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Causative organism and host response

Workshop 1—new tools for diagnosis and epidemiology

Our aim for the workshop was to review the state of the art of new tools for diagnosis and epidemiology studies of leprosy and to assess their potential impact on control programmes should they be implemented. We also identified those tools needing further development and testing prior to evaluating as a tool for leprosy control.

SEROLOGY TO IDENTIFY AT-RISK CONTACTS

After several years of extensive investigation it has become apparent that serology with PGL-1 has been found useful in identifying household contacts at high risk of developing multi-bacillary (MB) disease. Since MB disease is potentially the most significant reservoir of *Mycobacterium leprae* with potential for spreading the infection, workshop participants felt that control programmes should begin to explore this application. A 15-min dipstick assay for PGL-I antibody is now available and could be used in field conditions, for example, in small-scale LEC or SAPEL programmes. Important limitations of this test require that it not be applied as a mass screening tool among community contacts but as a specific test applied to 'close' contacts of MB index cases. This application ensures that the test is applied economically to a small group of contacts most likely to develop disease and who potentially represent a major link in the transmission of *M. leprae* among the community. Preliminary evidence suggests that aggressive antileprosy chemotherapy of PGL-I-positive household contacts can reduce the PGL-I antibody titre, while a single dose of ROM has little or no effect. Thus, aggressive prophylactic therapy of PGL-1 positive contacts has the potential to greatly reduce the force of infection in the community. The next step in this area is to define appropriate treatment interventions for this group of at-risk contacts.

MOLECULAR TEST FOR RIFAMPIN RESISTANCE

Tests for drug susceptibility have long been needed in leprosy. We now have one such test capable of detecting mutations associated with rifampin resistance in *M. leprae*. The test is based on DNA sequences found in the rpoB gene and is being tested as a survey tool in Nepal. This survey will establish the current level of rifampin resistance in the area which can be monitored in the future to determine trends in drug-resistance. Molecular studies to define the site for DDS resistance are underway but have not yet revealed the mechanism(s) of resistance. Should it turn out to be associated with mutations in the folate pathway as suspected, then a molecular test could be developed obviating the need for mouse foot pad testing for drug resistance. Other antibiotic gene targets, such as gyr A and B, are being

investigated as sites for resistance to the fluoroquinolones in anticipation of their use in shortening therapy for leprosy.

T-CELL ANTIGENS AND SKIN TEST REAGENTS

New developments in T-cell studies are allowing T-cell responses to *M. leprae* to be measured in large-scale field studies. These include the development of simple, whole blood culture assays to measure T-cell proliferation or cytokine production in response to antigen. These assays are currently being used in Nepal to test the antigenicity of new skin test reagents, and in Malawi to monitor changes in T-cell immunity induced by BCG vaccination in 700 volunteers. Such assays could be used to measure T-cell responses and their relationship to antibody responses in household contacts.

A new tuberculin-like skin test reagent for leprosy could be used to monitor the prevalence of preclinical infection in the community, to monitor interventions and to focus leprosy control efforts. Two initiatives to develop *M. leprae*-specific skin test reagents are underway. Cell wall and cytosolic antigen fractions have been produced in the first initiative. The fractions are depleted of carbohydrates and lipids and go into phase I testing in late 1998. Phase II and III trials are planned for Nepal. Another WHO initiative is screening synthetic peptides for *M. leprae* in a multicentre study to identify *M. leprae*-specific peptide epitopes and preliminary results have identified some promising candidates. Specificity testing must be met prior to advancing these reagents in the study protocol. It is anticipated that completion of the genome project may give rise to other *M. leprae*-specific proteins useful for testing as potential skin test reagents.

NASAL CARRIAGE OF *M. LEPRAE*

An important area gaining much interest involves defining rates of nasal carriage of *M. leprae* in leprosy endemic communities. PCR for *M. leprae* DNA and monoclonal antibody-directed staining of *M. leprae*-specific antigen have been used successfully for this purpose. Initial results range between 3 and 9% positivity in household contacts of MB and PB index cases. New large-scale studies need to be performed to determine the relationship between transient contamination of the nose, continuous carriage of the bacilli (colonization?) and development of lesions on the nasal mucosa. Results from these types of studies may be pivotal in determining maintenance of an *M. leprae* reservoir in the community and eventually how *M. leprae* is transmitted. Studies to improve the reliability of these types of assays need to be performed. For example, large-scale screening of uninfected individuals needs to be performed to establish realistic levels of false-positive rates using these very sensitive assays.

