

INTERNATIONAL JOURNAL OF LEPROSY
and Other Mycobacterial Diseases

OFFICIAL ORGAN OF THE INTERNATIONAL LEPROSY ASSOCIATION

EDITORIAL OFFICE

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VOLUME 57, NUMBER 1

MARCH 1989

EDITORIALS

Editorial opinions expressed are those of the writers.

T-Helper Cell Subpopulations and the Immune Spectrum of Leprosy

The World Health Organization estimates that leprosy afflicts approximately 13 million individuals.¹ This figure is expected to increase in the coming years due to the progressive increase in resistance of *Mycobacterium leprae* to sulfones.² Therefore, the development of a vaccine has been the prime goal of a number of research groups. However, little is known of the mechanism underlying the clinical and immunological spectrum of leprosy and, consequently, the outcome of a vaccination program is unpredictable.

We will attempt to correlate the immunological spectrum of leprosy with recent findings indicating that distinct helper-T (Th) cell populations produce different lymphokines.³ An hypothesis is proposed suggesting that the spectrum of leprosy reflects the balance between the Th-cell populations activated by the mycobacteria and the lym-

phokines produced by each one of them. In tuberculoid leprosy (TT), Th1, producing both interleukin-2 (IL-2) and gamma-interferon (IFN- γ), would be induced, leading to an efficient immune response. In contrast, in lepromatous leprosy (LL), the Th2 population would be preferentially activated, producing high levels of IL-5 which would inhibit secretion of IL-2 and IFN- γ .

Spectrum of leprosy; correlation with the immune status. The spectrum of leprosy is characterized, at the tuberculoid pole, by development of both T- and B-cell immunity against the mycobacteria which ultimately kill and clear the bacilli in the tissues. At the lepromatous pole, patients exhibit selective T-cell unresponsiveness to *M. leprae* with multiplication of the microorganisms in the skin. In contrast, the specific and polyclonal B-cell reactivity is augmented in these patients, with concomitant development of autoantibodies. The majority of the patients have, however, intermediate forms of the disease (borderline—BB, BL, BT) which, untreated, can shift toward either pole of the spectrum (reviewed in ⁴).

¹ Bloom, B. R. and Godal, T. Selective primary health care: strategies for control of disease in the developing world. V. Leprosy. Rev. Infect. Dis. 5 (1983) 765–780.

² Ridley, D. S. The histopathological spectrum of the mycobacterioses. In: *The Biology of the Mycobacteria*. Vol. 2. Ratledge, C. and Stanford, J., eds. London: Academic Press, 1983, pp. 129–171.

³ Mosmann, T. R. and Coffmann, R. L. Two types of mouse helper T-cell clone. Implications for immune regulation. Immunol. Today 8 (1987) 223–227.

⁴ Bloom, B. Learning from leprosy; a perspective on immunology and the Third World. J. Immunol. 137 (1986) i–x.

Specific T-cell unresponsiveness in lepromatous leprosy (LL). The specific T-cell unresponsiveness in LL patients is characterized by the absence of delayed-type hypersensitivity (DTH) reactions,⁵ *in vitro* lymphocyte proliferation,^{6, 7} and IFN- γ ^{8, 9} and IL-2 production¹⁰ (reviewed in ¹¹), in response to *M. leprae* antigens. In addition, macrophages from LL patients have a reduced capacity to release hydrogen peroxide (H₂O₂).¹² The knowledge accumulated during the past few years on the functional properties of IFN- γ and IL-2 makes it possible, however, to subdivide these defects into two major groups. Thus, since IL-2 is the main lymphokine required for the induction of a proliferative response in activated T cells, the absence of lymphocyte proliferation in LL can be ascribed to the lack of IL-2 production. Similarly, defective macrophage activation and DTH anergy are, most probably, a consequence of the inability to produce IFN- γ .

The conclusion that the failure to produce IFN- γ leads to DTH anergy was drawn from experimental evidence identifying IFN- γ with two lymphokines previously shown to mediate DTH, namely, macrophage-activating factor (MAF)¹³⁻²⁴ and migration in-

⁵ Rees, R. J. W. The significance of lepromin reaction in man. *Prog. Allergy* 8 (1964) 224-258.

⁶ Bjune, G., Barnetson, R., Ridley, D. S. and Kronvall, G. Lymphocyte transformation test in leprosy; correlation of the response with inflammation of lesions. *Clin. Exp. Immunol.* 25 (1976) 85-94.

⁷ Godal, T., Myrvang, B., Frøland, S. S., Shao, J. and Melaku, G. Evidence that the mechanism of immunological tolerance (central failure) is operative in the lack of host resistance in lepromatous leprosy. *Scand. J. Immunol.* 1 (1972) 311-321.

⁸ Noqueira, N., Kaplan, G., Levy, E., Sarno, E. N., Kushner, P., Granelli-Piperno, A., Vieira, L., Colomer Gould, V., Levis, W., Steinman, R., Yip, Y. K. and Cohn, Z. A. Defective γ interferon production in leprosy: reversal with antigen and interleukin 2. *J. Exp. Med.* 158 (1983) 2165-2170.

