Our studies raise two major questions: are the antibodies against the 5.1 antigen in endemic sera protective, and do the majority of them cross-react with sporozoites? Since we are now able to extract significant amounts of the 5.1 antigen from erythrocytic parasites, it should be possible to investigate these questions.

ELSA M. RADA
medicina
Apartado Postal 4043
Caracas 1010-A Venezuela

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BIOLOGIA MOLECULAR

Lymphocyte suppression in leprosy induced by unique *M. leprae* glycolipid

Vijay Mehra*, Patrick J. Brennan†, Elsa Rada‡, Jacinto Convit‡ & Barry R. Bloom*

* Albert Einstein College of Medicine, Bronx, New York 10461, USA
† Colorado State University, Fort Collins, Colorado 80523, USA
‡ National Institute of Dermatology, Caracas, Venezuela

Leprosy remains a significant medical and social problem in many developing countries. The varied forms of the disease form a spectrum[1]. At one pole, tuberculoid leprosy, patients develop high levels of cell-mediated immunity which results in the killing and clearing of bacilli in the tissues. At the lepromatous pole, patients exhibit a selective immunological unresponsiveness to antigens of *Mycobacterium leprae* so that the organisms inexorably multiply in the skin. We have suggested that in lepromatous leprosy one or a small number of unique antigenic determinants present on *M. leprae* might induce specific suppressor cells that inhibit the reactivity of helper T-cell clones capable of recognizing other specific or cross reactive determinants[2]. Although unique epitopes have been identified by monoclonal antibodies on a small number of *M. leprae* proteins[3], the only unique species of antigen present in *M. leprae*, and not on any other species of mycobacteria so far examined, is a phenolic glycolipid (gly-I)[4]. We show here that this unique antigen of *M. leprae* is capable of inducing suppression of mitogenic responses of lepromatous patients' lymphocytes *in vitro* and provide evidence that the suppressor T cells recognize the specific terminal trisaccharide moiety.

It remains unclear why the vast majority of people exposed to *M. leprae* develop no clinical disease, and why only a minority who do develop clinical disease become immunologically unresponsive to antigens of the organisms. Lepromatous patients who are anergic to antigens of *M. leprae* frequently show cell-mediated immunity to antigens of the tubercle bacillus although all known species of protein and glycoprotein antigens in *M. leprae* are either serologically identical or cross-reactive with those of BCG[5]. Some experimental support *in vitro* for the existence of lepromin-induced suppressor activity of mononuclear cells from lepromatous leprosy patients has appeared. Adherent cells from lepromatous patients, presumably monocytes, have been found to suppress mitogen and antigen responses *in vitro*[5–8]. Lepromin-induced suppressor T cells have also been implicated in some circumstances. We have previously reported that 84% of patients with lepromatous leprosy have a T cell bearing the OKT5/OKT8 phenotype which can be induced by lepromin to suppress responses of the patients or normal donors lymphocytes to the mitogen, concanavalin A (Con A)[6]. These suppressor cells (T_S) are active in patients with lepromatous and borderline leprosy, but not tuberculoid leprosy or in normal donors. A high percentage (approximately 50%) of the T8 cells from lepromatous patients expressed the activation markers, Fc IgG receptors and HLA-DR antigens[9]. Depletion of these T8 cells in a third of the patients with lepromatous leprosy permitted the remaining T cells to respond vigorously to lepromin, indicating that, in some patients, specifically reactive T cells were present, but unable to respond in the presence of the T suppressor cells.

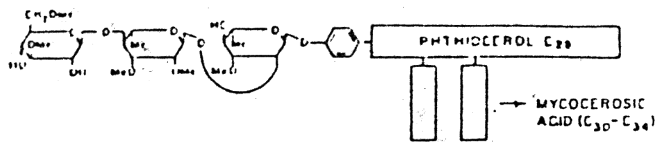

Fig. 1 Schematic structure of *M. leprae* phenolic gly-I. It is composed of 3,6-di-*O*-methylglucose, 2,3-di-*O*-methylrhamnose, 3-*O*-methylrhamnose linked to phenol-dimycocerosyl phthiocerol.

