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Scientific Commentary

THE US–JAPAN JOINT LEPROSY RESEARCH PROGRAM MEETING, SAN FRANCISCO, JUNE 28–30, 1999

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The US–Japan Cooperative Medical Sciences Program was founded in the 1960s by the then-President of the United States and the Prime Minister of Japan and, since then, has had the highest political support from both governments. Leprosy was among the first disease entities named as part of the overall program, and the US and Japanese leprosy research panels and their guests have met in the alternating countries every year for the past 34 years (in 1995, the separate leprosy and tuberculosis panels were amalgamated). These meetings of the joint US–Japan panels in the form of scientific conferences have become a highlight of the annual leprosy research agenda. Some of the major fundamental research developments in leprosy over the past 30 years have been first reported at this conference. These include: the early development of the drug regimens leading to present-day MDT and ROM; the early development of the mouse footpad; the recognition of sylvian leprosy in the armadillo and the development of this model of leprosy and, later, the Mangabey monkey model; the original work on the extension of hybridoma technology to leprosy and the development of banks of monoclonal antibodies; the first research on the application of genetic recombinant technology to *Mycobacterium leprae* and the production of 15–20 recombinant protein antigens; the discovery of the heat-shock proteins and of PGL-I, and the synthesis of corresponding neoglycoproteins and the development of ELISA systems; the major developments in the definition of the genome and proteome of *M. leprae*; all major developments in defining the cellular immune response in leprosy; the application of thalidomide to leprosy reactions and elucidation of its action mechanism etc.

The 34th US–Japan Leprosy Research Conference was held in San Francisco in conjunction with the US–Japan Tuberculosis Research Conference, June 28–30, 1999. Some of the highlights are as follows.

A. Rambukkana (Rockefeller University, New York, USA) described the latest chapter in his important work on the molecular basis of the interaction between the Schwann cell and *M. leprae.* Previously, he had described how the G domain of the laminin α_2 chain in the basal lamina that surrounds the Schwann cell-axon unit serves as an initial neural target for *M. leprae*. This time, he addressed the nature of the *M. leprae* surface molecules that bind

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to α_2 laminin. By using human α_2 laminins as a probe, a major 28 kDa protein in the *M. leprae* cell wall fraction was identified. Immunofluorescence and immunoelectron microscopy on intact *M. leprae*, using monoclonal antibodies against the recombinant protein, demonstrated that the protein is surface-exposed. Also, the recombinant protein was shown to bind avidly to α_2 laminins, the recombinant G domain of the laminin–α_2 chain, and the native peripheral nerve laminin. Thus, these data suggest that this 28 kDa protein functions as a critical surface adhesin that facilitates the entry of *M. leprae* into Schwann cells.

In subsequent discussion of this work, it was revealed that Dr Cristina Pessolani (Fio-Cruz, Rio de Janeiro) had also described a 28 kDa protein as a key bacterial ligand in *M. leprae*–Schwann cell interaction and had shown that this is a member of the histone-like protein family. It thus seems that the protein described by Dr Rambukkana is this HLP.

Dr Takeshi Yamada and colleagues (Nagasaki University, Japan) have also focused on this protein from a different perspective. They have been investigating the molecular basis of the slow growth of *M. leprae* and other mycobacteria and identified a 28 kDa protein (which they called MDPI) as the most abundant protein in *M. bovis* BCG. The protein was highly polymerized and localized in the nucleoid, 50S ribosomal subunit and cell surface. It interfered with replication, transcription and translation in *E. coli* cell-free systems, and was capable of transforming *E. coli* to slow growth. Sequence analysis also indicated a member of the HLP family. Thus, the 28 kDa HLP is apparently a major player in the pathogenesis and physiology of *M. leprae*. Its immunogenicity and diagnostic potential should now be examined.

Efforts to 'cultivate' *M. leprae* continue, but this time through genetic augmentation of the organism, a sensible plan in light of a genome that is small and very defective in gene density. Drs Scott G. Franzblau (GWL Hansen's Disease Center, Baton Rouge, LA, USA) and William R. Jacobs (Albert Einstein College of Medicine, New York, USA) have used a combination of freshly harvested, viable nude mouse-propagated *M. leprae* and a modified D29 mycobacteriophage vector to achieve phage infection of *M. leprae* and foreign gene expression. Therefore, the key preliminary work has been achieved as a prelude to constructing a shuttle cosmid vector, carrying DNA libraries from slow growing cultivable mycobacteria and capable of stable expression of foreign DNA in *M. leprae*, allowing, in future, perhaps, *in vitro* growth competence.