⁹ Kaplan, G., Weinstein, D., Steinman, R. M., Levis, W. R., Elvers, V., Patarroyo, M. E. and Cohn, Z. A. Analysis of *in vitro* T cell responsiveness in lepromatous leprosy. *J. Exp. Med.* 162 (1985) 917-929.

¹⁰ Haregewoin, A., Godal, T., Mustafa, A. S., Beleh, A. and Yemaneberhan, T. T-cell conditioned media reverse T-cell unresponsiveness in lepromatous leprosy. *Nature* 303 (1983) 342-344.

¹¹ Haregewoin, A., Mustafa, A. S., Helle, I., Walters, M. F. R., Leiker, D. L. and Godal, T. Reversal by interleukin-2 of the T-cell unresponsiveness of lepromatous leprosy to *Mycobacterium leprae*. *Immunol. Rev.* 80 (1984) 77-86.

¹² Nathan, C. F., Kaplan, G., Levis, W. R., Nusrat, A., Witmer, M. D., Sherwin, S. A., Job, C. K., Horowitz, C. R., Steinman, R. M. and Cohn, Z. A. Local and systemic effects of intradermal recombinant interferon- γ in patients with lepromatous leprosy. *N. Engl. J. Med.* 315 (1986) 6-15.

¹³ Roberts, W. K. and Vasil, A. Evidence for the identity of murine gamma interferon and macrophage activating factor. *J. Interferon Res.* 2 (1982) 519-532.

¹⁴ Schuetz, R. M. and Kleinschmidt, W. J. Functional identity between murine γ interferon and macrophage activating factor. *Nature* 305 (1983) 239-240.

¹⁵ Schreiber, R. D., Pace, J. L., Russell, S. W., Altman, A. and Katz, D. H. Macrophage activating factor produced by a T cell hybridoma: physicochemical and biosynthetic resemblance to γ -interferon. *J. Immunol.* 131 (1983) 826-832.

¹⁶ Murray, H. W., Rubin, B. Y. and Rothermel, C. D. Killing of intracellular *Leishmania dovani* by lymphokine-stimulated human mononuclear phagocytes: evidence that interferon- γ is the activating lymphokine. *J. Clin. Invest.* 72 (1983) 1506-1510.

¹⁷ Nathan, C. F., Murray, H. W., Wiebe, N. W. and Rubin, B. Y. Identification of interferon- γ as the lymphokine that activates human macrophage oxidation metabolism and antimicrobial activity. *J. Exp. Med.* 158 (1983) 670-689.

¹⁸ Rothermel, C. D., Rubin, B. Y. and Murray, H. W. Gamma-interferon is the factor in lymphokine that activates human macrophages to inhibit intracellular *Chlamydia psittaci* replication. *J. Immunol.* 131 (1983) 2542-2544.

¹⁹ Svedersky, L. P., Benton, C. V., Berger, W. H., Rindecknech, E., Harkins, R. N. and Paladino, M. A. Biological and antigenic similarities of murine interferon- γ and macrophage activating factor. *J. Exp. Med.* 159 (1984) 812-827.

²⁰ Le, J. and Vilček, J. Lymphokine mediated activation of human monocytes: neutralization by monoclonal antibody to interferon-gamma. *Cell Immunology* 85 (1984) 278-283.

²¹ Horwitz, H. A., Levis, W. R. and Cohn, Z. A. Defective production of monocyte activating cytokines in lepromatous leprosy. *J. Exp. Med.* 159 (1984) 666-678.

²² Kiderlen, A. F., Kaufmann, S. H. E. and Lohmann-Matthes, M.-L. Protection of mice against intracellular bacterium *Listeria monocytogenes* by recombinant immune interferon. *Eur. J. Immunol.* 14 (1984) 964-967.

²³ Talmage, K. W., Gallati, H., Sinigaglia, F., Walz, A. and Garotta, G. Identity between human interferon- γ and "macrophage-activating factor" produced by human T lymphocytes. *Eur. J. Immunol.* 16 (1987) 1471-1477.

²⁴ Edwards, C. K., III, Hedegaard, H. B., Zlotnik, A., Gangaharam, P. R., Johnston, R. B., Jr. and Pabst, M. J. Chronic infection due to *Mycobacterium intracellulare* in mice: association with macrophage release of prostaglandin E₂ and reversal by infection of indomethacin, muramyl dipeptide and interferon- γ . *J. Immunol.* 136 (1986) 1820-1827.

hibition factor (MIF).²⁵ Thus, IFN- γ enhanced the capacity of macrophages both to release H₂O₂ and to kill intracellular parasites (reviewed in ¹²); the latter phenomenon being, most probably, a consequence of the former, considering the well documented bactericidal effects of H₂O₂.²⁶⁻²⁸ In addition, when injected *in vivo*, IFN- γ induced histological changes resembling a DTH reaction.^{12, 24} More recently, T-helper cells belonging to the subgroup that produces IFN- γ (Th1, see below) were shown to transfer DTH, while those that do not produce this lymphokine (Th2) did not.²⁹ Finally, the correlation between the absence of IFN- γ production and the lack of *M. leprae*-specific DTH in LL was further supported by the experiments showing that IFN- γ injected into cutaneous lesions of LL patients induced a local cellular reaction resembling DTH and augmented the capacity of monocyte-derived macrophages to release H₂O₂.¹²

Mechanisms underlying specific unresponsiveness in LL. Four different mechanisms, namely, a) defective antigen presentation,³⁰⁻³² b) absence of antigen-specific

helper-T (Th) lymphocytes,^{33, 34} c) generation of specific suppressor cells,^{32, 35-39} and d) failure to generate nonspecific T-cell growth factors (TCGF),^{10, 11} in response to *M. leprae*, have been proposed to account for the specific immune defect in LL.

