1. INTRODUCTION

The Second Joint Meeting of the Scientific Working Groups (SWGs) on the Immunology of Leprosy (IMMLEP) and Immunology of Tuberculosis (IMMTUB) was held in Geneva, Switzerland, on 4-5 June 1984 and was attended by 33 experts from 12 countries and by seven members of the WHO Secretariat.

Welcoming the participants, Dr A.O. Lucas, Director, UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR), pointed out that there were many immunological features in common between leprosy and tuberculosis. He noted that one of the four sections of the meeting arose out of the establishment by IMMLEP of a bank of monoclonal antibodies that recognize Mycobacterium leprae; this was an example of the value of TDR in coordinating individual efforts.

2. SESSION ON MOLECULAR BIOLOGY

Dr B.R. Bloom described experiments to compare DNA from M. leprae with that from other microorganisms. These were undertaken after a published report had suggested that M. leprae DNA was very similar to that from some...
Corynebacteria originally isolated from biopsies from leprosy patients (leprosy-associated corynebacteria (LAC)). The method used was the measurement of binding between radioactive DNA probes and DNA bound to nitrocellulose filters in the so-called clot-blot procedure.

Strains within mycobacterial species could not be distinguished — for example, M. tuberculosis, M. bovis and BCG were not distinguishable. There was about 40% homology between some strains of M. tuberculosis and M. leprae, the most found between M. leprae and any other mycobacterium tested. In comparison, homology was a little higher than that reported for Escherichia coli and Shigella spp. No binding was detected between M. leprae DNA and DNA from LAC.

Dr R. Curtiss III had studied the properties of DNA from M. leprae. His results confirmed the published guanine + cytosine (G+C) content (56-57%), but indicated that the size of the genome measured by COT analysis* was 2.2 x 10^9, somewhat larger than had been reported earlier, although smaller than that of E. coli, M. vaccae and Mycobacterium strain Lufu. Assuming that the larger figure was correct, it was possible that the clone banks of M. leprae DNA already established were not quite complete. Dr Curtiss then described further progress in the cloning of DNA from M. leprae. He had a bank of "cosmids", which are plasmids with "cos" sites (cohesive ends) from lambda phage and inserted foreign DNA; the insertion consisted of ca 40 kb fragments obtained from high-molecular-weight M. leprae DNA. These had been used to infect irradiated E. coli "maxicells"; mycobacterial DNA had been expressed and some new proteins had been synthesized, but none reacted with anti-M. leprae sera. "Expression vectors", however, had allowed complementation of missing enzymes in mutant E. coli and two mycobacterial enzymes had been produced — citrate synthase and dehydroquinate synthase. Other defects had not been repaired, despite the probable presence of the necessary enzymes in M. leprae. In answer to questions, Dr Curtiss outlined the successful method, devised by Dr J. Clark-Curtiss, for obtaining high-molecular-weight DNA from M. leprae.

Dr R.A. Young had attempted to clone DNA from M. tuberculosis and M. leprae, using a vector known to be effective in obtaining expression of DNA from a variety of organisms. No production of mycobacterial proteins had been obtained, as measured with antisera, although the "libraries" of DNA almost certainly (M. tuberculosis), or probably (M. leprae), contained the complete genome. He had obtained "fusion proteins" — E. coli proteins extended by polypeptides specific to mycobacterial DNA fused to the E. coli DNA — which could be detected electrophoretically. His present view was that expression was occurring but that the products were being destroyed.

Dr J.D.A. van Embden described recently started experiments to clone DNA from BCG, using a system developed for Treponema pallidum. He had obtained no expression so far. He commented that both human subjects and rabbits showed a relatively uniform response to T. pallidum and produced a consistent range of antibodies. This was also true of rabbits vaccinated with BCG, but the human response was extremely variable. Such variability could cause difficulty in the use of antisera to detect proteins specified by cloned mycobacterial DNA.

* Product of the DNA concentration and the time of incubation (expressed in moles of nucleotides x sec/litre).
Finally, Dr P. Draper reviewed published methods for the production of spheroplasts from mycobacteria. These readily lysed forms would be a good source of mycobacterial nucleic acids. Most methods involved the use of glycine in the medium to weaken the wall structure, followed by treatment with lysozyme in the presence of sucrose to produce spheroplasts. Conversion rates were reported to be low with M. tuberculosis, but the method could probably be refined. M. leprae could not be grown in media, which was a problem, but, since suspensions of these bacteria had been shown to carry out various metabolic functions, it seemed worthwhile to subject them to treatments likely to weaken the wall.

