WORKSHOP 2: IMMUNOLOGY

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Toward the year 2000

Throughout history, there has never been an infectious disease that has been eradicated solely by chemotherapy, and it is unlikely that leprosy will be the first. Perhaps the greatest contribution of research in the immunology of leprosy has been its role at the cutting edge of basic immunology. Understanding protective immunity and immunopathology in leprosy has ramifications that stretch into the future, far beyond leprosy itself. It is essential to maintain the current research momentum to understand host-pathogen interactions in leprosy in order to develop the diagnostic tools and new immune-based therapies that are still needed in the treatment of disease. By the year 2000, many individuals in the world will still be infected with *Mycobacterium leprae,* and clinical disease will continue to emerge far beyond the turn of the century.

Immunology of the disease process

The aims of the Workshop were to discuss current research on the immunology of leprosy, to prepare a summary of research progress in the past 5 years, and to indicate areas that will continue to dominate future research directions.

Tuberculoid leprosy patients show strong T-cell-mediated immunity to *M. leprae,* and this is also seen in healthy contacts. Lepromatous leprosy patients have extremely high bacillary loads and widely disseminated lesions, with a striking absence of specific T-cell-mediated immunity to *M. leprae.* While T-cell-mediated immunity limits multiplication and dissemination of bacilli in tuberculoid leprosy, it does not provide protective immunity in patients and often leads to severe tissue damage. Immunology research seeks to understand the basis of protective immunity and those immune responses involved in tissue pathology, such as that seen in nerve damage.

Infection and disease

To establish infection in the host, *M. leprae* must be taken up by host macrophages, survive, and multiply. The development of this relationship between the macrophage and the leprosy bacillus leads ultimately to the clinical spectrum of disease.

In the past 5 years, it has become clear that the successful intracellular multiplication of *M. leprae* leads to a loss of normal macrophage function, impairing the process of antigen presentation and the subsequent activation of T cells. Within the last 2 years, methods have been developed to identify mycobacterial genes whose expression is induced in the microenvironment of the macrophage. Such gene products may be important in modulating macrophage function, such as intracellular pathways that are designed to inhibit bacterial multiplication. These studies should be extended to determine the genes of *M. leprae* that are induced in Schwann cells since these may differ from those expressed in macrophages.

Lipoarabinomannans (LAM) purified from *M. leprae* (Lep-LAM) are potent macrophage regulatory factors. Characterizations of LAM from other mycobacteria have

revealed unique chemical modifications in mannose capping that appear to correlate with function. For example, Ara-LAM from *M. tuberculosis* H37Ra, which is a rapid-growing avirulent strain, lacks mannose capping and leads to the induction of levels of the cytokine, tumor necrosis factor (TNF) which are 100–1000-fold greater than that induced by Man-LAM from virulent *M. tuberculosis* H37Rv. Since the induction of TNF may be central in resistance, Man-LAM may be a tuberculosis virulent factor, and may be important also as a leprosy virulence factor. All LAM species inhibit the production of interferon-gamma (IFN-γ). In murine macrophages, IFN-γ induces the intracellular synthesis of nitric oxides which directly inhibit mycobacterial multiplication. Human macrophages are more difficult to understand. The pathway leading to the arginine-dependent induction of nitric oxides has not been identified in human macrophages. However, treatment of lesions in lepromatous leprosy patients with IFN-γ appears to lead to the elimination of bacilli. The mechanism underlying this response is unknown.

A clear priority is to determine how human macrophages respond to infection with *M. leprae*. It is also becoming important to identify the differences in tissue-specific populations of macrophages, and also the kinetics of macrophage turnover.

Protein antigens of *M. leprae*

In the last 5 years the focus of work has been the definition of the immune repertoire of the host and the identification of specific antigens of *M. leprae*. Some 15–16 different protein antigens have now been identified by antibody reactivity. T-cell responses have been characterized to less than half of these proteins, and there are few studies reporting direct comparisons, making direct evaluations difficult. In general, individual patients are capable of responding to a broad range of these antigens. There is extensive crossreactivity of T-cell responses to individual proteins between different species of mycobacteria. No single *M. leprae*-specific protein antigen has been defined although a small number of species-specific T-cell epitopes have been recognized. As yet, few proteins have been capable of stimulating protective immunity against *M. leprae* infection in mice. Although certain cell-wall fractions and the 35-kDa and 10-kDa proteins may be capable of eliciting partial protection, other events induced at the time of exposure to these antigens may be more important in developing protective immunity.

There are now new methods which can be used to identify protein antigens expressed specifically within macrophages and secreted by mycobacteria. Since the *M. leprae* genome will now be completely sequenced, novel techniques are required to make use of the new genetic information that will become available. These may include the definition of T- and B-cell determinants based on sequence motifs. Once isolated, new proteins must be rigorously purified to avoid contaminants which may confound immunological studies.

As yet, no immune responses to defined antigens of *M. leprae* have been associated with the different patterns of immunopathology seen in clinical disease. This may indicate that quantitative rather than qualitative differences in immune responses are important.