Participants: T. Gillis, Chairman, P. Brennan (Rapporteur), Sang-Nae Cho, Maria DaGraca S. Cunha, Jim Douglas, Stella Van Beers, Francoise Portaels, Hazel Dockrell, Tranquilino Fajardo, Utpal Sengupta

Workshop 2—chemotherapy

The participants in the workshop agreed to the following:

Because the global prevalence of leprosy has decreased dramatically, treatment delivery

systems have to be adapted to the new reality, and it will be difficult to maintain everywhere the supervision of monthly doses of rifampicin. However because the present anti-leprosy drug regimens (WHO-recommended MDT) are so extremely effective and robust, these regimens should remain the treatment of choice for leprosy in national programmes. The robustness of the regimens and the systematic use of blister-packs enable less reliance on the direct supervision of monthly drug intake by the general health services.

Considering the effectiveness of the 2-year WHO-MDT for MDT leprosy, the changes in definition of PB and MB and the low BIs in the majority of MDT patients, shortening the duration of treatment of MB leprosy to 12 months is justified. Similarly, the use of single dose rifampicin-ofloxacin-minocycline (ROM) for the treatment of single lesion leprosy offers great operational advantage to national programmes. It should be understood that the current WHO recommendations represent minimal guidelines.

Except for the treatment of single lesion leprosy with ROM, use of the new drugs at the present time should be strictly limited to special circumstances, for example proven rifampicin resistance. The development of new drugs and regimens is encouraged and should continue to be a priority in the area of chemotherapy.

Drug resistance is not a problem at the current time and is not expected to increase in the future, even with shortening the duration of treatment of NO cases to 12 months, as long as the drugs are used in appropriate combinations. To replace mouse footpad inoculation, research should continue on molecular methods of detecting drug resistance.

Finally, it is crucially important for the survival of leprosy control programmes that the supply of drugs after the year 2000 be assured.

Participants: Jacques Grosset, Chairman, Scott Franzblau (Rapporteur), Robert Hastings, Paul Roche, D. V. A. Opromolla, Baohong Ji, Lou Levy, Diana Williams

Workshop 3—epidemiology/transmission/vaccines

The workshop attendees addressed the four major topic areas outlined and came to the following consensus opinion.

LEPROSY TODAY—PATTERNS AND TRENDS

Routine 'prevalence' data generated in recent years, in most countries of the world, have been greatly influenced by 'operational' factors (e.g. changes in ascertainment, diagnostic and classification criteria, treatment duration etc.). As such they may not, and often do not, reflect the underlying epidemiological situation, and can only be interpreted in the context of clear explicit information on these underlying factors over the time period covered by the data.

We recommend that all tables, figures and reports which purport to represent leprosy 'prevalence', 'incidence' or 'case detection' patterns or trends be accompanied with clear and explicit captions specifying the operational factors (ascertainment methods, case and classification definitions, treatment durations, etc.) employed during the entire period to which the data refer.

Leprosy frequency and patterns often differ greatly between various segments of populations. This heterogeneity at national, district and local level is not evident in crude summary statistics, which can thus lead to a distorted picture of the actual situation.

Whenever possible, an effort should be made to separate high prevalence populations from other group data, or at least to point out how crude data are effected by their inclusion (e.g. data from Asia, Africa and Latin America are heavily influenced by India, Ethiopia, Madagascar and Brazil, and national data for each of these areas are influenced by other area-specific operational/historical factors.

NEW INSIGHTS INTO THE NATURAL HISTORY OF LEPROSY

Evidence for zoonotic leprosy in armadillos of the southern United States is now overwhelming. It is no longer correct to claim there is 'no extra-human reservoir' of *M. leprae*. The relevance of primates in leprosy's natural history remains anecdotal but deserves more rigorous study including surveys in the wild and studies of human risk associated with primate contact. Any realistic consideration of leprosy eradication must contend with this issue.

