Memoranda
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Immunological research in tuberculosis: Memorandum from a WHO meeting*

This Memorandum discusses the application of modern immunological techniques to various problem areas in tuberculosis. The recent isolation of highly purified mycobacterial antigens will have important application in providing specific skin-test reagents for diagnosis, classification, and epidemiological investigations, and agents for use as immunogens and adjuvants. The development of monoclonal antibodies obtained by immunization of susceptible animals with partially purified antigens is considered a most promising approach to the identification and isolation of antigens.

In vitro *studies of the mechanisms of immunity and the effects of immunization in tuberculosis are needed. Several methods have been proposed recently which require further validation, e.g., through correlation of* in vivo *resistance with results* in vitro, *and comparison of different antigens in the* in vitro *tests.*

The specific antigens as well as the in vitro *tests of tuberculosis immunity would be readily applicable in clinical investigations of immunological parameters and the effects of immunization. New serological tests using purified antigens would also be of value in this regard.* In vitro *tests for cell-mediated immunity could be used to study the effect of various BCG vaccines in different populations, in order to investigate the role of genetic and environmental factors in determining the response to immunization. Specific antigens and serological tests should prove useful in the diagnosis of different forms of extrapulmonary disease, especially in children. A test that could distinguish between infection with* Mycobacterium tuberculosis *and sensitivity induced by BCG immunization or environmental mycobacteria would be very useful both in diagnosis and in epidemiological studies. Investigations are needed on the mechanisms of endogenous reactivation of disease and the breakdown of apparently quiescent disease, in order to try to identify high-risk groups.*

Tuberculosis is a major problem throughout the world. The prevalence is estimated to be 15–20 million cases of active tuberculosis, with an annual incidence of about 10 million and at least 3 million deaths each year.

The magnitude of the problem, coupled with uncertainty as to the efficacy of current control measures, especially BCG immunization, underlines the need to expand research on tuberculosis and to apply newly developed immunological and biological techniques. BCG immunization has long been considered an important measure for the control of tuberculosis, but the contradictory results of various trials of BCG, including the recent study in Chingleput, India, where it was apparently ineffective, have caused concern. Various hypotheses have been offered to explain these differences, some of which serve as the basis for the proposals in this Memorandum.

Despite the global importance of tuberculosis, in both developed and developing countries, the

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host-parasite relationship in the disease is still poorly understood. In particular, there is little information on possible mechanisms of killing or inhibition of growth of *Mycobacterium tuberculosis*. Furthermore, it is not clear what causes subclinical infection in some individuals to change into overt disease, although the complexity of lymphocyte-macrophage interactions in cell-mediated immunity is now becoming sufficiently well understood for application to the study of the disease process. It is not yet known how, and to what extent, the immune response to mycobacterial antigens is regulated; there are gaps in our knowledge of the chemistry and functional role of antigens, as regards not only *M. tuberculosis* but also BCG and other mycobacteria. These aspects of the infection must be better understood before an improved therapeutic strategy can be evolved.

In view of the defects in our knowledge, this Memorandum outlines avenues of research that might yield information leading to greater effectiveness of BCG immunization and, in the long term, to other more effective forms of prophylaxis. Measures are needed to protect against the change from subclinical to overt disease. It is hoped that the research proposed will lead to improvements in diagnosis and case-finding, and, possibly, to the development of effective chemoprophylaxis and treatment, which would, in turn, lead to control of the disease.

Problems are considered in three broad areas: (1) the isolation of mycobacterial antigens; (2) the use of *in vitro* methods to investigate immune responses to mycobacteria and host-parasite relationships; and (3) the clinical immunology of tuberculosis and BCG immunization.

ISOLATION AND PURIFICATION OF MYCOBACTERIAL ANTIGENS

Recently developed techniques for the purification of antigens have permitted the isolation of antigens and antibodies that characterize closely related micro-organisms. Thus it has become possible to identify infecting organisms and to produce vaccines with much greater precision than before.

Purified mycobacterial antigens have several important potential applications:

(*a*) as specific and sensitive skin-test reagents for diagnosis, classification, and epidemiological investigations;

(*b*) as reagents for serological investigations and tests of cell-mediated immunity *in vitro*;

(*c*) as immunogens, to boost immunity in later life, and perhaps in the long term and with suitable modification, as primary immunizing agents;

(*d*) as adjuvants;

(*e*) in studies of pathogenesis and of genetic factors influencing immune responses.

