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EDITORIAL

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Report of the Indo-European Community Joint Symposium "Leprosy and Other Mycobacterial Diseases," Lonavla, India, 6–9 November 1989

A symposium on "Leprosy and Other Mycobacterial Diseases," jointly sponsored by the Department of Science and Technology, India, and the European Community (EC), was held in Lonavla, India, from 6–9 November 1989. The symposium was coordinated by Drs. M. G. Deo and G. Nath (India) and Drs. S. H. E. Kaufmann and S. Gregoli (EC). Scientists from different parts of India and the EC discussed various aspects of research in the field of leprosy, tuberculosis, and other mycobacterial infections. The symposium brought together clinicians working in the field and scientists interested in immunology, molecular biology, chemotherapy, and pathology. Basic scientific questions and applied questions were covered. The full proceedings of this symposium will be published in Tropical Medicine and Parasitology.

Immunology. N. A. Mitchison (London) speculated on how the heterogeneity of the major histocompatibility complex (MHC) evolved in response to the evolutionary pressure caused by parasitic and infectious diseases. In addition, reduced variability of the T-cell receptor could make an individual more susceptible to infection due to the failure to recognize a major parasite antigen. Finally, a parasite could succeed in infecting the host because of genetically dominant unresponsiveness under the control of the MHC. In man, HLA-DQ seems to play a particular role in the activation of suppressor-T cells and, furthermore, mutual inhibition of TH1 and TH2 cell activities may further influence suppression.

P. Lagrange (Paris) discussed the genetic control of mycobacterial infections in mice. He emphasized that variability of the immune response to mycobacteria represents an important factor that may have a genetic basis. Using several inbred and outbred strains of mice, he analyzed genetic influences on cell-mediated immunity, natural resistance, acquired resistance, and immunopathology. These differences may have important implications for future vaccine design.

Stimulation of cellular immunity by mycobacteria is influenced by the route of immunization as well as by the growth rate of the microorganisms (R. S. Kamat, Bombay). Slow-growing mycobacteria were found to be immunogenic after immunization by the intraperitoneal and the intradermal routes; whereas fast-growing mycobacteria were only immunogenic after intradermal application. Treatment with poly I : poly C reversed unresponsiveness to

intraperitoneal immunization by rapid growers.

S. H. E. Kaufmann (Ulm) provided evidence that protection against mycobacteria includes both cytotoxic- and helper-T cells. Interferon-γ, an important macrophage activating lymphokine, is produced by both CD4 and CD8 T cells and both CD4 and CD8 T cells can express cytolytic activity. In addition, a high percentage of gamma/delta T cells recognizes mycobacteria and, accordingly, this T-cell set rapidly expands after *in-vitro* activation with mycobacteria. Gamma/delta T cells produce lymphokines and express cytolytic activity. In a granuloma, the balanced action of helper-T cells and cytolytic-T cells may provide the basis for a protective host response. Under less well-defined conditions, the lysis of target cells may have detrimental consequences. For example, the lysis of Schwann cells by cytolytic-T cells may contribute to nerve cell damage in leprosy. A novel method which allows the transfer of mycobacterial proteins into a soluble phase by electroelution after separation by 2D gels was described. These fractions can then be used directly for T-cell stimulation. Furthermore, evidence was presented that the 65-kDa heat-shock protein (hsp) is a dominant antigen of mycobacteria. However, since highly homologous cognates exist in host cells, autoimmune responses may develop. In support of this notion, it was shown that T cells of human donors after stimulation with mycobacterial components recognize peptides corresponding to epitopes shared by the mycobacterial and human hsp 65. Furthermore, T cells raised against peptides of mycobacterial hsp 65 recognize stressed macrophages and Schwann cells (S. H. E. Kaufmann). M. J. Colston (London) described experiments with synthetic peptides of hsp 65 to define the epitopes relevant to rheumatoid arthritis. Interestingly, the T cells recognized an epitope unique to the mycobacterial antigen and not shared with its human counterpart.

