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Editorial

THE POTENTIAL ROLE OF DNA TECHNOLOGY IN LEPROSY

Although *Mycobacterium leprae* was the first human pathogenic bacterium to be identified, now more than a century ago, like the latest human pathogen discovered, the AIDS virus, *M. leprae* poses quite immense modern scientific problems in the quest for a protective vaccine. *M. leprae* has yet to be successfully maintained in culture. For decades this has affected research in leprosy, but today the nine-banded armadillo is being used successfully as an animal host to grow large numbers of *M. leprae* organisms for study, and the new techniques of recombinant DNA molecular biology are beginning to provide an alternative source of the protein components of *M. leprae.* There are still, however, no suitable *in vivo* models in which the disease process seen in humans can be studied. This makes leprosy research unique, as difficult screening procedures must be employed to find new pharmaceuticals that kill *M. leprae*, and there remains no approach to the direct testing of bacterial products that might provide a protective vaccine against infection in humans. As a result of these difficulties, scientists have tended to concentrate their efforts in other fields of endeavour where more rapid research progress tends to be desirable for career development.

In the final analysis, leprosy like other infectious diseases, is a problem that the immune system of an individual must deal with. While no field of biological science has grown as rapidly and extensively as that of immunology in the last several decades, very fundamental issues of understanding remain. The infectious diseases of many areas of the world exist not only for economic reasons, but because protective vaccines against them have yet to be devised. The parts of infectious pathogens which are able to elicit immune responses (i.e. the antigens) must first be discovered but it is the small molecular regions within these antigens, termed epitopes, which lead to the activation of thymus-derived (T) and bone marrow-derived (B) lymphocytes responsible for cell-mediated and humoral immunity, respectively. These epitopes are the molecular structures which must be identified on pathogens and then harnessed for vaccine development.

The purpose of this editorial is to comment briefly on new research horizons that relate to the application of immunology and DNA technology to old

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problems in leprosy. In short, recombinant DNA work is now allowing the molecular definition of epitopes on protein antigens of *M. leprae* that function to stimulate the immune system. This molecular analysis might be regarded as the key to the development of a vaccine by helping to unlock the mysteries of the immunology of leprosy. What is the basis of immunological responsiveness to *M. leprae* antigens? What regulates the class of immune response, namely cell-mediated or humoral, elicited in response to immunization with these antigens? The exploitation of DNA technology must go hand-in-hand with the understanding of how the immune system works if we are to witness the elimination of leprosy as a disease throughout the world.

Leprosy and the immune system

The immunological response of an individual to *M. leprae* is important in the pathogenesis of leprosy. While it is not known why some individuals are susceptible to the development of clinical disease following infection, the immune response to the antigens of *M. leprae* ranges across the spectrum of clinical disease from well-developed cell-mediated immunity in tuberculoid disease, to an apparent lack of cell-mediated immunity in the lepromatous form of the disease. All evidence points to the activation of macrophages and thymus-derived (T) lymphocytes in the tuberculoid disease, leading to the elimination of pathogen. The high bacterial load found in patients with lepromatous disease appears to be correlated with an inability to mount effective cell-mediated responses to *M. leprae*.

It is important to remember that there are different forms of immunity in response to antigens. While cell-mediated immunity involves the activation of macrophages and T lymphocytes by antigen, humoral immunity results from the activation of B cells and the subsequent production of antibodies. Patients with leprosy, whether of tuberculoid or lepromatous form, appear to produce high levels of antibodies in response to *M. leprae* indicating that humoral immunity is not impaired. This raises several important points. First, cell-mediated rather than humoral immunity is required for immunological protection against clinical manifestation of disease. Second, both cell-mediated and humoral immunity appear not to be regulated by similar mechanisms. Any explanation of the lack of cell-mediated immunity in the lepromatous disease must consider why humoral immunity is left intact.[1,2]

Immunobiology of a vaccine

Much has been written of the use of inactivated *M. leprae* organisms as a vaccine, and the logistical problems of vaccinating individuals at risk of developing the

disease. These problems are mainly due to the low incidence of disease in a population and the long time it takes to exhibit clinical disease symptoms.

