planning ("Status of Biomedical Research and Related Technology for Tropical Diseases"; OTA-H-258, U.S. Govt. Printing Office, 1985) prepared by the Congressional Office of Technology Assessment indicates that in 1982 the National Institute for Allergy and Infectious Diseases spent $33 million for tropical medicine and approximately $3 million for projects on diarrheal diseases and acute respiratory illnesses, a large proportion of which was for influenza research directed at domestic problems. The Centers for Disease Control spends about $5 million on tropical disease research and medical entomology. The agency for International Development funds about $15 million and the Department of Defense about $12.5 million. The budget of the WHO Special Programme for Research and Training in Tropical Diseases has remained essentially constant at approximately $25 million for 4 yr, and in 1981 approximately one-third of its research funds, somewhat greater than the U.S. contributes to the program, was awarded to American scientists. The WHO program in diarrheal diseases awarded 25% of its research funds to American institutions, but the U.S. contribution represented only 1% of the program's total budget.

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