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Thoughts on the Immunology of Leprosy

Leprosy is a disease produced by *Mycobacterium leprae*, a seemingly obligate intracellular pathogen. *M. leprae* has a slow growth, and most of it takes place inside tissue macrophages. This, and the nature of its cell wall are key concepts. As skin and peripheral nerves are preferentially attacked, lesions are accessible to clinical and pathological examination. *M. leprae* does not grow *in vitro*; its exact identification has been based mostly on negative properties. Obtainment of antigens and their identification is difficult and is further hampered by the need of eliminating contaminating host tissue components. Another important concept is the current lack of a fully adequate language and sufficient knowledge to fully explain immunological events in leprosy. Great advances have been made, but a lot remains to be learned. Imagine the situation in 1940 when high resistance to *M. leprae* and low antibody levels in tuberculoid leprosy (and the opposite situation in lepromatous leprosy) had to be explained in the absence of concepts such as those of cellular immunity, role of lymphocytes, macrophage activation, or lymphokines. Our current efforts may be looked upon with similar eyes in 1990.

There are four aspects that will be mentioned here:

- a) Relationship of immunology to clinical and histologic features of leprosy.
- b) Vaccination and leprosy.
- c) Immunology and reactional states.
- d) Immunology and diagnosis of *M. leprae* and leprosy.

It is increasingly common to accept the idea that leprosy is a disease with diverse clinical types constituting a spectrum. It goes from the relatively highly resistant tuberculoid leprosy to the low resistance form of lepromatous leprosy. There are intermediate forms. Patients are usually stable, but there are ways by which they may move along the spectrum. Clinical forms have corresponding histologic features. The main point of the latter is that epithelioid cells and an admixture of lymphocytes indicate resistance whereas foamy histiocytes and no lymphocytes (except during reactional states) indicate lack of resistance¹. Clinical and histologic features are reflections of the immune status of the patient. Resistant individuals are able to develop cell-mediated immunity to *M. leprae* as witnessed by a positive Mitsuda reaction and mitogenesis and lymphokine production when their lymphocytes are incubated

¹ Ridley, D. S. Histological classification and the immunological spectrum of leprosy. Bull. WHO 51 (1974) 451-465.

with *M. leprae* or its fractions. This does not happen with lepromatous patients or their lymphocytes.

Antibody production does not seem to be involved directly in resistance because higher levels of them occur in lepromatous patients. It must be admitted, however, that we know little about the nature of antibodies that occur in LL, particularly to which structural components of the bacilli they are directed against.

There is growing evidence that lepromatous patients do not necessarily have an overall impairment of cell-mediated immunity (CMI)^{2,3,4,5}. Reports are not unanimous, but the truth seems to be that whatever overall impairment of CMI exists, it is secondary to the disease, either related to factors present in any chronic disabling disease (undernutrition, overcrowding, parasites) or by factors such as replacement of lymphoid tissue by granulomatous structures and blocking of lymphocyte circulation. This overall impairment may not occur in a given patient, or it may be of low intensity. It usually disappears when the general state of health improves and/or when bacillary load is diminished or wiped out by therapy. In contrast, anergy towards *M. leprae* (as measured by the tests employed), is persistent, and it may antedate the appearance of clinical disease⁶. Whether or not it may precede contact with *M. leprae* is a critical and unanswered question. The fate of *M. leprae* and of the patient is decided inside macrophages. Mac-

rophage control by lymphocytes seemed to be a single road a few years ago. It is not quite so any more. Macrophage function is affected by lymphokines, but macrophages may act on lymphocytes in diverse ways, not only by digestion and/or presentation of antigenic determinants in their surface, but also by many other means not always clearly understood^{7,8}. There is also evidence of control of macrophage and lymphocyte functions by serum factors attached or not to the surface of the macrophages and altered by them. The role of polymorphonuclear leukocytes, while not explored, has not been ruled out.

Another point, and an important one, is that lymphocyte populations are being subdivided more and more. The T, B and null classification is not sufficient any more. The exact pairing of lymphocyte receptors, lymphocyte types, and lymphocyte functions in man has not been achieved yet^{9,10}.

Control mechanisms are exceedingly important. The coincidence of low resistance, low levels of certain aspects of cell-mediated immunity, and high levels of (some) antibodies that may also modulate granulomatous responses is too common in certain clinical forms of intracellular and other granulomatous infections to be accidental. This occurs in certain forms of coccidioidomycosis, schistosomiasis, leishmaniasis, and paracoccidioidomycosis, to name but a few^{11,12,13,14,15,16}. The very detailed clin-

² Bullock, W. E. Leprosy: A model of immunological perturbation in chronic infection. *J. Infect. Dis.* **137** (1978) 341-354.