In order to facilitate progress in the application of the isolated antigens and to avoid duplication, it is essential to exchange information and to coordinate research in the various laboratories. To this end, a large single batch of hyperimmune serum and antigenic extracts of mycobacteria should be prepared and each demonstrable precipitinogen should be identified by a standard crossed immunoelectrophoretic procedure. Efforts should also be made to identify non-precipitable antigens, e.g., glycolipids and peptidoglycolipids. These reagents could then be used for the identification of mycobacteria and this work could be carried out by a designated laboratory service; alternatively, the reagents could be made available to individual investigators. In this context, it is most important to be able to identify *M. tuberculosis*, but other clinically relevant microbial species should also be included in the service.

The development of monoclonal antibodies, obtained by immunization of susceptible animals with partially purified antigens, provides a new approach to the identification and isolation of antigens. Furthermore, monoclonal antibodies can be coupled to insoluble substrates and used in the preparation of highly purified antigens. The use of current sophisticated physicochemical methods for the resolution of antigens should continue. Antigens to be injected into human subjects should be prepared so as to exclude contaminating proteins and other agents.

In preparing antigens, emphasis should be placed on the use of cell extracts from young actively growing cultures and on isolation procedures that minimize denaturation.

To facilitate standardization and the exchange of information, the following steps should be taken:

(*a*) a register should be made of all investigators and laboratories engaged in the preparation of antigens and antibodies;

(*b*) a laboratory workshop should be held to exchange and compare reagents—both antigens and antibodies—and to standardize terminology;

(*c*) a system should be instituted whereby reference preparations of antigens and antibodies are made available to qualified investigators.

Since antigens can be obtained in large amounts from microbial cultures, the use of recombinant DNA technology for antigen production appears to be unnecessary at present.

There is a need to expand research on the isolation of antigens, their specificity, laboratory evaluation of their clinical applicability and usefulness, their structural and functional characterization, and synthesis of clinically relevant epitopes and immunogens.

IN VITRO INVESTIGATIONS OF IMMUNITY, PATHOGENESIS, AND HOST-PARASITE RELATIONSHIPS

The control of tuberculosis by immunization will be greatly facilitated by an understanding of the mechanisms and regulation of antituberculosis immunity and by monitoring the effects of immunization on defined parameters of immunity *in vitro*. Such studies with material from human subjects, combined with results from experimental animals, should allow a more rational approach to the use of existing vaccines and the development of new ones.

In vitro studies of mechanisms of immunity should include the following:

(*a*) natural resistance: the occurrence and mechanism of restriction of mycobacterial growth by normal mononuclear phagocytes; the role of serum or plasma factors in modifying phagocytosis and the intracellular fate of mycobacteria; possible roles of other cells such as natural killer cells; analysis of differences among groups of people of different age, sex, ethnic background, etc.;

(*b*) acquired resistance: the nature of the lymphocytes that react with mycobacterial antigens; the nature of the lymphokines that influence macrophage accumulation and activation and bacterial growth; the nature of activation processes in macrophages; mechanisms of killing or growth inhibition of mycobacteria by macrophages; possible role of other cells; the influence of serum, plasma, or other soluble factors on intracellular processes and on extracellular mycobacteria;

(*c*) correlation of resistance *in vivo* with results of tests *in vitro*, including assays of potentially relevant lymphokines such as growth inhibition factor, macrophage activation and migration inhibition factors, and procoagulant inducing factor;

(*d*) comparison of different purified antigens *in vitro*;

(*e*) the search for and propagation of cell lines that produce relevant lymphokines.

Studies on the regulation of immunity should include the following:

(*a*) identification of cells that suppress responses (e.g., lymphocyte transformation and lymphokine production) to mycobacterial antigens *in vitro*;

(*b*) identification of antigens or preparations that selectively activate suppressor and other immunoregulatory cells *in vitro*;

(*c*) identification of soluble factors, such as serum or plasma factors, inflammatory mediators, cell products, and other specific or non-specific suppressor molecules that influence cellular effector mechanisms;

(*d*) identification of mechanisms of antigen presentation.

These tests will be especially valuable in investigating important details of human responses to immunization that are impossible to study in conventional field trials.