The first alternative was made unlikely by experimental evidence showing that monocytes from LL patients could present *M. leprae* to histocompatible sibling's lymphocytes in a proliferation assay.³⁴ On the other hand, recent findings, demonstrating that T lymphocytes from LL patients could be induced to proliferate in response to *M. leprae*, provided the cells were cultured *in vitro* in the absence of the mycobacteria for a period of 48 hours, challenged the concept that *M. leprae*-specific T cells were deleted in LL.⁴⁰

role of HLA-D-identical peripheral blood lymphocytes and macrophages in the *in vitro* modulation of lymphoproliferative responses. Clin. Exp. Immunol. **42** (1980) 203-310.

³³ Godal, T., Myklestad, B., Samuel, D. R. and Myrvang, B. Characterization of the cellular defect in lepromatous leprosy: a specific lack of circulating *Mycobacterium leprae*-reactive lymphocytes. Clin. Exp. Immunol. **9** (1971) 821-831.

³⁴ Stoner, G. L., Mshana, R. N., Touw, J. and Beleh, A. Studies on the defect in cell-mediated immunity in lepromatous leprosy using HLA-D-identical siblings. Absence of circulating suppressor cells and evidence that the defect is in the T-lymphocytes rather than the monocyte population. Scand. J. Immunol. **15** (1982) 33-48.

³⁵ Bjune, G. *In vitro* lymphocyte stimulation in leprosy: simultaneous stimulation with *Mycobacterium leprae* antigens and phytohaemagglutinin. Clin. Exp. Immunol. **36** (1979) 479-487.

³⁶ Mehra, V., Mason, L. H., Fields, Y. P. and Bloom, B. R. Lepromin induced suppressor cells in patients with leprosy. J. Immunol. **123** (1979) 1183-1188.

³⁷ Bullock, W. E., Carlson, E. M. and Gershon, R. F. The induction of immunosuppressive cell populations in experimental mycobacterial infections. J. Immunol. **120** (1978) 1709-1716.

³⁸ Stoner, G. L. Hypothesis: do phases of immunosuppression during *Mycobacterium leprae* infection determine the leprosy spectrum? Lepr. Rev. **52** (1981) 1-10.

³⁹ Modlin, R., Mehra, V., Wong, L., Fujimiyama, Y., Chang, W.-C., Horwitz, D. A., Bloom, B. R., Rea, T. H. and Pattengale, P. K. Suppressor T lymphocytes from lepromatous leprosy skin lesions. J. Immunol. **137** (1986) 2831-2834.

⁴⁰ Mohagheghpour, N., Gelber, R. H. and Engelman, E. T cell defect in lepromatous leprosy is reversible *in vitro* in the absence of exogenous growth factors. J. Immunol. **138** (1987) 570-574.

²⁵ Thurman, B. A., Brande, I. A., Gray, P. W., Oldham, R. K. and Stevenson, H. C. MIF like activity of natural and recombinant human interferon- γ and their neutralization by monoclonal antibody. J. Immunol. **134** (1985) 305-309.

²⁶ Elsbäck, P. and Weiss, J. A reevaluation of the roles of the O₂-dependent and O₂-independent microbicidal systems of phagocytes. Rev. Infect. Dis. **5** (1983) 843-853.

²⁷ Klebanoff, S. J. and Shephard, C. C. Toxic effect of the peroxidase-hydrogen peroxide-halide antimicrobial system on *Mycobacterium leprae*. Infect. Immun. **44** (1984) 534-536.

²⁸ Sharp, A. K., Colston, M. J. and Banerjee, P. K. Susceptibility of *Mycobacterium leprae* to the bactericidal activity of mouse peritoneal macrophages and to hydrogen peroxide. J. Med. Microbiol. **19** (1985) 77-84.

²⁹ Cher, D. J. and Mosmann, T. R. Two types of murine helper T cell clone. II. Delayed-type hypersensitivity is mediated by Th₁ clones. J. Immunol. **138** (1987) 3688-3694.

³⁰ Hirschberg, H. The role of macrophages in the lymphoproliferative response to *Mycobacterium leprae* *in vitro*. Clin. Exp. Immunol. **34** (1978) 46-51.

³¹ Salgame, P. R., Birdi, T. J., Mahadevan, P. R. and Antia, N. H. role of macrophages in defective cell-mediated immunity in lepromatous leprosy. I. Factors from macrophage affecting protein synthesis and lymphocyte transformation. Int. J. Lepr. **48** (1980) 172-177.

