Causative Organism and Host Response
Early Diagnosis of Leprosy

Paul R. Klatser (delivered by Stella van Beers)

Leprosy is an infectious disease in which symptoms are mainly determined by the host and the clinical presentation may vary within wide limits. Leprosy may have an acute, but usually a chronic onset, the latter developing insidiously. *Mycobacterium leprae* may be present in enormous numbers without its host showing any clinical signs and symptoms. The disease may thus be in an advanced state before any abnormalities are evident in the patient. Nevertheless, the diagnosis of leprosy is a key element in the control of this disease which is entirely based on timely detection followed by adequate treatment of patients.

Because of the lack of a single independent "gold standard," diagnosis is mainly made on clinical symptoms. For a relatively short period of time laboratory techniques had been introduced but have now been abandoned in most places where leprosy is still endemic. There are a number of findings that may be interpreted as an illustration of the present failure of diagnosis. In 1997 5.4% of the global new leprosy cases still presented with visible disability grade 2; it was 11.3% for the eight countries with the highest endemicity.

Assuming that the development of disability takes several years, it illustrates underdiagnosis in many situations. Thus, leprosy control programs face the problem of many leprosy cases remaining undetected, as may be evidenced by the large number of patients found in the leprosy elimination campaigns (LEC). Clearly, there is a need for an objective, easy-to-use diagnostic test. More so, because in the post-elimination era, leprosy will be a rare disease, with specific knowledge available at the central level and much less at the periphery. Over the past years several tests for diagnostic purposes have been described. Most of these tests got little appreciation from leprosy control programs for reasons that were not always clear.

Several studies have shown that serology with PGL-I is very useful in identifying persons at high risk of developing multibacillary (MB) disease, the main source of infection. A simple dipstick assay that can be used in the field is now available. Serological selection of high-risk groups as a means of early diagnosis and elimination of sources of infection makes sense, more so now that preliminary evidence indicates that chemoprophylactic treatment of seropositive contacts decreases their antibody titer. It has often been argued that intervention limited to contacts only deals with a small proportion of the problem, because the majority of incident patients appear among the so-called noncontacts. We now know that the vast majority of new patients, almost 80%, can be associated to contact with another leprosy patient, whereby the type of contact is not limited to household relationships but also includes neighbor and social relationships. This concept shows similarities with the "stone-in-the-pond" principle, describing tuberculosis transmission in concentric circles around a patient. This principle can be translated into a valuable and sustainable tool for leprosy control programs and elimination campaigns by focusing case detection and health promotion activities not only on household contacts but also on at least neighbors of leprosy cases.

In my view, serology is in a stage where it is warranted to study its introduction into control programs, especially for early detection of the most infectious form of the disease. Optimal operational procedures should be defined, and performance and cost-efficiency investigated. The skin tests which are now under development have the potential to detect most individuals infected with *M. leprae* and perhaps in conjunction with serology, will also be useful for the early diagnosis of disease. I expect these skin tests to be especially useful, contrary

to serological assays, for the early detection of paucibacillary leprosy patients. Other diagnostic assays which make use of other approaches, especially molecular amplification assays, have potentially numerous applications but are still at a level of sophistication, in terms of necessary skills and costs, which limits their application to well-equipped laboratories. Nevertheless, it would be regrettable if leprosy control would not benefit from the promising development in this field to miniaturize and simplify these techniques.

Both operational and technical approaches toward improvement of diagnosis are needed, and one or the other should not be excluded. As early diagnosis is concerned, there are developments which are very promising, both in terms of scientific merit and in potential usefulness for leprosy control. Let us together strive for these developments to continue.

Vaccine Trials Against Leprosy

M. D. Gupte

Several vaccine trials have been conducted in the past using BCG for prophylactic purposes based on the observation of lepromin conversion (Fernandez 1939). These studies essentially had problems with respect to design issues, sample size used to be small, control arms were not properly selected, and other such problems. However, four major studies were initiated in the 1960s from Uganda, Myanmar, New Guinea and India; as is widely known, the results are quite different with the range of 24% to 80%.