Regulation of immunity

In the past 5 years, emphasis has moved from the concept that there may be specific immunodominant proteins of *M. leprae* that are central to protective immunity, to the realization that the type of response of effector T cells to immunization or infection is likely to be more important for protection. The concept of suppression has become more firmly established but the mechanisms involved and the relationship to lepromatous leprosy remain to be determined. The complexity of the cell types that respond to infection or immunization has increased. These now include natural killer (NK) cells, T cells that express alpha/beta and gamma/delta T-cell receptors, and cytotoxic cells in both CD4+ and CD8+ subpopulations. Nonetheless, their role in protection and immunopathology is still unclear. The concept of T_H1-like and T_H2-like cells in both CD4+ and CD8+ T cell subpopulations in humans is widely accepted.

Current work is aimed at defining how different types of effector T cells are regulated and determining which cytokines are

the primary mediators of immune responses. There is a need to understand the complexity of the cytokine network and how it dictates the outcome of an immune response. The mouse is now used extensively as a model for the genetic deletion of pathways involved in specific immune reactions and these studies are providing new insight into the regulation of the immune system. Much can now be learned from studying the immunology of tuberculosis and other mycobacterial infections in parallel with leprosy.

Diagnostic assays

Serodiagnostic assays using *M. leprae*-specific molecules, such as PGL (NT-P/ND-O-BSA), the 35-kDa and the 36-kDa proteins, and LAM, have been carried out in the past to search for antibodies against these molecules. These studies clearly reveal that not all established cases of leprosy are detectable using these assays. FLA-ABS tests detect a proportion of subjects with subclinical stages of disease. Recent findings using recombinant proteins have detected far less numbers of leprosy cases as compared to the natural proteins. The findings of antibodies against the crossreactive 29-kDa/33-kDa antigens in leprosy sera show promise. However, their utility in the detection of early cases of leprosy requires further evaluation. Assays for detecting *M. leprae* antigens in leprosy sera have proved to be far less satisfactory compared to the antibody-based assays. It has been pointed out that it would be worthwhile to develop antibody based assays from slit-skin smears of early lesions of leprosy as a means of increasing the sensitivity of assays.

In reactions, a transient T-cell boost has been observed. Circulating immune complexes (CICs) and antigens like PGL, and 65-kDa antigen in the CICs have been demonstrated in a certain number of reactional cases. There has been no association of HLA-DR antigens with any of the types of these reactions. Antibodies to LSR2 peptides as well as TNF levels have been suggested as new predictors of ENL and require evaluation.

MSLA (Rees Ag/Convit Ag) and lepromin remain as DTH evoking antigens. The search for new, low molecular weight proteins like the 12-kDa and 10-kDa antigens for use as DTH-inducing antigens should be continued, and their further evaluation is necessary to prove that these are better evaluators of *M. leprae*-DTH than the existing antigens.

Combinations of *M. leprae* and BCG, and other mycobacterial species such as ICRC and *Mycobacterium w*, have been used as immunotherapeutic agents and have been taken to vaccine trials. The first survey using combinations of *M. leprae* and BCG in protection showed marginal benefits. *M. habana* has been taken as another agent for future vaccine trial.

Research priorities

Our research priorities build upon the considerable research achievements of the past 5 years and are divided into basic and applied categories:

Basic immunology.

1. Determining how human macrophages respond to infection by *M. leprae.*
2. Defining the cells and mediators that regulate the induction and suppression of effector T-cell and antibody responses in immune responses.
3. Continuing the search for new antigens of *M. leprae* and coordinating the comparative immunological analyses of standardized recombinant antigen preparations.
4. Using gene knockout technology to investigate cellular, intracellular and cytokine pathways that combine to provide protective immunity to mycobacterial infections in experimental models.
5. Initiating approaches to the investigation of the immunological basis of nerve damage.

Application of basic research.

1. Develop a superior DTH-evoking antigen which specifically detects *M. leprae* sensitization, and design *in vitro* correlates.
2. Continue to search for the various immunological tests that detect preclinical leprosy, diagnose early leprosy, monitor therapy, and detect relapse cases.
3. Emphasize the need for immunological markers for prediction of nerve damage, and for type 1 and type 2 reactions.
4. Work with microbiologists to find ways of determining the infectivity of *M. leprae* in the population.

Concluding comments

As an infectious disease, leprosy has plagued mankind for centuries. The coordinated efforts of many dedicated individuals from all walks of life have had a dramatic effect upon reducing the prevalence and incidence of disease in the world. The difficulties that result from the length of time it takes to develop clinical disease following infection and the lack of sensitive tests that detect preclinical and early stages of disease impose severe constraints on efforts to eradicate the disease. The slogan "eradication of leprosy by 2000 AD" is now beginning to place undue pressure on health and research workers alike. While health workers may begin to place less emphasis on the early signs of leprosy, to reduce patient lists, research workers are beginning to see the reluctance of funding bodies to continue supporting their activities since leprosy research is no longer a priority. At a time when diseases such as malaria, tuberculosis and AIDS are spreading, the dictum should revert to "control of leprosy by 2000 AD" rather than eradication. Immunology remains a very real force in the improvement of health for all, and its contribution to leprosy will be substantial.