Surprisingly, little attention today is directed toward the immunobiology of a vaccine. In short, is it possible to obtain protective immunity by immunization with whole inactivated *M. leprae* or purified antigens derived from the bacilli? There are two important aspects to this question. First, is there a genetic difference between individuals who are resistant and those who are susceptible to clinical disease following infection with *M. leprae*? Second, what is the immunological basis of the lack of cell-mediated immunity to antigens of *M. leprae* in lepromatous disease? The answers are of major importance in a search for a protective vaccine. If leprosy sufferers contract the disease because of a genetic defect in the immune repertoire or the recognition processes of T lymphocytes which then prevent these cells from adequately recognizing certain *M. leprae* antigens or mounting an effective response, then vaccination with inactivated organisms may be of little benefit to such individuals. Alternatively, if vaccines elicit humoral, but not cell-mediated immunity, protection is not likely to result.

Bacterial cells possess a large number of chemical structures which can act as epitopes for the immune system. It is a puzzle therefore, that there are people who may be genetically incapable of responding adequately to *M. leprae*, a bacterial cell which possesses many different epitopes. Conceivably, the immune system of a particular individual might not react with one, or several of the epitopes of the pathogen but this might not matter if there remain others that can be recognized. Yet lepromatous leprosy is a rather classic example where patients appear specifically unresponsive to the various antigens of *M. leprae* but exhibit essentially normal cell-mediated immunity to a range of other antigens.[2]

Immunologically, there are a number of ways of viewing this problem. First, to initiate either cell-mediated or humoral immune responses, helper T cells must be activated by specific antigenic determinants of *M. leprae*. The range of epitopes on *M. leprae* seen by helper T cells may be limited, and immune unresponsiveness may result from a defect in the recognition of these select epitopes. However, it must be remembered that in patients with lepromatous leprosy, levels of circulating antibodies that bind *M. leprae* antigens are high. It is not known whether the helper T cells that are required to initiate cell-mediated responses are identical to those that induce antibody synthesis in B cells. If they are identical, cell-mediated immune defects in leprosy sufferers are unlikely to result from a lack of helper T cells. If they are different, then it becomes important for the purpose of constructing a vaccine to identify the epitopes required to initiate cell-mediated and humoral immune responses. An additional complication however, is the puzzling existence of suppressor T cells capable of limiting cell-mediated immune responses to *M. leprae*.[2] What is the mechanism of a suppression which is apparently selective for cell-mediated, but not humoral immunity? Is there a selective suppression of helper activity for T cells but not B cells?

A second consideration is the effect of antigens on the immune response. It is known that the nature of an antigen can influence the type of immune response, ranging from cell-mediated to humoral immunity. Although it is not apparent what determines whether a delayed-type hypersensitivity response or whether IgM, IgG, IgA or IgE antibody synthesis results, these classes of immune responses are not equal in magnitude following immunization. There is quite clearly a spectrum of immunity in responses to different antigens which suggests that there is some degree of mutual exclusiv
results.[3] As a general rule, high cell-mediated immune responses to a given antigen are associated with low humoral antibody responses, and conversely, high antibody responses are associated with low cell-mediated responses. In the case of leprosy, if cell-mediated immunity is required for protection against the development of clinical disease, it is important that a vaccine be directed toward the induction of the appropriate class of protective immune response. Immunization of individuals with inactivated whole *M. leprae* may elicit good humoral rather than cell-mediated immunity and this may tend to preclude the later development of effective cell-mediated immunity. The engineering of protein antigens with known epitopes and numbers of epitopes might be more suitable for controlling the class of immune response required for protection.

Leprosy and recombinant DNA technology

To understand how immune responses to *M. leprae* are initiated, the antigens that are recognized by the T and B cells of the immune system must be identified. It is generally considered that the most common antigens recognized by T cells are proteins, while B cells, or antibodies, readily recognize a greater chemical diversity of antigens including proteins, carbohydrates and lipids. Isolating the antigens of a bacterial cell that are recognized by T cells is a difficult process as the assays required tend to be complex. On the other hand, a range of simple techniques now exist to follow the binding of an antibody to antigen. For this reason, the isolation of the antigens of *M. leprae* has initially employed the use of monoclonal antibodies which have yielded a range of proteins, carbohydrates and lipids.