³² Nath, I., van Rood, J. J., Mehra, N. K. and Vaidya, M. C. Natural suppressor cells in human leprosy: the

Regarding the remaining two alternatives, i.e., suppression and inability to produce TCGF, the question depends on whether these phenomena are, indeed, the defect or a reflection of it. Furthermore, the mechanism underlying suppression induced by cells from LL patients has not yet been analyzed. Unfortunately, when unresponsiveness mediated by CD8⁺ cells is observed, rarely are attempts made to exclude the possibility that the phenomenon was mediated by cytotoxic T lymphocytes (CTL). This would be particularly important when the indicator system includes a lectin (ConA, PHA, etc.), as was the case in most studies showing suppressor cells in specifically activated lymphocytes from LL. Thus, if suppressor cells are in reality CTL, the presence of a lectin will allow nonspecific killing of the responder cells. In fact, CD8⁺, *M. leprae*-specific, H-2-restricted CTL clones have been isolated from spleen cells of mice immunized with this mycobacterium.⁴¹ Consequently, a possible mechanism underlying the observed suppression induced by *M. leprae* is that CTL-mediated lysis of responder and/or antigen-presenting cells (APC) occurred in the cultures. However, this mechanism cannot be evoked in situations in which the response could be restored by either supplementing the cultures with IL-2 or preculturing the cells before addition of the antigen.^{8, 10, 11, 40}

Analysis of recent findings on subpopulations of Th cells and the lymphokines produced by each of them might, however, give an alternative explanation for the phenomena observed *in vitro* as well as for the regulatory mechanisms underlying the spectrum of leprosy.

Role of helper-T lymphocyte subpopulations on the immune response and pathogenesis of leprosy. Two subpopulations of Th cells, Th1 and Th2, have been defined on the basis of their surface phenotype (Ia⁺/Ia⁻), function, and capacity to produce different lymphokines. The major characteristics of the Th1 and Th2 subsets and the functional properties of the lymphokines

exclusively produced by each one of them have recently been reviewed.^{3, 43} Briefly, Th1 cells do not express class II (Ia) antigens and do not adhere to nylon wool. In contrast, Th2 are nylon-wool adherent and express Ia antigens. A number of lymphokines, such as IL-3, tumor necrosis factor (TNF), and granulocyte-macrophage colony stimulating factor (GM-CSF), are produced by both T-cell populations. However, IL-2 and IFN- γ are exclusively produced by Th1 cells, while only Th2 cells produce IL-4 and IL-5.^{3, 42, 43}

With the exception of some of the steps in the process of collaboration with the B cell, which was found to depend upon direct interaction between the Th and the B cell⁴⁴⁻⁴⁶, the functional properties of Th1 and Th2 cells correspond to the sum of the functional properties of the lymphokines that each one produces. Thus, IL-4 induces expression of IgG1 and IgE, and inhibits IgM and IgG2a, in B cells stimulated with lipopolysaccharide (LPS). In addition, it induces the expression of IgE-binding Fc receptors, the growth of mast cells, and increased expression of class II major histocompatibility complex (MHC) antigens in B cells and macrophages. At the T-cell level, IL-4 induces proliferation of activated cells and potentiates the generation of CTL. IL-5 potentiates both specific and polyclonal IgM

⁴² Mosmann, T. R., Cherwinski, H., Bond, M. W., Giedlin, M. A. and Coffman, R. L. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokines activities and secreted proteins. *J. Immunol.* **136** (1986) 2348-2357.

⁴³ Cherwinski, H. M., Schumacher, J. H., Brown, K. D. and Mosmann, T. R. Two types of mouse helper T cell clone. III. Further differences in lymphokine synthesis between Th1 and Th2 clones revealed by RNA hybridization, functionally monospecific bioassays, and monoclonal antibodies. *J. Exp. Med.* **166** (1987) 1299-1244.

⁴⁴ Tada, T., Takemori, T., Okumura, K., Nonaka, H. and Tokuhisa, T. Two distinct types of helper T cells involved in secondary antibody response: independent and synergistic effects of Ia⁻ and Ia⁺ helper T cells. *J. Exp. Med.* **147** (1978) 446-458.

⁴⁵ Swierkosz, J., Marrack, P. and Keppeler, J. Functional analysis of T cells expressing Ia antigens. I. Demonstration of helper T cell heterogeneity. *J. Exp. Med.* **150** (1979) 1293-1309.

⁴⁶ Rasmussen, R., Takatsu, K., Harada, N., Takahashi, T. and Bottomly, K. T cell-dependent hapten-specific and polyclonal B cell responses require release of interleukin 5. *J. Immunol.* **140** (1988) 705-712.

⁴¹ Chiplunkar, S., Libero, G. and Kaufmann, S. *Mycobacterium leprae*-specific Lyt-2⁺ T lymphocytes with cytotoxic activity. *Infect. Immun.* **54** (1986) 793-797.

and IgA production and induces maturation of eosinophils, proliferation of B cells stimulated with dextran sulfate and expression of IL-2 receptors (IL-2R) in both T and B cells (reviewed in ³ and ⁴⁷). Thymic as well as splenic CTL precursors (CTLp) require IL-5 to mature and to acquire lytic effector function⁴⁸ (and Ramos, T., submitted for publication). The properties of IL-2 have been extensively reviewed^{49, 50} and can be summarized as follows: IL-2 induces proliferation of activated T and B cells and potentiates CTL responses. IFN- γ has a potent antiviral activity and, as IL-4, induces increased expression of class II MHC antigens in macrophages, endothelial cells, fibroblasts,⁴⁷ and Schwann cells.⁵¹ However, regarding the Ig isotype, IFN- γ is an antagonist of IL-4 in that it inhibits expression of IgG1 and IgE and potentiates IgG2a.⁵² As already mentioned, IFN- γ activates macrophages and thereby mediates DTH reactions (reviewed in ¹²).

