Report of the Workshop on Recent Advances in the Immunology and Pathogensis of Leprosy

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Given the time limitations of a single "Immunology Workshop," a topic that deserves subdivision into a half dozen or more separate workshops for adequate discussion, our session was divided into 4 topics.

1. Genetics of susceptibility. There are four major areas that have been actively pursued in a search for a genetic predisposition to leprosy per se and to a particular form (TT or LL) of leprosy. Although **HLA-DR** associations have been described for the immunopathology and CMI of the polar forms of leprosy, susceptibility to leprosy seems to be controlled by some non-HLA gene. The possible contribution of the human homolog to **NRAMP1,** a murine gene important in early response to mycobacterial infection, has been examined in several endemic populations with no evidence for control of susceptibility. The binding of Vitamin D to its receptor (**VDR**) can regulate important immune responses, augmenting CMI ,and activating macrophage and a single base polymorphism has been described in TT and LL. A polymorphism in the Toll-like receptor 2 (**TLR**) which binds several mycobacterial ligands, has been reported to be linked to LL disease. In addition, polymorphism on the TNFα promoter region at –308 position (**TNF2**) may be associated to the intensity of the lepromin response and PB disease and could be a highly relevant marker as it links expression of a gene important to local inflammations and host resistance. Total genome scanning for susceptibility genes, without prior knowledge of the structure or function of the gene has revealed a significant linkage on **chromosome 10p13,** an area where the mannose receptor C type 1 for LAM maps. The implications of genetic associations with leprosy were discussed. Such association would help to identify key processes in host resistance and this knowledge could aid in determining therapeutic options, especially of reactions, and the design of a vaccination program.

2. Innate Immunity in Leprosy. Although an enormous amount of attention has been focused on the development of adaptive CMI responses during the course of infection, recent investigation into the mechanisms and modulation of "innate immunity" support speculation that the proceedings ongoing in the "black box" of indeterminate or preclinical leprosy, and perhaps played out on a background of genetic predisposition, might be the key immunoregulatory events that determine the subsequent path of disease into the clinical leprosy spectrum. Dendritic cells (DC), derived from mononuclear cells under the influence of IL-4 and GM-CSF, are very effective presenters of *M. leprae* antigen. Acting at the site of *M. leprae* invasion, DC may be the first cells to encounter the bacilli and likely play a key role in modulating the early innate immune response to *M. leprae.* Depending, in part, on the level of DC maturation, the uptake of bacilli by DC and interaction with components such as *M. leprae* cell membrane or PGL-1 and the subsequent local production of pro- and/or anti-inflammatory cytokines ([CK] IL-2, IL-12, etc. vs IL-4, IL-10, etc.) and the appropriate chemokines could regulate inflammation and manipulate the ensuing course of the adaptive CMI into a TH1 or TH2 response to *M. leprae.* Further investigation of DC function may provide clues to improving leprosy vaccine efficacy and understanding anergy in LL.

3. Microenvironment of the leprosy lesion. The balance between pro- and anti-inflammatory cytokines induced in response to bacterial products *in vivo* seems to be related to the induction and resolution

of inflammation in several diseases as well as in leprosy. Accumulated evidence has suggested the participation of cell mediated immune response both in RR and ENL. TNFα, IFNγ, and IL-12 mRNA's have been detected in the lesions and/or blood of reactional patients when compared to non reactional. Moreover, an identical mRNA cytokine profile has been observed in the lesions of patients during the course of reaction. Follow-up evaluation of cytokine mRNA expression in reactional skin in the dermis and epidermis indicated further that improvement of patients' clinical symptoms following *in vivo* administration of anti-inflammatory treatment (either prednisone, thalidomide, or pentoxifylline) was associated with the decreased expression of TNFα, IFNγ, and IL-12 mRNAs, whereas worsening of patients' clinical conditions correlated to the maintenance/induction of cytokine mRNAs levels, including IL-10 and IL-4. These cytokines might be a contributing factor in the deleterious effects classically attributed to TNFα. Thus, monitoring of cytokine mRNA expression *in situ* can allow early indication of occurrence and evolution of reactional inflammation in leprosy. In addition to TNFα, several other mediators are most likely involved in the induction of tissue damage. The remodeling of extracellular matrix components requires the action of proteases, among which are matrix metalloproteinases (MMPs), zinc-binding proteins that appear to be stimulated by cytokines, such as IL-1 and TNFα. Such proteases have long been implicated as potential contributory factors in the pathogenesis of inflammatory skin disorders. In leprosy, enhanced mRNA expression of both MMP-2 and MMP-9 (gelatinase proteins) have been detected only in the reactional leprosy tissue. Additionally, investigation of perforin mRNA carried out in biopsies showed expression of message in 50% of RR, 100% of ENL, but not in unreactional patients. The data suggest that cytotoxic molecules and MMPs may well be participating in the generation of the tissue damage detected during the reactions.