The protein antigens of *M. leprae* are of particular interest. While T cells and antibodies may see different epitopes or molecular regions on the same antigen, the small number of proteins found by antibody-binding techniques might be expected also to have epitopes which allow recognition by T cells.

Normally, the purification of antigens from *M. leprae* would require their extraction from bacilli—a major biochemical undertaking requiring large numbers of *M. leprae* organisms. However the advent of DNA technology has provided a major advance in the isolation and structural analysis of protein antigens recognised by antibodies. The proteins of *M. leprae* are all encoded by

the DNA of the organism. In a major step forward, Dr Richard Young and his collaborators have taken the DNA of *M. leprae*, broken it into smaller lengths and inserted these pieces of DNA into the DNA of a bacteriophage, producing a recombinant genome.[4] The advantage of this procedure is that the bacteriophage can be grown in another bacterial cell commonly used in the laboratory, *Escherichia coli*, and the production of protein from the small inserted pieces of *M. leprae* DNA can be regulated by the genes of the bacteriophage. Thus, the molecular biologist can manipulate the production of large amounts of *M. leprae* proteins in the laboratory. This, rather than the growth of *M. leprae* in armadillos, is the preferential source of proteins to use in the search for antigens found on *M. leprae*. Monoclonal antibodies can now be readily used to identify clones of *E. coli* growing in the laboratory which harbour the bacteriophage genomes containing DNA inserts originating from *M. leprae*. Once the correct recombinant DNA has been identified, it can be isolated and the nucleotide sequence determined. From an analysis of the genetic code, the amino acid sequence of the antigen can be deduced and the information used to discover the various epitopes that T cells and antibodies recognize.[4]

These spectacular advances now mean that it is feasible for laboratories to dissect the molecular structures on *M. leprae* that are responsible for immune responses and to define the epitopes best suited to stimulating a protective immunity.

Testing vaccines in model systems

The biggest dilemma faced by those whose research is aimed at producing a protective vaccine for leprosy lies in testing potential candidates. It is in this area that the most difficult decisions have yet to be made. It simply seems inappropriate in this decade to vaccinate a population and wait a number of years to see if there has been a statistically significant reduction in cases of disease in that population. Are there alternative approaches that are scientifically useful? The athymic mouse model, or testing immunized T cells by adoptive transfer into athymic mice, for their effects on the growth of *M. leprae* has been suggested as one approach. Another approach is to consider other mycobacterial infections as immunity to mycobacteria in general might be expected to result from common immunological mechanisms.

This latter approach offers testable models. *In vivo* testing of candidates for protective vaccines could then involve mycobacteria which readily infect and cause disease in laboratory animals. While immune response genes that govern T cell and B cell recognition will vary in each individual, as will the structure of the epitopes that elicit immune responses to different mycobacteria, basic immunological mechanisms that provide protection against infection by organisms such as *M. leprae*, *M. tuberculosis* and *M. marinum* might be similar, if not identical. Thus

studying immunity to the antigens of *M. tuberculosis*, for example, in laboratory animals may provide valued information in terms of establishing protection against the development of disease.

It is significant that recombinant DNA libraries containing *M. tuberculosis* DNA have been constructed, and that a range of monoclonal antibodies to the antigens of *M. tuberculosis* exist.[5] It would seem a logical step to apply the techniques of molecular biology to *M. tuberculosis* and isolate those protein antigens that elicit cell-mediated immunity. There may be curious structural overlaps between the antigenic proteins of *M. leprae* and *M. tuberculosis*. It would be practical to immunize laboratory animals with a range of such antigens and study the resulting immune responses together with the effects of infection of animals with viable *M. tuberculosis*. Successful protection against the development of disease must point to the path of a vaccine for leprosy. Ultimately however, the final decisions will rest on the results of field trials on humans, but by using a variety of testable models to expand our understanding of the immune response to mycobacteria, the time taken for the development of a suitable vaccine may be dramatically reduced.

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