If one then divides the immunological findings of LL into two groups, corresponding to the functional properties of the two subsets of helper-T cells, it becomes apparent that the immune impairment in LL, i.e. the absence of IL-2 and IFN- γ production, could be due to a defective response of the Th1 population while the enhanced B-cell-specific and polyclonal activation as well as generation of cytotoxic (suppressor) cells would be a consequence of overactivation of the Th2 population. In contrast, a bal-

anced activation of both populations, in TT, would lead to a successful defense against the mycobacteria.

Why then should the Th2-cell population be preferentially activated in LL patients? Three factors can be foreseen that would play a determining role in this context. a) T cells recognize foreign antigen in conjunction with cell-surface structures encoded by genes at the major histocompatibility complex (MHC). Generally, Th cells are MHC class II restricted, while CTL recognize the antigen in association with class I determinants. Thus, the proliferative response to *M. leprae* is HLA-DR restricted.⁵³ Therefore, the MHC-encoded alleles carried by the individual might influence the type of response induced. b) Alternatively, the MHC antigens present might preferentially associate with a particular mycobacterial antigenic determinant, and thus favor the activation of one or the other of the Th-cell populations. c) Finally, the mode of antigenic presentation will depend upon the route of infection and, in turn, this will, perhaps, determine the response obtained. We will briefly review evidence related to the different possibilities.

Genetic factors. In humans, an increased frequency of HLA-DR3 antigen has been reported in TT while the same allele was almost absent in LL.⁵⁴ Interestingly, DR3, per se, appears to function poorly as a restricting element for the proliferative response to lepromin of cells from TT patients.⁵³ However, analysis of the data⁵³ (and Table I in ⁵³) suggests that autologous APC (DR3/DR2) were far better than homozygous DR2 or DR3. This would be consistent with the restriction element being either a hybrid molecule or a determinant coded for in a different locus which is in linkage disequilibrium with DR.

Additional differences were reported for HLA-DQw1 which was found to be in-

⁴⁷ Ogarrá, A., Umland, S., Defrance, T. and Christiansen, J. B-cell factors are pleiotropic. *Immunol. Today* **9** (1988) 45-54.

⁴⁸ Takatsu, K., Kikuchi, Y., Takahashi, T., Honjo, T., Matsumoto, M., Haranda, N., Jamaguchi, N. and Tominaga, A. Interleukin 5, a T-cell-derived B-cell differentiation factor also induces cytotoxic T lymphocytes. *Proc. Natl. Acad. Sci. U.S.A.* **84** (1987) 4234-4238.

⁴⁹ Smith, K. A. T-cell growth factor. *Immunol. Rev.* **51** (1980) 337-357.

⁵⁰ Greene, W. C. and Leonard, W. J. The human interleukin-2 receptor. *Ann. Rev. Immunol.* **4** (1986) 69-95.

⁵¹ Wekerle, H., Schwab, M., Linington, C. and Meyermann, R. Antigen presentation in the peripheral nervous system: Schwann cells present endogenous myelin autoantigens to lymphocytes. *Eur. J. Immunol.* **16** (1986) 1551-1557.

⁵² Snapper, C. and Paul, W. E. Interleukin- γ and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. *Science* **236** (1987) 944-947.

⁵³ van Eden, W., Elferink, B. G., de Vries, R. R. P., Leiker, D. L. and van Rood, J. J. Low T lymphocyte responsiveness to *Mycobacterium leprae* antigen in association with HLA-DR3. *Clin. Exp. Immunol.* **55** (1984) 140-148.

⁵⁴ van Eden, W., de Vries, R. R. P., D'Amato, J., Schreuder, I., Leiker, D. L. and van Rood, J. J. HLA-DR-associated genetic control of the type of leprosy in a population from Surinam. *Hum. Immunol.* **4** (1982) 343-350.

creased in patients with LL.⁵⁵ Finally, family studies showed that the presence of a particular HLA DR or DQ did not confer susceptibility to the disease, instead it determined immune reactivity and thus the type of leprosy (reviewed in ⁵⁶).

In mice there is a clear linkage between non-MHC-encoded genes and susceptibility to the disease.⁵⁷ However, association with H-2 has not been found. Nevertheless, and because the number of strains analyzed so far has been limited, it is possible that, as in many other genetically controlled responses in mice, both H-2 and non H-2 genes control susceptibility to leprosy.

It is well known that different MHC determinants, either presented as alloantigens or associated with nominal antigen, will trigger different immune responses. A classical example is the preferential induction of CTL or Th cells in response to class I and class II, respectively. However, among the various class II determinants themselves, subtle differences can be found as to the type of response elicited. Thus, while activation of mouse-T lymphocytes with allogeneic cells bearing an I-A difference will induce both proliferation and CTL, disparity at the I-E locus will exclusively lead to generation of CTL.⁵⁸ Because generation of alloreactive CTL requires the presence of IL-5 (Ramos, T., submitted for publication), the latter results suggest that interaction with I-E will preferentially induce this lymphokine while recognition of the I-A molecule will lead to both Th1 and Th2 activation, production of IL-2, IL-4 and IL-5 and, consequently, generation of CTL as well as proliferation.