The vaccine trial from India has now been fully analyzed and the results are available. This was a study taken up along with BCG prophylaxis against tuberculosis in South India. It was a double-blind controlled trial with factorial design. Intake for the study was completed between 1968 and 1971; 210,337 individuals were skin tested with PPD-S and PPD-B and vaccinated with one of the selected vaccines. French and Danish strains of BCG in the dose of 0.1 mg and 0.01 mg were used. (In India the usual dose of BCG is 0.1 mg). Dextran was used as a control preparation. Allocation to various vaccine arms was made by individual randomization. Baseline survey for leprosy was taken up 5 years after vaccination during 1973 to 1976, and was restricted to only the vaccinated population; 191,696 individuals (91.4%) from the originally vaccinated cohort were examined. Subsequently, four resurveys were conducted at an interval of 2½ years covering the entire available population.

Observed protective efficacy according to the strain of BCG is given in Table 1. Both of the strains produced a similar type of protective efficacy around 24% to 25%. Observations are available for approximately 484,000 person-years for each of 0.1 mg, 0.01 mg and placebo arms. In the placebo arm there were 4238 cases; in 0. 1 mg dose of BCG, 3213 cases; in the 0.01 mg BCG arm, 3497 cases of leprosy. Consistent dose effect was seen: 0.1 mg dose of BCG gave a protective efficacy of 24.4% against 17.4% by 0.01 mg dose of BCG (Table 2). Females were generally better protected, but there was no statistically significant difference in the protective efficacy between males and females. Protective effi-

TABLE 1. *Leprosy incidence and protective effect of BCG vaccination by BCG strain.*

BCG strain	Incidence (0/00) in		Protective effect (%)
	0.1 mg BCG	Placebo	
Danish	6.64	8.82	24.7 (19.7–29.4)[a]
French	6.61	8.73	24.2 (19.1–29.0)

[a] 95% CI; Mantel Haenszel test $p > 0.2$.

TABLE 2. *Leprosy incidence and protective effect of BCG vaccination by BCG dose.*

	0.1 mg BCG	0.01 mg BCG	Placebo
No. cases	3,213	3,497	4,238
Person-years	484,864	482,735	483,143
Incidence (0/00)	6.6	7.2	8.8
Protective effect (%)	24.4 (20.9–27.8)[a]	17.4 (13.6–21.0)	—

[a]95% CI; Mantel Haenszel test p = 0.03

TABLE 3. *Distribution of new smear-positive cases of leprosy by age and "vaccine" frequency.*

Age (yrs.)	"Vaccine" group			Total
	0.1 mg BCG	0.1 mg BCG	Placebo	
0–14	9	11	9	29
15–24	10	8	12	30
25–44	4	15	10	39
45+	7	5	11	23
Total	40	39	42	121

cacy was observed against various clinical forms of leprosy to a varying extent. However, smear-positive cases of leprosy were not prevented by BCG (Table 3). Best protection was observed in the youngest age group of 0–4 years as observed in the first resurvey (57.8%). In the second resurvey it came down to 41.2%. In the third resurvey it was 32%, and in the fourth resurvey it was 17.8%. If one considers the cumulative effect, decline in the protective efficacy was from 57.8% between 5 to 7½ years and reached the level of 40.4% between 12½ to 15 years. As mentioned earlier, we do not have the information for the first 5 years following vaccination. However, baseline prevalence figures were 39.2 per 1000, 42.9 per 1000 and 46.5 per 1000 for the 0.1 mg dose of BCG, 0.01 mg dose of BCG, and the placebo, respectively. Thus, it was evident that there was some protective effect of BCG that could be observed at the baseline survey level. It was also seen that protective efficacy was not affected by PPD-S or PPD-B skin test positivity.

Thus, the BCG prophylaxis study in South India, provided the unique results of importance in several ways: a) BCG (0.1 mg) gave about 25% protective efficacy, which is not relevant from the public health point of view for protection against leprosy; b) smear-positive leprosy was not prevented; c) BCG efficacy was best seen in the youngest age group but substantially waned over a period of 15 years; and d) prior sensitization to *M. tuberculosis* or environmental mycobacteria as judged by PPD-S and PPD-B skin tests did not affect much the efficacy of BCG.

Subsequent to the availability of large quantities of *M. leprae* from armadillos, the next generation of vaccine studies became possible. Three large studies have been conducted using BCG + killed *M. leprae* as one of the arms in these studies. Results are already available for the studies from Venezuela and Malawi. It is claimed that in both these countries BCG gave substantial level of protection against leprosy. These observations did not emerge from prospectively controlled trials of BCG, but with the starting point of persons having a BCG scar and those who did not have a BCG scar. It was also observed in both these studies that the addition of *M. leprae* to BCG was not of much help in enhancing the efficacy of BCG in protection against leprosy. Results of the study from India are expected shortly.