4. Nerves and Reactions. The study of mechanisms of neuritis in leprosy and the roles Schwann cells (SC) may play in this were reviewed. SC are clearly a favorable site for maintenance of *M. leprae*, but *in vivo* they are not readily accessible to these (or any) bacilli, and some recent studies have suggested that *M. leprae* may initially enter nerves through the endothelium of endoneurial blood vessels. Several laboratories have now demonstrated that a number of SC surface molecules including laminin and fibronectin may bind *M. leprae* ligands including a fibronectin-binding protein, PGL-1, and a histone-like protein. Current evidence suggests that binding to SC cells by all of these ligands may not be specific to *M. leprae*, since other mycobacteria possess some of these ligands and binding of these organisms to SC has also been demonstrated. SC are able to express MHC class II molecules and can present *M. leprae* antigens, and may thus be victims of specific or cross-reactive immunologic injury. A number of studies have shown a role for cytotoxicity of a variety of T cell subsets on *M. leprae* infected target cells including SC. Several metabolic effects on SC have been described, but in most of these investigators were not able to verify the viability of the *M. leprae* in their models. Recent work with *highly viable M. leprae* freshly obtained from nude mice (foot pads) indicates that when SC are cultured with *M. leprae* at 33°C instead of 37°C, the bacilli are able to maintain viability for appreciable time, that some morphologic changes occur in infected SC, and that transcription of several genes is affected by this infection, but that the SC appear to remain myelinated and capable of normal interaction with axons *in vitro*. With increased emphasis on studies of nerve injury in leprosy, it is expected that SC will remain a focus of research, and these new data provide better models, more standardized systems for study, and promising avenues of investigation.

Immunocytochemical evaluation of CK production in nerve biopsies from patients with acute neuritis or reversal reactions complement other work showing that patients in reactions produce abundant CK but provided no evidence of a predominant TH1 or TH2 response. These findings thus failed to support the hypothesis that TH1 CKs predominate in reversal reactions and highlight the importance of defining the broad CK profile in leprosy reactions. This

will be especially important if drugs targeting specific CK are to be developed for leprosy neuritis or reactions.

Recommendations for future ILC workshops. Holding the workshops (WS) before the opening session was an improvement over the Beijing Congress, where WS were scheduled during the congress and often opposite competing oral and poster sessions. It is imperative that in the future WS organization must begin well in advance of the congress to allow invited participants to adjust their travel arrangements to attend a pre-meeting WS. Clearly, the topic of the "Immunology of Leprosy" cannot be handled in a single workshop.

Workshop participants: *Organizers,* James L. Krahenbuhl & Elizabeth Sampaio. *Speakers,* Warrick Britton, Diana Lockwood, Masahiko Makino, Indira Nath, Geraldo Pereira, Elizabeth Samapio, David Scollard. *Discussants,* Linda Adams, Patrick Brennan, P. K. Das, Howard Engers, Ben Naafs, Cristina Pesolani, Mariane Stefani, Celina Martelli, Euzenir N. Sarno.

Report of the Workshop on Social Science and Leprosy Entitled "Leprosy Stigma and Its Psychosocial Consequences"

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The two-day workshop focused on the nature, processes and consequences of stigma, paying specific attention to enacted and perceived stigma. The first objective was to identify ways in which current knowledge may be implemented in the field. The second objective was to identify gaps in knowledge and priorities for research. Participants were professionally involved in the fields of psychology, social psychology, anthropology, medicine, rehabilitation, nursing care, occupational therapy or social research and included representatives from IDEA and Morhan. Many participants have published work relevant to the discussion of leprosy-related stigma.

Problem statement. Leprosy-related stigma is found in countries world-wide, irrespective of whether leprosy is endemic, eliminated or eradicated. In the context of leprosy, stigma is known to have an adverse impact on efforts to achieve early detection, on treatment compliance and on every aspect of leprosy control.

The efforts of researchers and the energies of programme managers have concentrated on treatment and disease control while in comparison the social aspects of leprosy have been neglected. We now have a state of the art cure for *Mycobacterium leprae*, while stigma continues to permeate society and impact those affected, even to the point of exclusion on the basis of suspicion rather than diagnosis. While physicians make diagnoses and present solutions of MDT, those affected are concerned with the social consequences of the disease, the threat it carries to security, life chances and identity.

A much improved understanding of stigma is essential to all aspects of the care provided for people affected by leprosy. It will direct our provision of counselling and support services. It will allow us to design effective health education programs and materials. It will inform the evaluation of such interventions. It will highlight the need for rehabilitation interventions and provide effective support for advocacy.

The nature of stigma. Stigma is the response to an undesired "differentness," a departure from what is considered "normal" by society. Stigma is recognized in a society's restrictions on an individual in the form of isolation, exclusion, derogatory labelling, devaluation and many other forms of prejudice. It may be physically oriented or a response to blemishes of character or to race. It may result from ideas of culpability or from fears of contamination. Primarily stigma is a cultural phenomenon, an outworking of a society's world view, something that is learned. Everyone is capable of displaying stigma.