Recent PCR-based data on widespread presence of *M. leprae* in nasal cavities of individuals in endemic populations, and in environmental samples are potentially very important for our understanding of the natural history of leprosy. Some of these studies have, or appear to have, been influenced by appreciable numbers of false positives. To ensure credibility, such studies require rigorous controls to demonstrate high specificity of the assay used (preferably inclusion of large numbers of blind coded samples, from non-endemic populations, among the study samples). Presentation of such data by age, sex, contact status and area will enhance interpretability and credibility. Appropriate multivariate analysis should be carried out in order to ensure proper control of confounding factors.

The predominate portals of entry or exit of *M. leprae* are still unclear. Recent studies emphasizing nasal carriage and mucosal immunity reflect interesting hypotheses but are not yet convincing in themselves. If the presence of *M. leprae* in nasal cavities reflects transient carriage (the nose acting as an air filter), the data could also be consistent with skin as a portal of entry. Large, carefully conducted, long term studies will be required to solve this issue.

DOES CHEMOTHERAPY REDUCE TRANSMISSION?

Though it is logical to infer that effective chemotherapy must reduce the risk of infection with *M. leprae*, and consequent incidence of leprosy disease, at least to some extent, it is extremely difficult to demonstrate such an effect convincingly. Leprosy incidence is obviously strongly influenced by environmental or behavioural correlates of socio-economic development. Given that individuals may be infectious for long periods prior to diagnosis and treatment, the effect of even a good treatment programme on the overall leprosy incidence may be small. The issue of MDT's impact on leprosy incidence, though of obvious political importance, may well be beyond the reach of convincing epidemiological evidence.

VACCINES IN LEPROSY

The variability of BCG's efficacy between populations remains unexplained. The fact that BCG's effect in tuberculosis shows analogous variability enhances the importance of this issue for public health impact, and hence for research. The efficacy of BCG appears to decline with time. There are no data on whether BCG has any influence greater than 20 years after administration, either against leprosy or against tuberculosis. Since BCG has been given at

birth in most countries for the past 20–30 years, it is now possible to study the influence of BCG in infancy on adult disease incidence. The evidence from Venezuela, Malawi and Burma that repeated BCG enhances its protective effect against leprosy increases the potential importance of such studies. The ongoing trial of a second dose of BCG among school children in Brazil will provide important data on this very practical intervention.

Research into the immunology of leprosy and into leprosy vaccines should be linked to the major international research effort devoted to tuberculosis. Comparisons between the two infections/diseases will provide useful insights. Leprosy should be included as an outcome in any future trial of a tuberculosis vaccine. The current interest in post-exposure vaccines against tuberculosis could also have implications for potential leprosy interventions either in high risk populations or in therapeutic context.

Participants: Paul Fine (Chairman), R. Truman (Rapporteur), G. Bjune, K. V. Desikan, A. Diallo, V. K. Edward, M. D. Gupte, J. D. Habbema, S. Izumi, C. K. Job, C. M. Martelli, A. Meima, R. G. Reddy

Workshop 4—nerve damage and reactions

1. Nerve damage continues to be a major problem.
2. Nerve damage remains poorly understood.
3. Controlled trials of current and future therapies are urgently needed.

The participants discussed the epidemiology and pathogenesis of neuritis and reactions, and the currently recommended therapies.

Epidemiologically, MB disease and age (15–44 years) appear to be major risk factors for the development of reactions and nerve damage. The group noted the absence of good data relating to the relationship between reactions and endocrine alterations such as pregnancy and adolescence. Data were also presented showing that we may expect 40% of patients to now have their first reactional episodes after completing MDT. This has very important implications for management. Patients will need to be carefully warned about reactions advised to seek care promptly when symptoms develop. It was also noted with concern that neuritis may develop in some patients long after apparent cure.