Thus, the argument could be evoked that *M. leprae* associated with a particular DR or DQ allele would trigger, preferentially, one or the other Th-cell population or even favor the induction of a particular lymphokine.

Antigenic determinants in mycobacteria.

The major antigens of *M. leprae* and *M. tuberculosis* have been identified using monoclonal antibodies. One of these antigens, the 65-kDa protein, is one of the major mycobacterial antigens and its immunogenicity for both T and B cells has been extensively studied (reviewed in ⁵⁹). Several groups have reported isolation of human T-cell clones reactive with the 65-kDa antigen ⁶⁰⁻⁶⁵ and 14 B-cell epitopes have been defined using monoclonal antibodies.^{66, 67}

⁵⁹ Young, D. B., Ivanyi, J., Cox, J. H. and Lamb, J. R. The 65 kDa antigen of mycobacteria—a common bacterial protein? *Immunol. Today* **8** (1987) 215–219.

⁶⁰ Mustafa, A. S., Gill, H. K., Nerland, A., Britton, W. J., Mehra, V., Bloom, B. R., Young, R. A. and Godal, T. Human T-cell clones recognize a major *M. leprae* protein antigen expressed in *E. coli*. *Nature* **319** (1986) 63–66.

⁶¹ Ottenhoff, T. H. M., Klatser, P. R., Ivanyi, J., Elferink, D. G., Wit, M. Y. L. and de Vries, R. P. *Mycobacterium leprae*-specific protein antigens defined by cloned human helper T cells. *Nature* **319** (1986) 66–68.

⁶² Emmrich, F. and Kaufmann, S. H. E. Human T-cell clones with reactivity to *Mycobacterium leprae* as tools for characterization of potential vaccines against leprosy. *Infect. Immun.* **51** (1986) 879–883.

⁶³ Emmrich, F., Thole, J., van Embden, J. and Kaufmann, S. H. E. A recombinant 64 kilodalton protein of *Mycobacterium bovis* bacillus Calmette-Guérin specifically stimulates human T4 clones reactive to mycobacterial antigens. *J. Exp. Med.* **163** (1986) 1024–1029.

⁶⁴ Oftung, F., Mustafa, A. S., Hussan, R., Young, R. A. and Godal, T. Human T cell clones recognize two abundant *Mycobacterium tuberculosis* protein antigens expressed in *Escherichia coli*. *J. Immunol.* **138** (1987) 927–931.

⁶⁵ Lamb, J. R., Ivanyi, J., Rees, A. D. H., Rothbard, J. B., Howland, K., Young, R. A. and Young, D. B. Mapping T cell epitopes using recombinant antigens and synthetic peptides. *EMBO J.* **6** (1987) 1245–1249.

⁶⁶ Gillis, T. P. and Buchanan, T. M. Production and partial characterization of monoclonal antibodies to *Mycobacterium leprae*. *Infect. Immun.* **37** (1982) 172–178.

⁶⁷ Buchanan, T. M., Nomaguchi, H., Andersson, C. D., Young, R. A., Gillis, T. P., Britton, W. J., Ivanyi, J., Kolk, A. H. J., Closs, O., Bloom, B. R. and Mehra, V. Characterization of antibody-reactive epitopes on the 65-kilodalton protein of *Mycobacterium leprae*. *Infect. Immun.* **55** (1987) 1000–1003.

⁵⁵ de Vries, R. R. P., Serjeantson, S. W. and Layrisse, Z. Leprosy. In: *Histocompatibility Testing 1984*. Berlin: Springer-Verlag, 1985, pp. 362–367.

⁵⁶ Jeannet, M. Class II HLA antigens in autoimmune and immunemediated diseases. In: *HLA Class II Antigens; A Comprehensive Review of Structure and Function*. Berlin: Springer-Verlag, 1986, pp. 489–514.

⁵⁷ Closs, O. and Haugen, O. A. Experimental murine leprosy. I. Clinical and histological evidence for varying susceptibility of mice to infection with *Mycobacterium lepraemurium*. *Acta Pathol. Microbiol. Scand. [A]* **81** (1973) 401–410.

⁵⁸ Beretta, A., Demoyen, P. L. and Larsson, E.-L. The cytotoxic T cell response to Ia antigens: lack of correlation between the level of specific cytotoxicity obtained in primary MLR and the frequency of specific CTL precursors. *Scand. J. Immunol.* **24** (1986) 643–657.

The gene encoding the 65-kDa protein has been cloned from both *M. leprae* and *M. tuberculosis*,^{68, 69} and the amino-acid sequences, which constitute six of the epitopes recognized by B cells and two by Th cells, have been determined using recombinant DNA sublibraries constructed from portions of the 65-kDa gene^{65, 68} or by using synthetic peptides.⁶⁵

In all of these studies, however, immunogenicity at the T-cell level was defined as the capacity of the antigen to induce proliferation and thus, most likely, IL-2 production. According to the present hypothesis, it is possible that some of these antigenic determinants will, when associated with MHC antigens carried by LL patients, preferentially induce the Th2-cell population. Alternatively, other determinants that did not induce a response in cells from TT patients might be immunogenic for individuals with LL. Our hypothesis being correct, this possibility becomes relevant when selecting antigenic determinants potentially useful for vaccination purposes.