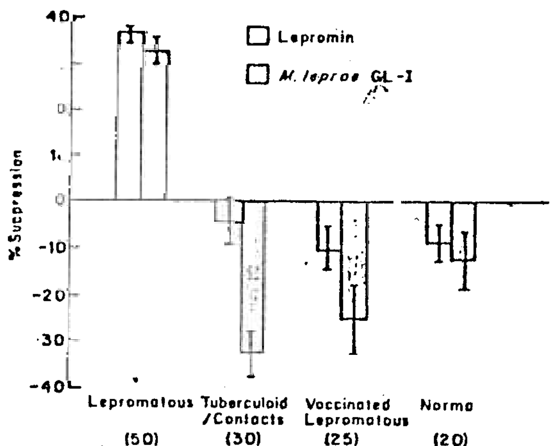

Fig. 2 Suppression of mitogenic responses of peripheral blood lymphocytes of leprosy patients and normals to Con A in the presence of *M. leprae* specific phenolic gly-I and Dharmendra lepromin. Peripheral blood mononuclear cells were obtained from heparinized blood by Ficoll-Hypaque centrifugation. The suppression of mitogenic response of lymphocytes to Con A, induced by lepromin and phenolic glycolipid, was assayed as described earlier[2]. Briefly, 2×10^5 lymphocytes were cultured in triplicate in RPMI 1640 containing 10% heat inactivated pooled human AB serum with (1) no additions, (2) 2.5 $\mu g\ ml^{-1}$ Con A, (3) Con A plus Dharmendra lepromin (1:10), 0.5 $\mu g\ ml^{-1}$ *M. leprae* phenolic gly-I in the form of liposomes or control liposomes. Liposomes were prepared by the method of Six *et al.*[12]. A mixture containing 2.0 mg sphingomyelin, 0.73 mg cholesterol, 0.065 mg dicetylphosphate and 0.23 mg *M. leprae* gly-I in 125 µl of Tris-NaCl buffer (*p*H 8.0) was sonicated for 1 h with glass beads. The cultures were labelled on day 2 with 1 µCi ^{3}H-thymidine (specific activity 5 Ci $mmol^{-1}$) per well and harvested 18 h later. The data were analysed by analysis of variance and Duncan's multiple range test. Significance was accepted at the $P < 0.05$ level. Numbers in parenthesis represent the number of subjects studied in each group.

posomes alone. These data indicate that the suppressor cell has rather exquisite specificity for the terminal trisaccharide found in *M. leprae* gly-I. This was confirmed by testing a series of monoclonal antibodies for their ability to inhibit suppression induced by lepromin or the phenolic glycolipid (Table 1). Monoclonal antibody specific for the terminal disaccharide of the *M. leprae* glycolipid completely inhibited the suppression induced by gly-I, and significantly reduced that by lepromin. A monoclonal antibody to an *M. leprae* specific epitope on a polypeptide of molecular weight 68,000 weakly diminished suppression of the intact lepromin, but was without effect on the purified glycolipid. Monoclonal antibodies to a cross-reactive mycobacterial surface antigen, and to an antigen present in Dharmendra lepromin were also without effect. These results indicate that the phenolic gly-I is at least one of the major suppression-inducing determinants of *M. leprae* in this assay.

It remains to be ascertained whether the phenolic glycolipid and T cells capable of suppressing mitogenic responses are effective in suppressing specific T-cell responses to *M. leprae* antigens in appropriate patients. Nevertheless, it has been possible to associate the lepromin-induced *in vitro* suppressor activity described here with the course of disease by examining a small number of patients with lepromatous leprosy who have been successfully vaccinated with a mixture of killed *M. leprae* and live BCG[13]. As shown in Fig. 2, there is an almost total reduction in both lepromin-induced and gly-I-induced suppression in patients showing clinical improvement following immunotherapy. These results suggest that T_S cells assayed here and the unique phenolic glycolipid of *M. leprae* may be relevant to the clinical unresponsiveness of patients with lepromatous leprosy.