In addition to some of these prospective studies, case control studies using a BCG scar as evidence for vaccination have also been conducted. It is well known that a BCG scar may disappear to a substantial extent, particularly in infants. Another possibility that is to be considered is the "take" for BCG and the subsequent resulting scar might be indicative of protection against leprosy, as was seen in the past lepromin conversion studies results from Myanmar. Therefore, even though the results from case-control studies demonstrate substantial protection against leprosy, at least for some clinical forms, these studies are not comparable and not as much reliable as the prospective cohort studies.

With the *M. leprae* genome project nearing completion and the *M. tuberculosis* genome project nearly completed, the prospects for a new generation of leprosy vaccines have brightened. There is a growing need for collaboration and interdependence between TB and leprosy immunology research. In the face of these developments,

several new questions emerge. It is generally felt that leprosy is already declining all over the world. Do we need a leprosy vaccine at all at this stage? It is interesting to note that even though leprosy prevalence is coming down substantially, new case detection in countries where MDT programs are implemented did not show a parallel decline. It is certain that various countries will not be able to maintain vertical leprosy programs with substantial expenditure indefinitely. Therefore, there will be a growing need and demand for integrated health services with a resultant danger of dilution of the program. As of now, there are no indications of a resurgence of leprosy, and we hope that such kind of danger may not be there. However, an effective leprosy vaccine may be needed in certain countries, at least in certain areas, in the near future.

The other question is regarding the feasibility of vaccine trials against leprosy and the various ethical aspects. As has been mentioned, incidence rates have been showing changes. Estimates based on "incidence" rates using one single resurvey are quite fallacious. The "new cases" from the first resurvey would be a mixture of missed prevalent cases and the real new cases. Our observations indicate that in the South Indian situation, the incidence of leprosy is perhaps coming down substantially. Whether this is a natural decline or because of MDT, or some other known or unknown factors, is difficult to say. But the fact is leprosy incidence is going to be rather low in time to come. Diagnostic techniques for leprosy, particularly the early forms, are insensitive, and this would lead to missing of prevalent cases. When these cases are counted as incidence cases there is a danger of underestimation of vaccine efficacy. Similarly, if we are looking at a situation of lower specificity for diagnosis during resurveys, this would also lead to underestimation of vaccine efficacy. It is almost certain that precipitation of a clinical form of leprosy to a certain extent does occur following an immunologically potent antileprosy vaccine. There are no methods as of now to identify separately each of these components of leprosy which would affect assessment of vaccine efficacy. Thus, one would have to consider very large sample sizes and a very long duration for follow up. The long duration of follow up would also lead to losses of cohort to a substantial extent. It is, therefore, obvious that the routine forms of antileprosy vaccine trials conducted so far would be a very difficult proposition. The need for developing and validating surrogate measures of protection cannot be overemphasized.

The ethical issues of concern are whether BCG can be denied, if some experts think that BCG is "the vaccine" against leprosy? Of course, from the results from the Indian study, it is quite obvious that BCG has limitations. The next question is could we think of using a placebo arm? If we tell the volunteers that they would be given the placebo, how many of them would accept to join the study? Can the consent be real informed consent? With a growing enlightenment about human rights and awareness about ethical issues, prospective vaccine studies are going to be difficult. It has to be fully borne in mind by each of the investigators that the ultimate responsibility for the vaccine trial would solely rest on the organizers of these studies.

The *Mycobacterium leprae* Genome Project

Stewart T. Cole

Everything that we need to know about the leprosy bacillus, from its biology to its behavior, is encoded in its genome. Through the concerted use of DNA sequencing and bioinformatics, a vast body of information can be deduced from the genes and this will further all aspects of biomedical research on *Mycobacterium leprae*. The startpoint for the genome project was the collection of cosmid clones ([3]) from the

Institut Pasteur that covered most of the ~3 Mb chromosome. In the first phase of sequencing, ~1.7 Mb of genomic sequences were generated in conjunction with the Genome Therapeutics Corporation of Waltham, Massachusetts, U.S.A. ([4, 8]), and this dataset was later extended by the Sanger Centre, Hinxton, England. At the time of writing, an estimated 0.3 Mb of sequence remains to be generated to achieve completion.

When the first cosmid carrying *M. leprae* DNA was analyzed, it was immediately apparent that only 50% of the potential coding sequence was actually used ([5]). As more cosmid sequences became available, this atypical trend was confirmed. The situation in *M. leprae* is thus radically different from that seen in all other bacteria where >90% of the genome codes for proteins. This raised the possibility that the chromosome may have mutated on a large scale resulting in some of the unusual properties of *M. leprae,* such as its remarkably long generation time and lack of growth in the laboratory.