Cells present in lesions of LL and TT patients were characterized immunologically by H. D. Flad (Borstel). The proportion of T-cell subsets and cells expressing activation markers varied markedly in LL and TT granulomas and, interestingly, granulomas of TT and BT patients had cells positive for interleukin-1, tumor necrosis factor-α, interferon-γ, and interleukin-2, which were rare to absent in lesions of LL patients. Also, evidence for the local proliferation of T cells in TT granulomas was obtained. It was concluded that components of *Mycobacterium leprae* may influence the localization and function of infiltrating cells, and thus contribute to the characteristic microenvironment within the lesions in the various forms of leprosy.

I. Nath (New Delhi) described the local immune reactions after intralesional injections of recombinant interferon-γ. The time kinetics of erythema and induration at the injected lesions indicated responses in 6 of 10 subjects at 24 hr. Maximum reactions occurred at 48 hr and remained stable for more than 5 days. A fall in the bacillary index of the injected lesions was observed in 4 of 12 patients. Interferon-γ also caused a mild-to-moderate increase in the ability of intralesional esterase-positive cells to generate H_2O_2 and O_2^-. Some patients showed bacterial clearance in the injected lesions at 2 months after infection.

In order to understand the mechanisms underlying the elimination of *M. tuberculosis,* Rajiswamy (Madras) examined macrophage functions in healthy controls and patients with confirmed pulmonary tuberculosis. She found no differences in H_2O_2 production by cultured macrophages from the two groups. Interestingly, alveolar macrophages from pulmonary tuberculosis and peritoneal macrophages from abdominal tuberculosis showed significantly higher H_2O_2 production than did controls, indicating that these macrophages had been activated at the site of infection. Still, the macrophages were not able to kill tubercle bacilli in the *in vitro* systems employed.

N. F. Mistry (Bombay) showed that live, not killed *M. leprae* are able to enter Schwann cells. In a mixture of live and killed bacilli, viable organisms were preferentially taken up. Although Schwann cells produced reactive oxygen metabolites, they failed to kill the ingested bacilli. Lymphokines did not stimulate the expression of gene products of the MHC on murine Schwann cells. After long-term coculture of lymphocytes and Schwann cells, a cytotoxic factor was produced which killed the lymphocytes which were then ingested by the Schwann

cells. Short-term culture of *M. leprae*-containing Schwann cells and lymphocytes resulted in lymphocyte proliferation.

Evidence was presented by P. R. Mahadevan (Bombay) that delipidified cell components of *M. leprae* can restore the capacity of macrophages to kill *M. leprae* through reactive oxygen intermediates. Mice preimmunized with this complex were able to kill *M. leprae in vivo,* and their peritoneal macrophages were bactericidal for *M. leprae* probably through the production of reactive oxygen metabolites. The complex imparted the capacity to kill *M. leprae* to macrophages of multibacillary patients, although it failed to induce a significant lepromin conversion. This delipidified cell-wall-antigen complex of *M. leprae* thus represents a possible candidate for a vaccine.

S. G. Gangal (Bombay) discussed the *in-vitro* reactivity to ICRC bacilli and *M. leprae* of lymphocytes from LL patients before and after vaccination with ICRC. T lymphocytes from many LL patients showed increased proliferative responses and interferon-γ production 6 to 10 months after vaccination. In clinically multidrug-therapy unresponsive patients, the bacillary index decreased following vaccination while antigen-specific T-cell responses increased. Thus, vaccination with ICRC bacilli stimulated T cells in LL patients to respond to ICRC and *M. leprae* antigens.

Molecular biology. Molecular biology techniques provide helpful tools for the isolation and characterization of mycobacterial proteins as well as for the diagnosis of pathogenic mycobacteria. DNA sequences which are specific for *M. leprae* or *M. tuberculosis* have been identified. The sensitivity of direct hybridization of these sequences is generally low but can be increased through the use of the polymerase chain reaction (PCR). The application of PCR to specifically detect small numbers of tubercle and leprosy bacilli in clinical materials was reviewed by P. W. M. Hermans (Bilthoven). He described a unique sequence of the 36-kDa protein of *M. leprae* which was used to detect small numbers of bacteria in clinical material by PCR. The potential of restricted fragment length polymorphism (RFLP) for evaluating the strain differences of *M. leprae* was stressed by V. M. Katoch (Agra).