If the selective unresponsiveness of lepromatous leprosy patients is due to the action of suppressor cells induced by the phenolic glycolipid or other antigens, it would appear that successful immunization, either with *M. leprae* plus live BCG or with presumably cross-reactive culturable mycobacteria can overcome this unresponsiveness and engender positive skin test reactivity, histological upgrading of the quality of the lesions and more rapid clearing of antigens. The recent report by Abebe *et al.*[14] indicating that the unresponsiveness to *M. leprae* antigens could be partially overcome *in vitro* by the addition of T-cell conditioned medium containing interleukin-2 (IL-2) would suggest that the mechanism of action of such suppressor cells would be to block either the production of, or responsiveness to, lymphokines by lepromin-responsive T_H cells. Even so, the factors governing the development of T_S cells and the lepromatous form of the disease remain unclear: genetic constitution, defective antigen presenting cells and the route of infection have been suggested as possible predisposing factors.

It has long been problematic whether T cells are capable of recognizing polysaccharide antigens or sugars. Earlier studies on antibody responses to pneumococcal III polysaccharide indicated a role for T suppressor cells *in vivo* although there was no evidence for recognition of SSS-III by T cells[15]. Clearly antibody responses to hapten coupled-ficoll[16] and the streptococcal A carbohydrate[17] have been found to be T-cell dependent. Delayed type hypersensitivity to tuberculocarbohydrates have been reported[18]. Recently, cell-mediated immunity to *Bacillus fragilis* has been shown to be induced by the capsular polysaccharide and, curiously, is mediated by a major histocompatibility complex unrestricted, Lyt2 T cell in the mouse[19]. These findings indicate that some carbohydrate moieties can be recognized by T cells, perhaps preferentially by T cells of the suppressor phenotype.

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Intrathymic presentation of circulating non-major histocompatibility complex antigens

B. A. Kyewski, C. G. Fathman* & H. S. Kaplan

Cancer Biology Research Laboratory, Department of Radiology,
* Division of Immunology, Department of Medicine, Stanford University School of Medicine, Stanford, California 94305, USA

Intrathymic selection of T-cell specificity has been shown t(o be) influenced by self-major histocompatibility complex (MH(C)) antigens encoded by radioresistant thymic stromal cells[1,2]. (The) role of non-MHC antigens in intrathymic T-cell differentiati(on,) in particular induction of antigen-specific tolerance, has b(een) unclear[3–5] and the access of non-MHC antigens to the thy(mus) is controversial. Here we present evidence that circulating n(on-MHC pro)tein antigens enter the thymus and are presented by thy(mic) stromal cells. At least three distinct types of stromal cells (are) thought to be associated with intrathymic lymphopoiesis[6-]... after intravenous (i.v.) injection of antigen only I–A/E-posit(ive) medullary dendritic cells, but not I–A/E-negative macropha(ges) or I–A/E-positive cortical epithelial cells co-purified with a(nti)gen-specific stimulation of cloned T-helper cells *in vitro*. A(nti)gen presentation by thymic stromal cells was dependent on (the) dose of antigen injected and the time interval after injectio(n).

Three types of direct cell–cell lymphostromal interacti(ons) have been identified as sites of intrathymic lymphopoiesis: th(ey) involve associations of thymocytes with (1) thymic macropha(ges) (MØ), (2) thymic medullary dendritic cells (DC), and (3) corti(cal) epithelial cells[6–12]. Two types of multicellular lymphostro(mal) complexes which represent the *in vitro* correlates of these int(er)actions *in vivo* have been separately isolated and purified: (1) MØ and DC form rosettes with thymocytes (thymocyte rosett(es,) T-ROS) and (2) epithelial cells completely enclose the asso(ci)ated thymocytes (thymic nurse cells, TNC)[10–12]. The T ce(lls) associated with all three types of stromal cells are specifica(lly) enriched in actively dividing cells[12]. The recognition sign(als) responsible for the binding and activation of the stromal ce(ll-) associated thymocytes have not been defined. From previo(us) indirect evidence in chimaeras[1,2], it is surmised that self-MH(C) determinants, at least in part, specify these interactions. He(re) we analyse a possible role of blood-borne non-MHC antige(ns) in these interactions.

Highly purified T-ROS are significantly enriched in antige(n-) presenting cells (APC) compared with unselected thymocyt(es.) As few as 1×10^3 T-ROS elicit a stronger response than 1×1... unselected thymocytes, indicating a 100–1,000-fold enrichme(nt)