They will also be useful for studying the pathogenesis of tuberculosis, for instance:

(*a*) in correlating mycobacterial virulence *in vivo* and *in vitro*;

(*b*) in identifying and studying macrophage and lymphocyte products affecting pathogenesis, such as mycobacterial growth-enhancing factors;

(*c*) in studying mycobacterial growth in macrophages in the presence of products of lymphocytes stimulated by different purified antigens, in order to identify those that stimulate protective immunity.

The above studies are now possible because, in addition to standard *in vitro* methods for studying cell-mediated immunity, new methods and reagents are available, including assays for immunity against the tubercle bacillus, assays for immune suppression induced by mycobacterial antigens, reagents for analysis of subsets of human lymphocytes, specific microbicidal molecules, partially purified human lymphokines, and continuous cell lines of macrophages. The anticipated availability of purified mycobacterial antigens makes exploration of these *in vitro* systems both relevant and compelling.

CLINICAL IMMUNOLOGY OF TUBERCULOSIS AND BCG IMMUNIZATION[a]

Studies are needed to investigate the efficacy of immunization against tuberculosis, to improve diagnostic procedures, and to explore changes in immunological parameters during the course of the disease. Such studies, using modern immunological and histochemical methods, are likely to yield important new information. Among the new techniques that should be of particular value are:

(*a*) the use of skin-test reagents containing pure species-specific antigens obtained by physicochemical methods or by means of monoclonal antibodies;

(*b*) new serological tests, perhaps using newly purified antigens;

(*c*) *in vitro* tests of cell-mediated immunity that can

[a] For any research involving human subjects it is most important that experiments are planned and conducted in accordance with ethical principles, including the provisions of the Declaration of Helsinki (as revised by the 29th World Medical Assembly, Tokyo, Japan, 1975) and the additional requirements, if any, of the country in which the work is carried out.

differentiate between the effects of different cell populations or that give results that correlate with protective immunity.

Studies to explore the reasons for the failure of BCG immunization in the Chingleput study and elsewhere and to evaluate the influence of environmental mycobacteria should be given a high priority. In these studies, *in vitro* tests for cell-mediated immunity should be carried out on subjects before and after immunization and might also be done on those who were immunized at birth. A comparison should be made of the responses in subjects in a community where BCG immunization has had little or no protective value and in one where excellent protection has been demonstrated. If an appropriate immigrant population were also included, the effects of genetic and environmental factors might be separated. The role of environmental mycobacteria could be explored using new specific skin-test reagents and specific and broad-range antigens in *in vitro* tests for cell-mediated immunity. Such a study would be an excellent basis for correlating the results of *in vitro* tests with the degree of protection provided by immunization.

Similar studies, with tests before and after immunization, could be used to compare BCG substrains and regimens and to test new immunizing agents. They should be designed to explore the influence of the proportion of dead bacilli within a BCG preparation on its immunizing capacity, the waning of immunity, the effect of revaccination, and the effect of previous infections with atypical mycobacteria. Such studies could be incorporated in present BCG immunization programmes.

Another high priority area is the development of improved methods for diagnosing forms of tuberculosis that are currently difficult to identify, e.g., tuberculous meningitis, miliary tuberculosis, primary complexes in children, bone and joint disease, urinary tract disease, pleurisy, and pericarditis. An efficient serological test and a specific skin test that could distinguish a BCG-induced reaction from one induced by *M. tuberculosis* would be particularly useful. The latter would also be valuable for calculating the risk of infection, a most important epidemiological parameter.

Although some immunological studies have already been carried out, the changes that occur in cell-mediated and humoral immunity during the course of tuberculosis should now be investigated using the new and better methods. Tests should be carried out at different times during the course of the disease, i.e., at diagnosis, during the first months of chemotherapy, and during the follow-up period when bacilli are dormant in the lesions. Little is known about the reasons for endogenous reactivation of disease or the breakdown of apparently quiescent disease following chemotherapy. Studies on both these subjects are advisable with the aim of defining high-risk subjects who might be treated by chemoprophylaxis and immunotherapeutic agents. The successful application of such a programme would greatly improve the control of tuberculosis. Studies on any of these aspects should take into account such factors as age, sex, appropriate genetic markers, nutritional status, associated illness, and socioeconomic status.

It is likely that new immunotherapeutic agents will soon be developed; these might be substances affecting specific cell populations (for instance suppressor T cells) or drugs that modulate the immune response. It will be of great importance to study the use of such agents at the same time as chemotherapy or with the object of killing dormant organisms after chemotherapy or natural infection.

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