Interpreting the features of the noncoding regions of the genome was initially difficult since no suitable comparisons could be performed. However, a strong indication that gene inactivation had occurred was provided by scrutiny of the katG loci ([2, 6]) of *M. tuberculosis* and *M. leprae.* In *M. tuberculosis,* katG encodes catalase-peroxidase, a heme-containing enzyme that potentiates the toxic effect of isoniazid. Comparison of the corresponding regions revealed the presence of numerous mutations in the *M. leprae* katG gene that abolish its activity. This undoubtedly explains why the leprosy bacillus produces no catalase-peroxidase and displays high level resistance to isoniazid.

The recent completion of the genome sequence of *M. tuberculosis,* comprising 4,411,529 bp ([1]), now enables us to perform systematic, genome-wide comparisons that will be of immense value for interpreting the genetic organization and function of *M. leprae.* Preliminary studies reveal extensive relatedness and conservation of blocks of genes of very similar sequence and arrangement. However, in *M. leprae* these are often separated by DNA segments that appear to have contracted, consistent with the smaller genome size, and to have degenerated in sequence. These show lower levels of similarity and correspond to pseudogenes, the vestiges of genes that still function in *M. tuberculosis.* Yet again, *M. leprae* differs radically from other bacteria since few pseudogenes have been found in microbial genomes. While adapting to its intracellular niche, the leprosy bacillus apparently dispensed with genes conferring a limited competitive advantage and this downsizing may have led to the loss of one, or more, metabolic functions that affect growth rate.

Comparison of the genes found in these two mycobacterial genomes has led to the identification of coding sequences in *M. leprae* that have no counterparts in *M. tuberculosis.* The corresponding proteins offer great potential as reagents for use in diagnostic skin tests and may even be involved in neuropathy ([7]).

REFERENCES

1. Cole, S. T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., Gordon, S. V., Eiglmeier, K., Gas, S., Barry, C. E., III, Tekaia, F., Badcock, K., Basham, D., Brown, D., Chillingworth, T., Connor, R., Davies, R., Devlin, K., Feltwell, T., Gentles, S., Hamlin, N., Holroyd, S., Hornsby, T., Jagels, K., Krogh, A., McLean, A., Moule, S., Murphy, L., Oliver, K., Osborne, J., Quail, M. A., Rajandream, M.-A., Rogers, J., Rutter, S., Seeger, K., Skelton, J., Squares, R., Squares, S., Sulston, J. E., Taylor, K., Whitehead, S. and Barrell, B. G. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature **393** (1998) 537–544.
2. Eiglmeier, K., Fsihi, H., Heym, B. and Cole, S. T. On the catalase-peroxidase gene, katG, of *Mycobacterium leprae* and the implications for treatment of leprosy with isoniazid. FEMS Microbiol. Lett. **149** (1997) 273–278.
3. Eiglmeier, K., Honoré, N., Woods, S. A., Caudron, B. and Cole, S. T. Use of an ordered cosmid library to deduce the genomic organization of *Mycobacterium leprae.* Mol. Microbiol. **7** (1993) 197–206.
4. Fsihi, H., De Rossi, E., Salazar, L., Cantoni, R., Labò, M., Riccardi, G., Takiff, H. E., Eiglmeier, K., Bergh, S. and Cole, S. T. Gene arrangement and organization in a ~76 kilobase fragment encompassing the oriC region of the chromosome of *Mycobacterium leprae.* Microbiology **142** (1996) 3147–3161.
5. Honoré, N., Bergh, S., Chanteau, S., Doucet-Populaire, F., Eiglmeier, K., Garnier, T., Georges, C., Launois, P., Limpaiboon, P., Newton, S., Nyang, K., del Portillo, P., Ramesh, G. K., Reddy, T., Riedel, J. P., Sittisombut, N., Wu-Hunter, S. and Cole, S. T. Nucleotide sequence of