I. Nath (New Delhi) screened the λgt11 library of *M. leprae* with sera from lepromatous leprosy patients and identified four clones that produced *M. leprae* fusion proteins of 135 kDa and were different from those already known. The antigenicity of one recombinant protein was analyzed in more depth. It induced proliferative responses in T cells from healthy contacts and tuberculoid patients but not in T cells from lepromatous patients. Of the lepromatous patients' sera which showed positive reactions with leprosin, 50% reacted with this recombinant clone.

J. S. Tyagi (New Delhi) described the use of cDNA probes derived from *M. tuberculosis* and *M. segmatis* for examining the organization of tRNA genes in nine mycobacterial species. While the tRNA gene sequences were conserved, the gross genomic organization of the tRNA genes was divergent in the species studied, except for the *M. tuberculosis* complex. Different tRNA pools seem to exist in the slow-grower *M. tuberculosis* and the fast-grower *M. segmatis.*

Antigens. When cultured in Sauton, zinc-deficient medium, *M. bovis* BCG secretes a 32-kDa protein abundantly (J. DeBruyn, Brussels). The relevant gene has been cloned and sequenced, and the amino-acid sequence deduced. The gene encodes for 336 amino acids, including a signal peptide of 42 amino-acid residues. Sequence analysis revealed 72.8% homology with a cognate in *M. tuberculosis.* The 32-kDa protein has fibronectin-binding activity. It stimulates cell-mediated immune responses in BCG-sensitized experimental animals. Tuberculin-positive individuals respond to this antigen, and 75% of the tuberculosis patients with active disease show T-cell proliferation in response to it *in vitro.* Serum antibodies in tuberculosis patients and healthy subjects differ significantly, and some evidence was obtained for a positive correlation between antibody levels and the extent of disease. The diagnostic value of this protein, therefore, will be further evaluated.

M. J. Colston (London) described the identification of a gene encoding a 28-kDa protein of *M. leprae* showing a high degree of sequence homology with a known superoxide dismutase in man and other species. He suggested co-evolutionary pressure as a

cause for generating such a similar molecule in the host and the intracellular parasite.

S. V. Chiplunkar (Bombay) studied T- and B-cell responses of leprosy patients to antigenic fractions of the ICRC bacilli and *M. leprae*. Sera from LL patients precipitated a 21-kDa antigen from ICRC sonicate. The sera of TT, BT, and tuberculosis patients, as well as of normal donors, failed to precipitate this antigen. Interestingly, the 21-kDa antigen from *M. leprae* was precipitated by all sera across the clinical spectrum of leprosy.

Monoclonal antibodies and their reactivity to *M. tuberculosis* were described by L. Ljungqvist (Copenhagen). By immunosorbent affinity chromatography, nine proteins ranging in molecular weights from 17 to 81 kDa were purified. Proteins of 17 to 19 kDa, 38 kDa, and 17 kDa appeared to be of interest for diagnostic purposes.

Screening of a genomic *M. tuberculosis* library with monoclonal antibodies allowed the identification of a 3.9-kDa DNA fragment which was shown to encode a 35-kDa protein present only in *M. tuberculosis* (G. Damiani, Genoa).

Microbiology. M. Silva discussed his electron microscopy studies on normal and altered mycobacteria. According to him, freezing and thawing of *M. leprae* even once, could result in loss of viability. He, therefore, prefers to use fresh preparations of *M. leprae* in such studies.

Chemotherapy. Multidrug therapy and the development of drug resistance pose particular problems for the treatment of *M. leprae* and *M. tuberculosis* (J. K. Seydel, Borstel). Certain combinations, such as clofazimine and dapsone, act antagonistically *in vitro* as well as *in vivo*. On the other hand, combinations of rifampin and thiacetasone are highly synergistic and also active in partially resistant strains of *M. tuberculosis* and *M. avium*. A new, highly effective inhibitor (K130) of *M. leprae* has been developed using modern computer-assisted methods of drug design. This compound is presently undergoing chronic toxicity studies.