Antigenic presentation. It is well established that the route of administration of an antigen will determine the type of immune response induced. Thus, injection of an antigen intravenously often leads to tolerance, while the same antigen given subcutaneously will induce immunity. This is indeed the case with most mycobacteria, and it is generally accepted that the subcutaneous route favors appropriate processing of the antigen.⁷⁰

The mechanism by which the mode of antigenic presentation would favor activation of one or the other of the Th-cell populations or production of a particular lymphokine has not yet been defined. However, results indicating that different lymphokines have distinct requirements for activation provide an operational basis for such a mechanism. Thus, while IL-5 was secreted

in mixed lymphocyte cultures (MLC) induced by metabolically inactivated (ultra-violet irradiated) allogeneic cells, production of IL-2 and IL-4 in the same cultures was below detection level (Ramos, T., submitted for publication). These results suggest that even within the same T cell subpopulation (Th2, IL-4/IL-5) one lymphokine may be preferentially induced. The decision as to which factor shall be synthesized may lie on the local environment, i.e., the type of antigen-presenting cell (APC) present, the density of the MHC determinants expressed on these cells, and their state of activation.

Regulatory network of lymphokines. It has not yet been established whether the Th1 and Th2 populations represent different stages of differentiation of the same cell lineage or belong to two independent lineages. Nevertheless, the possibility that these two cell populations are reciprocally regulated is suggested by the findings that lymphokines produced by one population have an antagonistic effect on the activities induced by the other. Thus, both *in vivo* and *in vitro*, IFN- γ stimulated the expression of immunoglobulin of the IgG2a isotype and inhibited the production of IgG1 and IgE, while IL-4 promoted the expression of IgG1 and IgE and inhibited IgG2a.^{52, 71} Data are not available on the mechanism by which the mutual regulation of the different interleukins operates. It is, however, tempting to speculate that high levels of one lymphokine, for instance IL-5, would inhibit release of the others, while appropriate amounts would induce their production.

In support of this hypothesis are the findings that intravenous injection of high doses of bacille Calmette-Guérin (BCG) causes a nonspecific loss of cell-mediated immunity in mice, which, as in LL, was ascribed to the presence of suppressor cells. In this case, however, soluble factors produced by T cells and macrophages were found to be respon-

⁶⁸ Mehra, V., Sweetser, D. and Young, R. Efficient mapping of protein antigenic determinants. *Proc. Natl. Acad. Sci. U.S.A.* **83** (1986) 7013-7017.

⁶⁹ Shinnick, T. M. The 65-kilodalton antigen of *Mycobacterium tuberculosis*. *J. Bacteriol.* **169** (1987) 1080-1088.

⁷⁰ Closs, O. Experimental murine leprosy: growth of *Mycobacterium lepraemurium* in C3H and C57/B1 mice after footpad inoculation. *Infect. Immun.* **12** (1975) 480-489.

⁷¹ Coffman, R. L., Seymour, B. W. D., Lebman, D. A., Hiraki, D. D., Christiansen, J. A., Shrader, B., Cherwinski, H. M., Svelkoul, H. F. J., Finkelman, F. D., Bond, M. W. and Mosmann, T. R. The role of helper T cell products in mouse B cell differentiation and isotype regulation. *Immunol. Rev.* **102** (1988) 5-28.

sible for the phenomenon.⁷² Subsequently, the absence of mitogen-induced lymphocyte proliferation was shown to be a consequence of an inhibition of IL-2 production, mediated by a T-cell-derived suppressor factor. The interesting aspect of these findings, in the present context, is that the suppressor factor in this case had a molecular weight of 50–70,000.⁷³ This molecular size together with the recently published evidence that mice immunized with BCG produce high levels of IL-5⁷⁴ strongly suggest that the 50–70-kDa suppressor factor was IL-5 itself.

One last piece of evidence that is consistent with the presence of activated IL-5-producing cells in leprosy comes from experiments showing that, in mice, *M. lepraemurium* induces a polyclonal antibody response with particular high numbers of IgA plaque-forming cells (Silva, P., personal communication).

In conclusion, we would like to suggest that the spectrum of leprosy reflects the balance between the Th-cell populations activated by the mycobacteria. At the tuberculoid pole, induction of both Th-cell subsets will lead to an efficient immune response and, consequently, to elimination of the mycobacteria. At the lepromatous pole the Th2 population will be preferentially activated, producing high levels of IL-5 which will inhibit production of IL-2 and IFN- γ , thus preventing an adequate immune defense against the pathogen.

Role of cytotoxic T lymphocytes on the pathogenesis of leprosy. In 1974 Zinkernagel and Doherty^{75, 76} showed that mice

infected with lymphocytic choriomeningitis virus (LCMV) developed cytotoxic T lymphocytes (CTL) that could only kill syngeneic targets infected with the same virus. Independently, Shearer⁷⁷ showed that a similar phenomenon occurred *in vitro* with syngeneic hapten (TNP)-modified lymphocytes.