the first cosmid from the *Mycobacterium leprae* genome project : structure and function of the Rif-Str regions. Mol. Microbiol. **7** (1993) 207–214.
6. NAKATA, N., MATSUOAKA, M., KASHIWABARA, Y., OKADA, N. and SASAKAWA, C. Nucleotide sequence of the *Mycobacterium leprae* katG region. J. Bacteriol. **179** (1997) 3053–3057.
7. RAMBUKKANA, A., SALZER, J. L., YURCHENCO, P. D. and TUOMANEN, E. I. Neural targeting of *Mycobacterium leprae* mediated by the G domain of the laminin-a2 chain. Cell **88** (1997) 811–821.
8. SMITH, D. R., RICHTERICH, P., RUBENFIELD, M., RICE, P. W., BUTLER, C., LEE, H.-M., KIRST, S., GUNDERSEN, K., ABENDSCHAN, K., XU, Q., CHUNG, M., DELOUGHERY, C., ALDREDGE, T., MAHER, J., LUNDSTROM, R., TULIG, C., FALLS, K., IMRICH, J., SMYTH, A., TORREY, D., DRILL, S., AVRUCH, A., ENGELSTEIN, M., BRETON, G., MADAN, D., NIETUPSKI, R., SEITZ, B., CONNELLY, S., MCDOUGALL, S., SAFER, H., DOUCEFTE-STAMM, L., EIGLMEIER, K., BERGH, S., COLE, S. T., ROBISON, K., JAEHN, L., GRYAN, G., JOHNSON, J., CHURCH, G. M. and MAO, J. Multiplex sequencing of 1.5 Mb of the *Mycobacterium leprae* genome. Genome Res. **7** (1997) 802–819.

Potential Application of Molecular Biology to Leprosy Research

Thomas P. Gillis

Much debate over the last few years has centered on whether basic research should be encouraged in light of recent trends in prevalence rates for leprosy. Strategic planning by the World Health Organization (WHO) has indicated that they will direct their research initiatives toward applied research, primarily to field operations. While operational research is needed to focus our current knowledge in this disease, fundamental research must continue as a means of providing enhanced understanding of leprosy, with our ultimate goal being global eradication of leprosy. At a minimum, we need a much fuller understanding of the mechanisms of protective immunity, the mechanisms associated with nerve damage and the epidemiology associated with spread of disease and risk of infection.

Neurologic aspects of leprosy. Nearly one third of the patients with leprosy develop disabilities as a result of nerve damage. Multidrug therapy (MDT) does not guarantee absence of nerve damage and attendant sensorimotor loss. We are still without a comprehensive model for pathogenesis of nerve damage. Recent work by Rambukkana and colleagues has opened a door in this area. Their work describes the probable nature of the neural tropism of *M. leprae* focusing on the molecular characteristics of the leprosy bacillus and its affinity for a specific form of laminin found on Schwann cell-axon units. The specific region of the laminin-2 isoform (G domain of the alpha-2 chain) appears to be responsible for *M. leprae*'s unique and specific targeting of the Schwann cell. An interesting correlation raised by this finding is that this form of laminin is restricted to the basal lamina of Schwann cells, striated muscle and the trophoblast of the placenta. These tissues have long been recognized as sites where *M. leprae* can be found in tissues apart from professional phagocytic cells, such as macrophages.

Molecular immunology of leprosy. With our improved understanding of what constitutes the lesion in its final stages, it would seem prudent that we need to begin investigating "early lesions" to describe molecular events which precede and possibly direct the outcome of disease including type 1 and type 2 reactions.

Leprosy vaccines. An ideal leprosy vaccine should be administered once and should induce sustained protective immunity from early childhood through young adulthood. Studies are needed to exploit breakthroughs in vaccine development such as: evaluating protein subunit vaccines, so-called "naked" DNA vaccines or redesigned BCG using recombinant DNA techniques.

"Naked" DNA vaccines are comprised of an especially designed bacterial plasmid carrying a bacterial gene which, when injected into eukaryotic cells, allows expression of the bacterial protein antigen. Expression of the bacterial antigen then gives rise to an immune response in the vaccinated host. The initial success of this approach was very much a surprise to the vaccine field, but the approach is gaining general acceptance as a potential alternative to vaccination under certain circumstances.

BCG has always been an appealing candidate for leprosy control due to its demonstrated efficacy and attractive native properties, such as a high degree of safety in all age groups, persistence in the intracellular environment and good adjuvant properties. Many investigators believe these attributes can be improved upon and have successfully expressed foreign genes derived from other human pathogens in BCG.. Auxotrophic mutants provide an approach for producing a "crippled" BCG that retains immunogenicity and may prove safer for implementation in immunocompromised individuals.