In discussion, it was pointed out that a difference in G+C content, as exists between M. leprae and other mycobacteria, did not necessarily indicate that they were not related. Three explanations were offered for the failure to detect expression of mycobacterial DNA in the form of antigenically active proteins. First, there was a limited range of antibody specificities in human sera; especially in leprosy sera, the major antigens recognized seemed to be surface components, particularly lipid and carbohydrate. Various "specific" antisera were available and might be tried, and antisera might be prepared by immunization of animals with cell-free extracts in order to eliminate surface-directed antibodies. Second, degradation of "foreign" protein seemed to be universal in living cells; the ability to do so was essential for survival and so could not be eliminated completely by use of, for example, protease-deficient mutants. Third, techniques which use detergents to denature or solubilize proteins destroy conformational antigenic determinants, so that primary-sequence determinants would mainly be detected.

3. WORKSHOP ON MONOCLONAL ANTIBODIES

The monoclonal antibodies in the IMMLEP bank at the Centers for Disease Control, Atlanta, GA, USA, had been tested for specificity by a wide variety of methods. Drs M. Abe, T.M. Buchanan, M. Harboe, J. Ivanyi and A.H.J. Kolk presented their results to the meeting; a written report was received from Dr W. Britton of the Clinical Immunology Centre, University of Sydney, Sydney, New South Wales, Australia.

The collaboration had been an unqualified success. Twenty-two monoclonal antibodies that recognize M. leprae, from seven laboratories, had been tested blind in six laboratories in the remarkably short period of two months. Two of the samples were duplicates, so that 24 samples were tested in all. These could be divided into 11 groups of antibodies according to specificity: five were crossreactive and directed against carbohydrate/lipid moieties (protease-resistant); four were crossreactive and directed against 55-65 kilodalton (kD) proteins (protease-sensitive); three were M. leprae-specific and directed against 55-65 kD proteins; one was M. leprae-specific and directed against a 12 kD protein; one was M. leprae-specific and directed against an 18 kD protein; one was M. leprae-specific and directed against a 36 kD protein; one was M. leprae-specific and directed against a 35 kD protein; one was "sticky" and its use was not practicable.

It was decided for the present to keep 17 of the antibodies and to test certain of them for epitope specificity by cross-competition tests. In addition, the antigens recognized by two of the antibodies would be tested for protease-sensitivity. When these tests were completed, a range of antibodies that react with independent epitopes would be chosen to stock the IMMLEP monoclonal antibody bank. There should be 10 to 15 antibodies, including reagents specific for M. leprae and also those that crossreact with other mycobacteria.
The results of the Workshop are to be tabulated and published as a brief paper,* signed by the participants of the Workshop and the contributors of the monoclonal antibodies. It was decided for the moment not to attempt to devise a system of nomenclature. The method of defining the nature of the antigen recognized by the antibody -- whether lipid, carbohydrate or protein -- has not yet been agreed.

Some problems and pitfalls remained, such as the existence of prozones in radioimmunoassays and in the monoclonal-antibody-based enzyme-linked immunosorbent assay (ELISA). There seemed to be batch-to-batch variation in the antigen preparations of M. leprae when tested with the same monoclonal antibody by ELISA or by radioimmunoassay. Some soluble antigens required special coating conditions (e.g. pH 5, high salt) to bind to the plastic plates used in these types of assay. The immunofluorescence assay probably failed to identify monoclonal antibodies against internal bacterial components.

Potential uses of the reagents included immunodiagnosis, identification of M. leprae-specific proteins produced by genetic engineering techniques and the characterization of those antigenic components of M. leprae that are recognized by T-cells.

Dr J. Roder outlined the advantages of developing human monoclonal antibodies: the range of specificities obtained would be found in human sera; the reagents would be suitable for injection into man; and the B-cells used would be typical of those seen in a "live" infection rather than those produced by immunization with special reagents under artificial conditions. He also outlined the technical advantages of his experimental system. He had obtained clones producing antibodies which recognize M. leprae antigens, apparently including glycolipids. Most of the glycolipid-specific antibodies, however, crossreacted with other mycobacteria.

Dr N. Mohagheghpour, using a similar experimental system, had obtained five human monoclonal antibodies which recognize antigens in cell-free extracts of M. leprae. It was possible that all recognized the same antigen, which was probably a wall antigen, but not the phenolic glycolipid.