Pathology and clinics. The clinicopathological spectrum of leprosy, ranging from an insignificant dermal lesion of indeterminate leprosy to a disseminated form in lepromatous leprosy, was described by G. Ramu (Sakkottai). He referred to the classical work of Dharmendra and Chatterjee showing that a negative Mitsuda reaction is a good indicator of host susceptibility to the lepromatous form of the disease. Although many attempts have been made to develop an early diagnosis of leprosy, none has been found to be satisfactory in the field. Nerve involvement as a prominent feature in all forms of leprosy was discussed by H. Srinivasan (Agra). Nerve damage may exhibit three modes of clinical presentation: insidious, episodic, or catastrophic. Although infection of Schwann cells is the first step, progression in nerve damage is probably governed by immunological reactions that are poorly understood. A. Mukherjee (New Delhi) discussed evidence that the route of bacillary invasion of nerve tissue is through the epineural blood vessels. *M. leprae* seems to have a particular affinity to Schwann cells in its natural environment. In addition, *M. leprae* also seems to have a special predilection for vascular and endothelial cells.

The early interaction between nerve cells and the immune system in leprosy are studied by R. Mukherjee (New Delhi) in a primate model. Antineural antibodies were found in 95% of leprosy cases but not in tuberculosis cases assessed in an ELISA system using human-nerve sonicate as antigen. Antineural antibodies were detectable quite early after *M. leprae* injection in rhesus monkeys, even before the appearance of overt signs of disease. Thus, the system may be useful for the early immunodiagnosis of leprosy.

Diagnosis. The availability of purified *M. leprae* antigens and of monoclonal antibodies of well-defined specificity has restimulated attempts to develop test systems for the early diagnosis of leprosy. V. P. Bharadwaj (Agra) discussed currently used serological techniques in the epidemiology of leprosy, ranging from conventional methods to modern techniques including the fluorescent leprosy antibody absorption tests and ELISA. R. Paranjpe (Madras) reviewed the need for diagnosis of the various forms of tuberculosis, evaluated the antibody- and antigen-based immunodiagnostic assays, and described the reactivity of monoclonal antibodies generated at the Tuberculosis Research Centre, Madras, India. G. V. Kadival (Bombay) showed the usefulness of

radioimmunoassays for antigen detection in sputum samples in pulmonary tuberculosis and in samples from the cerebrospinal fluid. A monoclonal antibody against a 38-kDa antigen was found to be particularly interesting. U. Sengupta (Agra) discussed the utility of several *M. leprae* antigens and monoclonal antibodies, including phenolic glycolipid and monoclonal antibodies against the lipoarabinomannan. None of the serological assays tested so far was found sufficiently efficient in diagnosing the early forms of leprosy.

Genetics. Predisposing genetic factors in leprosy and the possible genetic interrelationship between tuberculoid leprosy and pulmonary tuberculosis were discussed by N. K. Mehra (New Delhi). In family studies, an association of HLA-DR2 in TT patients and a nonrandom segregation of HLA haplotypes in families were observed. Apart from HLA genes, other genes seem to be involved in determining susceptibility to mycobacterial infections. A study performed in the South Indian population and concerned with HLA associations in tuberculosis patients was discussed by R. M. Pitchappan (Madurai). Individuals of HLA A10, B5, B8 and DR3 seem to represent high-risk groups for pulmonary tuberculosis.

Vaccination. Vaccination trials with the ICRC bacillus were described by M. G. Deo (Bombay). The ICRC bacillus is a leprosy-derived mycobacterium which is probably a member of the *M. avium/intracellulare* complex and shows extensive crossreactivity with *M. leprae.* Vaccination with the ICRC bacilli induces lepromin conversion in a majority of lepromatous patients and in 95% of healthy subjects. The ICRC vaccine is currently being tested in a phase III prophylaxis trial on healthy household contacts of leprosy patients. So far, 30,000 contacts have been vaccinated and will be followed for the next 10 years. Patients clinically nonresponsive to multidrug therapy, after ICRC vaccination showed a progressive drop in the bacillary index as well as clinical improvement.

A second vaccine against leprosy, *Mycobacterium w* (Mw), was introduced by G. P. Talwar (New Delhi). Mw also shares many antigens with *M. tuberculosis* and *M. leprae.* Immunotherapy with killed Mw brought about significant improvement in clinical features of 300 multibacillary cases 6 to 12 months after entry into the trials. Bacillary clearance was faster in the group who were drug-treated and vaccinated as compared to controls who were only on drug treatment.

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