³ Faber, W. R., Leiker, D. L., Nengerman, I. M., Zeijlemaker, W. P. and Schellekens, P. Th. A. Lymphocyte transformation test in leprosy: Decreased lymphocyte reactivity to *Mycobacterium leprae* in lepromatous leprosy, with no evidence for a generalized impairment. *Infect. Immun.* **22** (1978) 649-656.

⁴ Rea, T. H., Quismorio, F., Harding, B., Friou, G. and Levan, N. Quantitative dinitrochlorobenzene (DNCB) responsivity and phytohemagglutinin (PHA) induced lymphocyte transformation in patients with lepromatous leprosy. *Int. J. Lepr.* **44** (1976) 250-255.

⁵ Ulrich, M., Salas, B. and Convit, J. Lymphocyte transformation with phytomitogens in leprosy. *Int. J. Lepr.* **40** (1972) 4-9.

⁶ Price, M. A., Enders, E. M., Anders, R. F., Russell, D. A. and Dennis, E. S. Cell-mediated immunologic status of healthy members of families with a history of leprosy. *Int. J. Lepr.* **43** (1975) 307-313.

⁷ Allison, A. C. Mechanisms by which activated macrophages inhibit lymphocyte responses. *Immunol. Rev.* **40** (1978) 3-27.

⁸ Unanue, E. R. The regulation of lymphocyte functions by the macrophage. *Immunol. Rev.* **40** (1978) 227-255.

⁹ Chess, L. and Schlossman, S. F. Human lymphocyte subpopulations. *Adv. Immunol.* **25** (1977) 213-241.

¹⁰ Snell, G. D. T cells, T cell recognition structures and the major histocompatibility complex. *Immunol. Rev.* **38** (1978) 3-69.

¹¹ Boros, D. L. Granulomatous inflammations. *Prog. Allergy* **24** (1978) 183-267.

¹² Fava-Netto, C. The immunology of South American blastomycosis. *Mycopathol. Mycol. Appl.* **26** (1965) 349-358.

¹³ Fava-Netto, C. The serology of paracoccidioidomycosis. Present and future trends. In: *Paracoccidioidomycosis. Proceedings of the First Pan American Symposium (PHO/WHO)*, Medellin, Columbia. PAHO Scientific Publication No. 254. Washington: Pan American Health Organization, 1972, pp. 209-213.

¹⁴ Gohman-Yahr, M., Convit, J. and Pinardi, M. E.

ico-pathological analysis done in leprosy may not be easily achieved in diseases that also attack organs not as readily accessible as the skin, but the main features are there. The basic derangement of the immune system ought to be the same in all corresponding clinical forms of the diseases mentioned, but it is specific in that it applies *only* to a given causative organism (e.g., patients with diffuse anergic leishmaniasis may develop a positive lepromin reaction and may be resistant to leprosy). In other words, the phenomenon may be better explained not by a passive anergy but by an active one where the antigen or organism is specifically recognized by certain lymphocyte populations that act in a suppressive way^{17, 18}. A more precise explanation will have to wait for more knowledge.

The immune response is genetically ruled. Efforts have been made to characterize genetic markers that would identify lepromatous patients. Current emphasis has been based on knowledge obtained from mice. In these animals there is a relatively well known relationship between Ir genes and the MHC. Initially, the effort was directed to find the "formula" of the lepromatous patient expressed in HLA antigens; this was not too fruitful. Another way is to establish an "HLA formula" that would reflect a genetic composition such that patients would react in a given way ("weak responders") not only in leprosy but in other diseases, i.e., a genetically-determined, HLA-expressed overall pattern of reaction. This is intellectually more satisfying, but no conclusive results are available. The human system is very complex; it is not impossible that different antigenic formulae may apply to different ethnic

groups. The code may be redundant and degenerate, and there is further complicating evidence that resistance to intracellular parasites and development of delayed hypersensitivity (as measured by intradermal testing) may be coded by different genes in different loci^{19, 20, 21, 22, 23, 24, 25, 26, 27}.

My feeling is that there will not be found a genotype such that it would definitely condemn somebody to become lepromatous if he or she contacts *M. leprae*. We may find one that would favor a given type of response under a given set of circumstances. Vaccination against leprosy is conceivable only if this second alternative is true. In a rational world, one would start research on vaccination only when factors that determine immune response are well known. This is not the story of successful vaccines, however. Current efforts in leprosy vaccination mainly aim at the use of killed *M. leprae* together with adjuvants applied to nonlepromatous individuals to induce a preemptive immune response or to channel it towards a desirable goal. The subject has been covered in several authoritative reviews^{28, 29, 30}. My point is that the

Aspectos inmunológicos en la Leishmaniasis. An. Bras. Derm. 52 (1977) 325-332.