The knowledge of this phenomenon has largely contributed to the understanding of the mechanism by which the immune system clears the organism of potentially pathological invaders. On the other hand, it has also given insight into the possible deleterious consequence of such an aggressive mechanism of defense. An example of the latter is lymphocytic choriomeningitis, where the organic lesions observed are due to the T-cell reaction itself, leading to destruction of virus infected cells of the central nervous system (CNS).⁷⁸ A similar mechanism has been postulated for chronic hepatitis B.⁷⁹ More recently, cytotoxic T cells in blood from patients with acquired immune deficiency syndrome (AIDS) were shown to lyse CD4+ T cells expressing HIV II protein.^{80, 81}

Some of us (Silva, M., *et al.*, unpublished) have observed that in the later stages of disease in mice infected with *M. lepraemurium* free bacteria could be found in the peritoneal exudate which seemed to originate from disrupted macrophages. One of the interpretations of this phenomenon has been that

sensitized T lymphocytes in lymphocytic choriomeningitis. *Nature (Lond.)* **251** (1974) 547–548.

⁷⁷ Shearer, G. M. Cell-mediated cytotoxicity to trinitrophenyl-modified syngeneic lymphocytes. *Eur. J. Immunol.* **4** (1974) 527–533.

⁷⁸ Doherty, P. C. and Zinkernagel, R. M. T-cell-mediated immunopathology in viral infection. *Transplant. Rev.* **19** (1974) 89–120.

⁷⁹ Hoffmann, R. M., Pape, G. R., Rieber, P., Eisenburg, J., Döhrmann, J., Zachoval, R., Paumgartner, G. and Riethmüller, G. Cytolytic T cell clones derived from liver tissue of patients with chronic hepatitis B. *Eur. J. Immunol.* **16** (1986) 1635–1638.

⁸⁰ Walker, B. D., Chakrabarti, S., Moss, B., Paradis, T. J., Flynn, T., Durno, A. G., Blumberg, R. S., Kaplan, J. C., Hirsch, M. S. and Schooley, R. T. HIV-specific cytotoxic T lymphocytes in seropositive individuals. *Nature* **328** (1987) 345–348.

⁸¹ Plata, F., Autran, B., Martins, L. P., Wain-Hobson, S., Raphael, M., Mayaud, C., Denis, M., Guillon, J.-M. and Debré, P. AIDS virus-specific cytotoxic T lymphocyte in lung disorders. *Nature* **328** (1987) 348–351.

⁷² Collins, F. M. and Watson, S. R. Suppressor T cells in BCG-infected mice. *Infect. Immun.* **25** (1979) 491–496.

⁷³ Colizzi, V., Feeluga, J., Garrean, F., Malkovsky, M. and Asherson, G. L. Suppressor cells induced by BCG release non-specific factors *in vitro* which inhibit DNA synthesis and interleukin-2 production. *Immunology* **51** (1984) 65–71.

⁷⁴ Tominaga, A., Matsumoto, M., Haranda, N., Takahashi, T., Kikuchi, Y. and Takatsu, K. Molecular properties and regulation of mRNA expression for murine T cell-replacing factor/IL5. *J. Immunol.* **140** (1988) 1175–1181.

⁷⁵ Zinkernagel, R. M. and Doherty, P. C. Restriction of *in vitro* T-cell-mediated cytotoxicity in lymphocyte choriomeningitis within a syngeneic and semiallogeneic system. *Nature (Lond.)* **248** (1974) 701–702.

⁷⁶ Zinkernagel, R. M. and Doherty, P. C. Immunological surveillance against altered-self components by

metabolical inactivation (or exhaustion) of macrophages due to the bacterial load would lead to intracellular accumulation of *M. lepraemurium* and, consequently, to cell disruption. According to the hypothesis proposed above and because IL-5 would favor generation of CTL, an alternative interpretation for these findings would be that CTL induced by *M. lepraemurium* presented on the cell surface of macrophages were responsible for the lysis of the infected cells. This hypothesis implies that CTL would be present from the onset of disease, their destructive effect being masked by the constant influx of monocytes from bone marrow. Exhaustion of precursors due to both increased output and invasion of the bone marrow by the bacteria would, in late stages of the disease, allow detection of the phenomenon.

This mechanism could also contribute to the inability of the immune system to cope with this infection. Thus, CTL defense against virus infection is effective because it prevents propagation of the infection by lysing the infected cells before assembly of the virus components occurs.⁸² If, in contrast, CTL against *M. lepraemurium* are triggered by bacterial components processed and presented by the macrophage, lysis of the phagocytic cell will only lead to further spreading of the disease. Furthermore, assuming that the defense against my-

cobacteria is better achieved by the non-specific limb of the immune system, i.e., macrophages, granulocytes, etc., continuous destruction of such cells would lead to further impairment of the already deficient defense system.

We think that analyzing, experimentally, the questions raised above is important because the hypothesis being correct, the answers will not only give new insight into the pathogenesis of diseases such as leprosy but will also add to the understanding of the mechanisms underlying regulation of T-cell activation.

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