Widespread resistance to dapsone in the 1970s was the catalyst for the development of multiple drug therapy (MDT) for the treatment of leprosy. However, to date, researchers have not been successful in characterizing the molecular basis of dapsone resistance. Two laboratories have now conducted crucial preliminary experiments (Dr Y. Kashiwabara, Leprosy Research Center, Tokyo, Japan, and Dr Diana Williams, GWL Hansen's Disease Center). An analysis by others of sulphonamide resistance in *E. coli* has shown an association with dihydropteroate synthase (DHPS), a key enzyme in the folate biosynthetic pathway, encoded by the *folp* gene. Dr Williams has shown that *M. leprae* possesses two *folp* homologs (*folP1* and *folP2*). DDS resistance was not associated with mutations in *folP2* from two high-level DDS-resistant strains of *M. leprae*. However, mutations were observed within a highly conserved region of *folP1* in two of these high-level DDS-resistant *M. leprae* clinical isolates. In addition, this *folP1* homolog has been shown to encode a functional DHPS which itself is highly sensitive to DDS. These new data thus support early predictions that DDS resistance in *M. leprae* is associated with alterations in folate metabolism and that one possible mechanism of resistance is due to mutations in *folP1*.

Resistance to fluoroquinolones is becoming widespread, at a time when ofloxacin, one of the fluoroquinolones, is being used more and more frequently in the form of ROM (rifampin, ofloxacin, and minocycline) in leprosy control programs. Dr Y. Kashiwabara has determined the sequences of the QRDR (quinolone resistance determining region) of *gyrA* (the gene encoding the A subunit of gyrase, the site of action of the fluoroquinolones) in 13 clinical isolates of *M. leprae*, and demonstrated that eight of them showed mutations in this region. Importantly, five of the eight also showed mutations in the *rpoB* gene (the gene encoding the B subunit of RNA polymerase, the site of action of the rifamycins), suggesting that exposure to one or the other of the two drugs can lead to resistance to both, a new worry as we develop alternative drug regimens for leprosy.

The type of molecular epidemiology that is now being applied to *M. tuberculosis* isolates and tuberculosis in general has not been possible with leprosy, because *M. leprae* is devoid of the type of variable but relatively stable genetic polymorphism associated with the IS6110 insertion sequences in the *M. tuberculosis* chromosome. If other forms of DNA polymorphism could be identified in the *M. leprae* genome, the lessons that could be derived from its application would be profound in terms of tracking sources of infection, examining the relationship between non-symptomatic carriage of *M. leprae* and disease, probing the possibility of environmental sources of *M. leprae*, and differentiating between reactivation and new infection. Dr Y. Kashiwabara has now found some evidence of such polymorphism, albeit limited. The sequences of the *rpoT* gene from many *M. leprae* isolates were compared, allowing the classification of isolates into two broad categories. One group had three tandem repeats of a six-base-pair (AGATCG) sequence, and the other group had four tandem repeats. Isolates from Japan and Korea had the four-tandem repeat profile, whereas isolates from South-East Asian and Latin American countries had the three-repeat pattern, indicating that this genetic characteristic could be used to trace the origins of infections and the evolution of disease.

The role of various cytokines and different T-cell subsets in leprosy pathogenesis and immunity to leprosy has long been a favorite topic of US–Japan participants. The curious balance between acquired resistance and pathogenesis is seen in granulomatous infiltration, a consequence of the marshalling of the acquired response to essentially contain bacilli, but with pathological sequelae. In the hands of Dr Linda Adams (GWL Hansen's Disease Center), mice genetically incapable of producing a functional inducible NO synthase (iNOS) showed markedly enhanced granuloma formation, and these types of granulomas were composed primarily of $CD4^+$ cells and multinucleated giant cells. Thus, iNOS has an unexpected role in leprosy granulomatosis. Among the newer cytokines to be involved in the leprosy immune response are IL-12 and IL-10. According to Dr Robert Modlin (University of California, Los Angeles, CA, USA), some key lipoprotein ligands of *M. leprae* bind to the toll-like receptors in macrophages, evoking the dual response of NO production and IL-12 evocation, two new major players in counteracting infection. We have long been very conscious of the role of IFN-γ in the type-1 protective immune response in leprosy. Apparently, part of the mechanism of this effect is to up-regulate type-1 cytokine expression and down-regulate IL-10, one of the type-2 cytokines (Drs Y. Fukutomi and M. Matsuoka, Leprosy Research Center, Tokyo, Japan). The newest players in these events are the chemokines. *M. leprae* induces elevated levels of MCP-I, MIP-1α, and MIP-1β expression, and it is now believed that chemokines will prove to be important in regulating granuloma formation and other immune responses in leprosy (Dr Linda Adams).

Preliminary results were also reported by Dr T.P. Gillis (GWL Hansen's Disease Center)

on the application of DNA vaccines to an animal model of leprosy. A recombinant construct of the antigen 85A injected intradermally proved to be the most promising with strong IgG1 and Ig2a antibody responses and increased IFN-γ and IL-2 production. However, protection studies in the mouse footpad infection model were disappointing.

With the amalgamation of the US–Japan Leprosy and Tuberculosis Panels in 1995, a fear of leprosy research workers within the US–Japan Cooperative Medical Sciences Program was that leprosy research would be engulfed by the tuberculosis research juggernaut. This fear has proved to be unfounded. Basic research in leprosy is thriving, notably in Japan, where the Leprosy Research Center has been incorporated into the prestigious, well-endowed National Institute of Infectious Diseases. The formal combination of both panels is clearly benefiting leprosy research.