The group noted success in the use of sensitive tests to evaluate sensory function in many centres. However, it is important that the reliability, diagnostic cut-off, specificity and sensitivity of these tests is carefully considered. Scoring systems derived from these tests should be developed in a logical manner, such as ensuring that scores are recorded for individual nerves. Functional outcome is also an important measure that needs to be considered as well as motor and sensory function. It was also noted that occupation and resultant mechanical nerve stress may have affected outcome.

Nerve injury may occur in three phases:

1. Localization of *M. leprae* to nerve, followed by
2. Active neuritis, and
3. Late nerve damage

Evidence was presented that armadillo nerves may be a useful model for lepromatous nerve involvement. Tuberculoid type nerve damage seems to occur in murine nerves directly injected with *M. leprae*.

Studies from Mumbai indicate that viable *M. leprae* can be recovered from the nerves of patients who have completed MDT. The clinical significance of this finding is not yet known.

The immunological basis of reactions and neuritis was briefly reviewed, and several lines evidence indicate that TNFα may play a key role in these processes. Other cytokines may also have critical roles in reactions. Several previous Congress workshops have discussed the difficulty in distinguishing between a late reaction and relapse in nerve. This remains a clinical and pathologic challenge.

In its consideration of current treatment of reactions, the group expressed concern that there is an absence of data from controlled clinical trials relating to doses of corticosteroids and duration of treatment. There was also concern that the doses and duration of treatment recommended by the WHO 7th Expert Committee (Geneva, June 1997) are too low and too short.

Multicentre trials are currently in progress in India to determine the optional length of treatment with corticosteroids. A randomized control trial of prophylactic corticosteroids to prevent reactions and nerve damage in new MB patients is being done in Bangladesh and Nepal.

The workshop discussed the need to evaluate currently available immunosuppressants as second-line treatment for patients who do not respond to corticosteroids. Multicentre trials are also needed to define the role of neurolysis in the management of acute neuritis. All of the above mentioned multicentre trials are required in order to generate high quality evidence for the best treatment of leprosy patients. Funding such trials should be a high priority.

CONCLUSION

The workshop participants expressed confidence that this combination of careful and appropriate patient evaluation, studies on pathogenesis, and high quality clinical trials will lead to improved care for leprosy patients.

Participants: D. Lockwood (Chair); D. Scollard (Rapporteur), W. Britton, R. Jerskey, R. Barnetson, B. Naafs, Dr Antia, A. Anderson, S. Suneeta, P. Saunderson, Dr Shetty, E. Sarno, E. Sampaio

Workshop 5—pathogenesis and lessons from leprosy

Scientifically, the opportunities for studying pathogenesis in leprosy could not be more timely. The availability of the complete sequence of the M tuberculosis genome and the considerable inroads that have been made in to sequencing the *M. leprae* genome, mean that we will be able to identify genes associated with particular biological properties by sequence comparison. Techniques for genetic exchange between mycobacteria will make it possible to test for gene functions in a way which is not possible with the non-cultivable *M. leprae*. Additionally, novel approaches for developing new animal models (gene knockout and transgenic animals) developing at a rapid pace; these will prove invaluable for testing hypotheses relating to control of infection and immunopathological mechanisms.

We believe that it is important to continue to address questions of pathogenesis for two broad reasons. Leprosy is a paradigm for intracellular infections. Comparative pathogenesis studies will provide important information for understanding not only leprosy, but infectious

processes in general. There are many important lessons that can be learned from the study of leprosy. Secondly, the consequences of the host–pathogen interaction remain a clinical problem for the leprosy patient for many years after bacteriological cure has been achieved. Rapid advances have been made in the pharmaceutical and technological fields for developing novel approaches to such things as wound healing, the treatment of immunopathological conditions, and other infections. However, these industries are not interested in leprosy and it will be up to us to exploit the developments for the treatment of leprosy patients. An understanding of the mechanisms involved in leprosy will enable us to make informed decisions as to which are likely to be useful for the leprosy patient.