¹⁵ Phillips, S. M. and Colley, D. G. Immunologic aspects of host responses to schistosomiasis: Resistance, immunopathology and eosinophil involvement. Prog. Allergy 24 (1978) 49-182.

¹⁶ Swatek, F. E. The epidemiology of coccidioidomycosis. In: *The Epidemiology of Human Mycotic Diseases*. Al-Doory, Y. ed. Springfield, Illinois: Charles C Thomas, 1975, pp. 74-102.

¹⁷ Eichmann, K. Expression and function of idiotypes in lymphocytes. Adv. Immunol. 26 (1978) 195-254.

¹⁸ Turcotte, R. Suppressor cells in experimental murine leprosy. Int. J. Lepr. 46 (1978) 358-363.

¹⁹ Greiner, J., Schleiermacher, E., Smith, T., Lenhard, V. and Vogel, V. The HLA system and leprosy in Thailand. Hum. Genetics 42 (1978) 201-213.

²⁰ Najakima, S., Kobayashi, S., Nohara, M. and Sato, S. HLA-antigen and susceptibility to leprosy. Int. J. Lepr. 45 (1977) 273-277.

²¹ Nakamura, R. M. and Tokunaga, T. Strain difference of delayed-type hypersensitivity to BCG and its genetic control in mice. Infect. Immun. 22 (1978) 657-664.

²² Poulter, L. W. Systemic immunological reactivity in the absence of delayed-type hypersensitivity during murine leprosy. Cell. Immunol. 46 (1978) 117-127.

²³ Rea, T. H., Levan, N. E. and Terasaki, P. I. Histocompatibility antigens in patients with leprosy. J. Infect. Dis. 134 (1976) 615-618.

²⁴ Skamene, E., Kongshavn, P. A. L. and Sachs, D. H. Resistance to *Leisteria monocytogenes* in mice: Genetic control by genes that are not linked to the H2-complex. J. Infect. Dis. 139 (1979) 228-231.

²⁵ Stoner, G. L., Touw, J., Belehu, A. and Naafs, B. *In vitro* lymphoproliferative response to *Mycobacterium leprae* of HLA-D identical siblings of lepromatous leprosy patients. Lancet 2 (1978) 543-547.

²⁶ Stoner, G. L. Ir genes and leprosy. Int. J. Lepr. 46 (1978) 217-220.

²⁷ Takada, H., Sada, M., Ozawa, S. and Seikiguchi, S. HLA and mycobacterial infection: Increased frequency of B-8 in Japanese leprosy. Tissue Antigens 11 (1978) 61-64.

²⁸ Convit, J. and Ulrich, M. General ideas concerning a vaccine against leprosy: A basis for discussion

number of possible ways is great, favorable results may or may not be achieved, and intensity of efforts will depend on the availability of funds and on whether a very effective, easy to give, and cheap chemotherapeutic agent(s) becomes available in the future. Among other avenues of vaccine research we could mention the use of attenuated live preparations of *M. leprae* or of other related mycobacteria that would critically cross-react with *M. leprae*. Everything depends on the knowledge about antigenic components and chemical constitution of mycobacteria and on development of methods to inoculate *M. leprae* in animals and to grow these bacilli *in vitro*. This last is the most important hurdle to overcome.

At the beginning, I stressed the importance of the cell wall; it is antigenically and chemically related to the cell wall of other mycobacteria and acid-fast organisms. It is this cell wall that gives *M. leprae* the property of adjuvanticity and that may explain many features of reactional states, even maybe their existence. Reactional states are not necessarily present in other intracellular diseases immunologically akin to lepromatous leprosy (diffuse anergic leishmaniasis, for instance). Without delving into this subject I would like to point out the following:

- a) Tissue damage may be produced by diverse mechanisms reciprocally related or not.
- b) Adjuvanticity also implies immune deviation.
- c) Autoantibodies, cytotoxic or not, may be induced by *M. leprae* cell walls acting as adjuvant.
- d) Explanation of reactional states solely by humoral factors (circulating immune complexes and/or Arthus phenomenon) is at best incomplete³¹, and

adjuvant disease of the rat may provide a better model for study^{32, 33}. On the other hand, the Lucio phenomenon may be better explained by humoral factors³⁴.

Tissue damage in reactional leprosy may be due to activation of some lymphocyte population by cell walls of *M. leprae*. These lymphocytes may directly induce tissue damage or else they may liberate soluble factors that could induce the appearance and activation of cells of the granulocytic series.