The question remains then, can BCG be redesigned to better express *M. leprae*-relevant protein antigens, resulting in an improved BCG vaccine for leprosy? It is very likely that this area of investigation will be pursued vigorously and should this approach or another yield a potent vaccine for leprosy, an even larger question will be facing us: who will pay for testing the vaccine in humans? Alternative funding sources may be the answer. It seems prudent that groups involved in vaccine research should keep their goals in line with current trends in tuberculosis (TB) vaccine development. Newly developed TB vaccines should be designed with combined protective efficacy to include leprosy. This will better insure the potential of testing a new vaccine with the sponsorship of tuberculosis-funding agencies.

Summary of Causative Organism and Host Response Workshop Reports

James L. Krahenbuhl, Moderator

Whether or not the leprosy elimination target is met in all endemic countries by the year 2000, the multidrug therapy (MDT) program will have greatly reduced worldwide prevalence. However, our Workshop chairmen were asked to ignore the prevalence-based leprosy "elimination" program 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 *Mycobacterium 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 do not know how infection is transmitted? Can infection be detected, diagnosis made earlier? Is there a nonhuman 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 program. 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 programs, while there are still leprosy treatment and research centers that can coordinate 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 centers 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 and 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 and address the future of worldwide leprosy.

Report of Workshop on New Tools for Diagnosis and Epidemiology

Thomas P. Gillis, Chairperson
Sang-Nae Cho, Rapporteur

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 programs 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-I has been found useful in identifying household contacts at high risk of developing multibacillary (MB) disease. Since MB disease is potentially the most significant reservoir of *M. leprae* with potential for spreading the infection, Workshop participants felt that control programs should begin to explore this application. A 15-minute dipstick assay for PGL-I antibody is now available and could be used in field conditions, for example, in small-scale LEC or SAPEL programs. 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 titer, while a single dose of rifampin-ofloxacin-minocycline (ROM) has little or no effect. Thus, aggressive prophylactic therapy of PGL-I-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 initia-

tives 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 multicenter 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 paucibacillary (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.

Report of Workshop on Chemotherapy

J. H. Grosset, Chairperson
Scott Franzblau, Rapporteur

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 rifampin. However, because the present antileprosy drug regimens (World Health Organization-recommended multidrug therapy; WHO/MDT) are so extremely effective and robust, these regimens should remain the treatment of choice for leprosy in national programs. 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 2-year WHO/MDT for MDT leprosy, the changes in definition of paucibacillary (PB) and multibacillary (MB) and the low bacterial index (BI) 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 rifampin-ofloxacin-minocycline (ROM) for the treatment of single-lesion leprosy offers great operational advantage to national programs. 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 rifampin 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 MB cases to 12 months, as long as the drugs are used in appropriate combinations. To replace mouse foot pad inoculation, research should continue on molecular methods of detecting drug resistance.

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

Report of Workshop on Epidemiology/Transmission/Vaccines

Paul E. M. Fine, Chairperson
Richard Truman, Rapporteur

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 the national, district and local levels 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 affected 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 *Mycobacterium 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 the 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 nonendemic 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 behavioral 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 program 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 Myanmar 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.

Report of Workshop on Pathogenesis and Lessions from Leprosy

M. J. Colston, Chairperson
Linda Adams, Rapporteur

Scientifically, the opportunities for studying pathogenesis in leprosy could not be more timely. The availability of the complete sequence of the *Mycobacterium tuberculosis* genome and the considerable inroads that have been made into 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 noncultivable *M. leprae.* Additionally, novel approaches for developing new animal models (gene knockout and transgenic animals) are 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 leprosy and 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 will 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, 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 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.

Report of Workshop on Nerve Damage and Reactions

Diana Lockwood, Chairperson
David M. Scollard, Rapporteur

- Nerve damage continues to be a major problem
- Nerve damage remains poorly understood
- 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, multibacillary (MB) disease and age (15–44) 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 expect 40% of patients to now have their first reactional episodes after completing multidrug therapy (MDT). This has very important implications for management. Patients will need to be carefully warned about reactions and 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 centers. However, it is important that the reliability, diagnostic cut off, specificity and sensitivity of these tests be 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 *Mycobacterium 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 immunologic basis of reactions and neuritis were briefly reviewed, and several lines of evidence indicate that TNF-alpha 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.

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

The Workshop discussed the need to evaluate currently available immuno-suppressants as second-line treatment for patients who do not respond to corticosteroids. Multicenter trials are also needed to define the role of neurolysis in the management of acute neuritis. All of the abovementioned multicenter 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.