XIII INTERNATIONAL LEPROSY CONGRESS REPORTS OF THE WORKSHOP COMMITTEES

WORKSHOP 1: IMMUNOLOGY

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In the last 5 years, it is clear that advances in basic immunology are rapidly expanding our understanding of the immunology of leprosy. The Workshop arbitrarily divided the field into five general areas which we have attempted to summarize.

Immunogenetics. Additional evidence has accumulated that HLA-genes control the type of leprosy that develops in infected, susceptible individuals. Different HLA-alleles are associated with the respective leprosy types, but susceptibility to leprosy per se is not under HLA control. No association has been found between HLA and erythema nodosum leprosum (ENL). The mechanisms of the HLA influence in leprosy may be via differential binding of processed antigenic peptides by the polymorphic domains of HLA molecules. As a possible example, helper-T-cell clones recognize different epitopes on the *Mycobacterium leprae* 65-kDa protein that are segregated according to the Class II restrictor element used (DR1, 2, 3 and 5, but not DR4, 6, 7 or 8). It is not clear yet how antigen epitope specificity is related to protective or pathological responses. Future studies should include a search for a human counterpart to the murine *Bcg* R/S phenotype/gene and understanding the mechanism of the association between epitope specificity and MHC restriction element specificity and their relationship with protection, immunopathology and vaccine efficacy.

***M. leprae* antigens and molecular biology.** In recent years, seven protein (10, 65, 36, 35, 28, 18 and 12 kDa) and two glycolipids (PGL-I, LAM-B) antigens have been identified from *M. leprae.* Much detail is now available. In general, a large number of *M. leprae*-specific and crossreactive epitopes have been identified, many down to the molecular level. One of the most studied, for example, is the 65-kDa protein. It has now been shown to contain one specific and ten crossreactive T-cell epitopes. Virtually all of these proteins are being expressed from recombinant DNA libraries. The gene (and amino acid) sequences are now complete for the 65-kDa protein as well as the 18-kDa protein and major portions of the 10-kDa protein. In addition to the *M. leprae* antigenic epitopes which have been identified by these techniques, there is evidence that many more T-cell epitopes exist on other *M. leprae* proteins. Goals in the study of molecular biology of *M. leprae* continue to be production and identification of *M. leprae* antigens for: a) the development of immunodiagnostic tests (serology and skin tests); b) dissection of the immune response to *M. leprae* (e.g., identify antigens impor-

tant in protective cell-mediated immunity and hypersensitivity, pathologic immune responses, reactions or autoimmunity); c) understanding the structure and function of *M. leprae* which may shed light on how the organism resists killing by the immune system of some individuals; and d) development of a subunit antileprosy vaccine.

Macrophages. Evidence is lacking that failure in macrophage (M) function is the basis for host susceptibility to leprosy. Examples in which activated M successfully cope with *M. leprae* were discussed and contrasted with experimental models where *M. leprae*-infected M became defective in afferent and efferent functions. The anatomical source of M being studied was emphasized. Caution was expressed about solely studying readily obtainable (blood, peritoneal cavity) M. Interest should be focused more on M from the leprosy lesions themselves. Collectively, *in vivo* and *in vitro* mouse studies and clinical trials of local immunotherapy in lepromatous leprosy patients suggest that killing and clearance of *M. leprae* from lepromatous lesions likely depends on the influx of new M into the lesions rather than activation of resident *M. leprae*-burdened M. Future studies should address: a) the importance of antibody in the phagocytosis of *M. leprae* by M; b) clarification of early events in phagocytosis (phagosome acidification, fusion with lysosomes); c) whether *M. leprae* do escape from the phagosome into the cytoplasm; d) kinetics of M traffic into the lepromatous lesion; e) importance of infected M as target cells for cytotoxic T-cell lysis or destruction by new M; and, finally, f) the mechanisms of *M. leprae* entry into nonphagocytic cells should be studied and the importance of these infected host cells in pathogenesis explored.

Cell-mediated immunity (CMI). Lymhocytes can be divided functionally into helper, cytolytic, and suppressor subclasses and by phenotype and genetic (MHC) restriction into CD4+, Class II restricted and CD8+, Class I restricted subgroups. Generally CD4+ T cells are helpers and CD8+ are cytotoxic. Exogenous antigens preferentially induce CD4+ T cells, while newly synthesized or endogenous antigens induce CD8+ T cells. Killed *M. leprae* should induce CD4+ helper T cells. Intracellular bacteria, including *M. leprae,* can activate CD8+ T cells to lyse antigen-primed M. CD4+ T cells as well as CD8+ T cells may express cytolytic activity that could result in the release of *M. leprae* from host cells of low microbicidal potential (ineffective M, Schwann cells, somatic cells) and thus could function in protection. Reversal reaction-type phenomena occur locally after PPD, interferon-gamma, or IL-2 are infected into the skin lesions of lepromatous leprosy patients. *M. leprae*-specific suppressor-T-cell clones have been described. In tuberculoid leprosy skin lesions, CD8+ cells appear to be cytolytic and in lepromatous leprosy lesions, suppressive. The role of distinct suppressor-T cells in the pathogenesis of unresponsiveness in lepromatous leprosy is not clear. Different T cells both produce and respond to different interleukins. The types, quantities and interactions of these different interleukins may play a role in the development of an individual's type of leprosy. Future studies should continue to explore: a) the mechanisms of ineffective CMI in lepromatous leprosy; b) which immunomechanisms contribute to protection and which to disease; c) the traffic of mononuclear cells into leprosy lesions; and d) the characteristics of the cellular infiltrate in leprosy lesions including (1) functional studies on cells isolated from these lesions, (2) studies using CD4+ cell markers of maturity and antigen exposure (CD45R and CD45), and (3) studies using CD8+ cell markers for cytolytic capability (CD28).

Serology. Over the past 5 years, four types of antigens have been evaluated in leprosy serology: a) PGL-I; b) *M. leprae*-specific epitope monoclonal antibody inhibition assays; c) antibody assays to synthetic peptides of specific and crossreactive epitopes on *M. leprae* proteins; d) the crossreactive LAM-B of *M. leprae.* Assays utilizing PGL-I and its synthetic analogs have had the most widespread application. With these assays virtually 100% of lepromatous leprosy patients but only approximately 30% of paucibacillary (PB) patients are positive. Antibody levels are positively correlated with the bacterial index (BI) in untreated multibacillary (MB) patients and fall (together with BI) in treated MB patients. Anti-

LAM-B and monoclonal antibody inhibition assays fall more sharply. Antibody assays are not helpful in predicting reactions. Several prospective studies of contacts of MB patients have identified an increased relative risk of developing clinical leprosy in seropositive individuals. Future studies should include: a) further exploration of synthetic peptides; b) refinement of techniques for monitoring patients on chemotherapy; and c) further evaluation of antigen-detection systems in clinical specimens using both immunological and DNA probe techniques, such as the polymerase chain reaction.

The future. In addition to a number of specific areas requiring attention which have been mentioned, there are several broad recommendations for the next 5 years. Field trials of new potential leprosy vaccines should be based upon additional basic knowledge, together with information from ongoing vaccine trials. Attempts should be made to integrate leprosy research into more general research areas in order to expand the number of scientists and the variety of skills required to develop second-generation vaccine(s). In the shorter term, the immunopathology of leprosy (including a possible role for autoimmunity), the pathogenesis and possible immunomodulation of ENL and reversal reactions and, particularly, neural reactions deserve high priorities.