It was noteworthy that both Dr Roder and Dr Mohagheghpour found that their human monoclonal antibodies had low affinity for the antigens recognized. This phenomenon was not understood and was not true of mouse monoclonal antibodies which recognize M. leprae antigens.

4. SCREENING OF T-CELL LINES AND CLONES

Dr S. Kaufmann discussed experiments with mouse T-cell clones and T-cell hybridomas. The clones were established from lymph-node cells of mice immunized with irradiated M. leprae in Freund's incomplete adjuvant. In the presence of H-2 I-A-compatible accessory cells, either M. leprae or BCG, but not an unrelated antigen, was able to stimulate the cloned T-cells. This suggested that the cells were of the helper/inducer type and recognized a crossreacting mycobacterial antigen. In vivo, cloned T-cells could induce delayed-type hypersensitivity (DTH) and bactericidal mechanisms when transferred locally together with the homologous antigen. In vitro, after stimulation with antigen, they stimulated secretion of lymphokines, including interleukin 2 (IL2), macrophage-activating factor (MAF) and interferon (IFN).

It seemed that immunization of mice with live BCG might lead to the generation of autoreactive T-cells. Some T-cell hybridomas derived from lymph-node cells of BCG-immunized mice were stimulated by autologous cells alone, while others required both antigen (BCG or PPD) and accessory cells. Interestingly, IL2 secretion by some hybridomas was induced by accessory cells alone, but was further increased by addition of antigen (PPD), indicating that these cells might be both auto- and antigen-reactive.

Dr J. Louis had also obtained T-cell clones from mice immunized with BCG in incomplete Freund's adjuvant. The cells were Thy1+, L3T4+ and Lyt2−, and were stimulated by mycobacterial antigen but not by unrelated antigen. Upon antigenic stimulation, the T-cells produced IFN and MAF. Some clones could provide help to B-cells in a carrier-hapten system and induced DTH reactions after local transfer. At least one clone could provide help but could not confer DTH, indicating heterogeneity among these clones.

In studies with Leishmania-specific T-cell clones, it had been found that T-cells which mediated DTH reactions would actually enhance multiplication of Leishmania in the mouse footpad.

Dr M.J. Colston had obtained T-cell clones from mice immunized intradermally with irradiated M. leprae. The cells showed M. leprae-dependent proliferative responses, but also responded to other mycobacterial species and strains. Unfortunately, production of MAF and IL2 by these cells was stimulated by accessory cells alone, independently of mycobacterial antigen. Different immunization and in vitro culture protocols were now being tested in an attempt to obtain M. leprae-specific T-cell clones. Preliminary data indicated that T-cells from mice chronically infected with M. leprae might respond to soluble M. leprae antigen.

To summarize, the results of Drs Kaufmann, Louis and Colston indicated that monoclonal T-cells and T-cell hybridomas from mice may provide useful tools for the functional characterization of mycobacterial antigens and for the investigation of the role of T-cells in infections with M. leprae and M. tuberculosis.

Dr T. Godal described human, BCG-reactive T-cell clones. Peripheral blood cells from PPD-responsive individuals were cloned in the presence of feeder cells, BCG and IL2. Native peripheral blood cells from some donors reacted with both BCG and unrelated antigens (e.g. tetanus toxoid) but, after cloning, BCG-reactive T-cells were no longer stimulated by such unrelated antigens. Different strains of BCG had the same stimulatory activity. Soluble antigens from BCG and M. tuberculosis stimulated all the clones, although not all reacted with antigens from M. phlei and M. avium. Thus, crossreactivity was also a feature of the human system.

At an early stage of culture in vitro, some T-cell clones were able to amplify BCG-induced T-cell responses, but many were suppressive. On increasing the time of culture, most became suppressive. This suppression also affected Con A-induced responses, indicating that it was nonspecific. The reason for this "drift" in function was unknown; the possible involvement of the feeder cells or of BCG was discussed.

Dr B.R. Bloom proposed the hypothesis that M. leprae has a specific suppression-stimulating component and described studies with peripheral blood cells from lepromatous leprosy patients, which were able to suppress Con A-induced T-cell proliferation. Suppression was specifically induced by the phenolic glycolipid of M. leprae, whereas the expression of suppression was nonspecific. The cells involved were OKT4+ and OKT8−. Because attempts to clone OKT8+ glycolipid-specific T-cells had failed, T-cell hybridomas were established. Ten percent of the hybridoma clones from lepromatous leprosy patients constitutively produced a nonspecific suppressor factor.
Dr Y. Koide had analysed the genetic restriction of human PPD-reactive T-cells. Three monoclonal antibodies specific for distinct Ia molecules were used to try to block PPD-induced proliferation. Only two of the antibodies were able to do this, although all three bound to Ia molecules.