We would regard the following as priority areas:

1. Completion of the genome sequencing project and comparative genomics with related organisms. This will enable us to understand what is biologically unique about *M. leprae* and hence to provide clues for the molecular basis of its pathogenicity.
2. Proteomic analysis, which will complement the genomic approach, will help us to understand which proteins are important for survival within the infected host. Once these proteins have been identified, further genetic studies can be undertaken.
3. New animal models, including transgenic and knockout mice, will play an important role in exploring pathogenesis. For example, mice with specific immunological deficiencies will enable us to determine important pathways in host immunity. These studies require highly specialized facilities and expertise, such as those available in mouse foot pad laboratories, which are in danger of being lost; in order to exploit these new models, it is important that these be maintained.
4. Molecular approaches to characterizing the interaction between *M. leprae* and the Schwann cell will enable us to further understand the unique pathogenic mechanism of *M. leprae*, and will complement clinical studies on nerve damage.
5. Host response to *M. leprae* is still poorly understood. The role of such factors as host genetics in determining susceptibility to infection and/or immunopathology will provide important pointers to the mechanisms involved.
6. New approaches to investigating the molecular details of immunological recognition could have important practical applications for detecting infection.
7. We believe that it is important that an integrated approach to the study of pathogenesis should be encouraged. A great deal can be learned by drawing on the expertise available in related fields such as neurobiology, immunology and molecular biology.

Participants: Jo Colston (Chairman), Linda Adams (Rapporteur), Christina Pessolani, Tom Ottenhof, Stewart Cole, Yasuo Fukutomi, Rabia Hussain, Delphi Chatterjee, James Krahenbuhl.

Summary

Whether or not the leprosy elimination target is met in all endemic countries by the year 2000, the MDT programme will have greatly reduced worldwide prevalence. However, our workshop chairmen were asked to ignore the prevalence-based leprosy 'elimination' programme and focus on recommendations for a long term, incidence-based eradication target where transmission is blocked. They were asked to be concerned with basic leprosy research goals in the post 2000 era.

The members of our workshops are actively productive workers, committed to their special interests. They are fully cognizant of the obstacles faced daily in working with leprosy and *M. leprae*, the requirement for clever experimental design even with the availability of the powerful tools of molecular biology which can now be brought to bear on some of the research obstacles. They are also aware of our lack of understanding about leprosy and *M. leprae*. How do you block transmission if you don't know how infection is transmitted? Can infection be detected, diagnosis made earlier? Is there a non-human reservoir host, a carrier state, an environmental source? What is the basis of *M. leprae*'s predilection for nerves, the mechanisms underlying reactions? What needs to be targeted to treat reactions? Can a vaccine play a role?

There is nothing startling in the workshops' recommendations. Other individuals and groups of experts have made the same suggestions, with slightly varying priorities. What one can read between the lines of these reports, is a sense of urgency to get as much done as soon as possible. Worldwide interest in leprosy will soon be diminished, not by design but as a consequence of the laudable success of the MDT programme. The experiment is still underway, but chemotherapy alone, killing bacilli in the detectable human host, does not appear to be the answer to blocking transmission.

A number of goals must be addressed while there are still intact national and international leprosy programmes, while there are still leprosy treatment and research centres that can co-ordinate and facilitate the necessary trials for early diagnosis, early detection of reactions, evaluation of immunosuppressive regimens for reactions. A key recommendation is concerned with the means of measuring progress. A clear and explicit means of reporting incidence, prevalence and 'case detection' should be implemented to avoid a distorted picture of worldwide leprosy.

These recommendations are non-controversial. What should be done is clear. The uncertainty is in determining who will do the work. Who will fund the laboratories engaged in this work? Look around you. There are fewer scientists attending this Congress but browsing the abstracts and attending our sessions and posters clearly revealed to me that fewer of us are doing far better work than in the past. Alternative sources of funding will help. Tuberculosis research is enticing researchers away from leprosy in the developed countries but is visibly sustaining leprosy research in many centres in developing countries. Formation of alliances was a key goal of this Congress. I asked my colleagues from Carville to identify in their own discipline, dedicated people, committed laboratories that will sustain their leprosy research efforts over the next 5, 10 or more years. These are the people with whom we wish to collaborate, form alliances, share resources and expertise, address the future of worldwide leprosy.

JAMES L. KRAHENBUHL
(Moderator)