Immunological methods have been used for the diagnosis of disease and of subclinical infection since the dawn of bacteriology. Several roads may be taken, and they are not mutually exclusive. One is the use of an intradermal test to detect delayed hypersensitivity to a given set of antigens. Sensitivity would be present in patients with leprosy or individuals sensitized to the bacillus. This road is *a priori* feasible because it has been successfully used in other mycobacterial diseases such as tuberculosis and because *M. leprae* induces mitogenesis *in vitro* in lymphocytes from persons with the disease or presumably sensitized to *M. leprae*. As resistance and delayed hypersensitivity may not be mediated by the same cell population, it does not necessarily follow, although it is likely, that an intradermal test would be negative in lepromatous patients. The problem is the lack of an appropriate antigenic preparation. The likely sources would be protoplasmic fractions from armadillo-grown *M. leprae* or from cross-reacting mycobacteria. The technical problems are great, and

during the Eleventh International Leprosy Congress. (editorial) *Int. J. Lepr.* **46** (1978) 61–63.

²⁹ Godal, T. The rationale behind a leprosy vaccine research program. *Int. J. Lepr.* **45** (1977) 61–63.

³⁰ Godal, T. Is immunoprophylaxis in leprosy feasible? *Lepr. Rev.* **49** (1978) 305–317.

³¹ Tung, K. S. K., Kimi, B., Bjorvatn, B., Kronvall, G., McLaren, L. C. and Williams, R. E. Discrepancy between Clq deviation and Raji cells tests in detection of immune complexes in patients with leprosy. *J. Infect. Dis.* **136** (1977) 216–221.

³² Gohman-Yahr, M., Requena, M. A., Vallecalle-Suegart, E. and Convit, J. Autoimmune diseases and thalidomide. II. Adjuvant disease, experimental allergic encephalomyelitis and experimental allergic neuritis of the rat. *Int. J. Lepr.* **42** (1974) 266–275.

³³ Gohman-Yahr, M., Convit, J., Rodríguez-Ochoa, G., Aranzazu, N., Villalba-Pimentel, L., Ocanto, A. and Gómez, M. E. Significance of neutrophil activation in reactional lepromatous leprosy. Effects of thalidomide *in vivo* and *in vitro*. Activation in adjuvant disease. *Int. Arch. Allergy Appl. Immunol.* **57** (1978) 317–332.

³⁴ Quismorio, F. P., Rea, T., Chandor, S., Levan, N. and Friou, G. Lucio's phenomenon: An immune complex deposition syndrome in lepromatous leprosy. *Clin. Immunol. Immunopathol.* **9** (1978) 184–193.

I do not foresee short-term breakthroughs in this respect.

The use of antibodies for diagnosis depends on the individualization of an antigen-antibody reaction characteristic of leprosy and *M. leprae* and not occurring with other related organisms (mycobacteria) with which contact may be likely. The problem here is not lack of antibody response in all forms of leprosy or in methods to detect this but rather in lack of a definite or specific antigen. This specific antigen need not be related to antigens involved in resistance or in adjuvanticity. There is some evidence that a protein antigen may be specific to *M. leprae*^{35, 36}. Its isolation needs an adequate supply of unaltered organisms from armadillos or else the culture of *M. leprae in vitro*.

In summary, the main avenues for progress would be:

- a) A more accurate definition of immunologically active cells; this includes their characterization by reliable methods and understanding of their functions and of control mechanisms.
- b) A better insight of adjuvants. This would include knowledge of their chemical structure and understanding of the way by which they act.

³⁵ Caldwell, H. D. and Buchanan, T. M. Immunological and structural integrity of surface protein antigens of mycobacteria during separation from armadillo liver tissue. *Int. J. Lepr.* **47** (1979) 469-476.

³⁶ Caldwell, H. D., Kirchheimer, W. F. and Buchanan, T. M. Identification of a *Mycobacterium leprae* specific protein antigen(s) and its possible application for the serodiagnosis of leprosy. *Int. J. Lepr.* **47** (1979) 477-483.

- c) Obtainment of antigens from *M. leprae* and their immunological and chemical characterization.
- d) If all or most of the above is achieved, then manipulation and direction of the immune response is possible. Current reports of the use of cyclophosphamide to eliminate suppressor lymphocytes³⁷ and of indomethacin to suppress their function are examples of this. All this may be far from us.

I would like to point out that the problems that we face are basically identical to those of the immunology of cancer and of autoimmune disorders. It is not that research in leprosy is not up to the times and cannot answer pertinent questions; it is that the questions now posed are key biological questions not completely answered by anyone.

—Mauricio Goihman-Yahr

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³⁷ Glaser, M. Regulation of specific cell-mediated cytotoxic response against SV-40-induced tumor associated antigens by depletion of suppressor T cells with cyclophosphamide in mice. *J. Exp. Med.* **149** (1979) 774-779.