PPD-reactive T-cell clones were then established. Some were restricted by one of two HLA-DR antigens, one required compatibility with both HLA-DR antigens and one appeared to be restricted by non-HLA-DR antigens. Antigenic stimulation of this last clone was blocked by the monoclonal antibody which recognized an epitope distinct from DR and MBl molecules. Furthermore, PPD-induced proliferation of DR-restricted T-cell clones could be inhibited by antibodies to DR molecules.

Dr T.H.M. Ottenhoff had obtained T-cells which reacted with mycobacterial antigens but did not express alloreactivity after short-term culture in vitro. Peripheral blood cells from donors were cultured with PPD for five to seven days and afterwards expanded in medium containing T-cell growth factor. Several PPD-reactive lines were established. Accessory cells sharing two HLA-DR determinants were required for maximal responses; those sharing only one determinant gave moderate responses, but in the absence of DR compatibility only marginal responses were observed. It appeared that the restriction element was closer to the D region than to the DR region. Two new restriction elements, MT2 and LB-Q1, involved in antigen presentation, were described. Several T-cell lines were stimulated when they shared one or other of these loci with the accessory cells, but most required identity at both.

T-cell lines with reactivity to M. leprae were established from borderline tuberculoid (BT) and tuberculoid (TT) leprosy patients. Some of the lines showed crossreactivity with PPD, but some seemed to be specific for M. leprae.

To summarize, studies with human T-cells indicate that their long-term culture may not depend absolutely on the availability of autologous accessory cells. It may be feasible to establish human T-cell clones for functional characterization of the M. leprae antigens involved in the immune response of leprosy patients.

5. RESISTANCE TO, AND PATHOGENESIS OF, EXPERIMENTAL TUBERCULOSIS

Dr F.M. Collins discussed mouse models of tuberculous infection. He stressed that minor variations in experimental technique grossly affected the results obtained and made their application to human tuberculosis difficult. Intravenous BCG protected against intravenous M. tuberculosis, although with increasing time after vaccination there was an increasing delay in the ability of the host to control the growth of the pathogen. Different strains of BCG grew at different rates and to different extents in mice, but all protected well against an aerosol challenge with M. tuberculosis. Immunity could be transferred passively, but only if the recipient mouse was sublethally irradiated or thymectomized. (Such treatment was unnecessary in similar experiments with rats.)

More recent experiments had used subcutaneous BCG and aerosol-delivered M. tuberculosis. Vaccination was successful against two virulent strains of M. tuberculosis but ineffective against M. avium. It appeared that efficacy of the vaccination depended on the growth rate of the pathogen — M. avium grew more slowly in mice than did M. tuberculosis. In the mouse, at least, virulence seemed to be associated with the ability of the pathogen to continue growth in the lungs, even after growth in the liver and spleen had been suppressed by the host.
During discussion, it was made clear that there was little information about the relation between growth rate in macrophages and virulence. In the larger HeLa cells there were known to be major differences in growth rates.

Dr D. Smith showed examples of tests of the relative efficacy of vaccines against tuberculosis, using different experimental systems. There was little agreement between the various models; it seemed possible to obtain any desired result by altering the conditions of the experiment. How should a model applicable to the protection of human subjects against tuberculosis be selected? Attempts to compare laboratory assays with field trials seemed to be invalidated by the results of the South India BCG trial, in which vaccines giving clear protection in animals gave none in man.

Dr Smith proposed a "physiological model", that is, one in which the nature of the vaccination and of the infection were matched as closely as possible to those in man. The essential property of a vaccine against tuberculosis seemed to be its ability to control the bacillaemic phase of the infection, during which M. tuberculosis was able to spread to "privileged sites" (the upper part of the lungs, in man) where multiplication was not readily checked. He considered that a guinea-pig model, with intradermal vaccination and a small aerosol challenge, most closely fitted the requirements.

In discussion, the need for a test of protective immunity was stressed. It was clear that delayed-type hypersensitivity did not correlate well with protection. Unlike the typical animal experiment, vaccination in man was required to be effective over many years. There was no way (apart from observing the incidence of the disease) of telling which individuals were protected.

Closing the meeting, Dr T. Godal thanked the participants for their contributions. There had been exciting progress in the fields reviewed, and he looked forward to continuing developments.

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