07 de junho / June 7th

Conferências / State of Art

Mycobaterium leprae: O significado dos nossos conhecimentos sobre sua composição e antigenicidade
Mycobacterium Leprae: the significance of our knowledge of its composition and antigenicity

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There has been a clear exodus in recentyears of many key investigators from the area of leprosy research to that of tuberculosis, with the result that leprosy research has suffered. However, I, personally, am determined to continue for the rest of my career with the study of the molecular definition of M. *leprae* because I regard it as a very important subject for a number of reasons, as I will demonstrate.

A discussion of the nature of the leprosy bacillus can be divided into five topics: discussion of the extracellular components of *M. leprae;* the cell wall of M. *leprae;* the proteins of *M. leprae;* the genome of *M. leprae;* and, finally, the future of basic molecular biology in the context of present-day leprosy.

There are several obvious reasons for studying the organism in detail. One is to understand the interaction with the immune system in the hope that new diagnostic tools can be developed, particularly for non-obvious types of leprosy. Another is to develop a vaccine, and still another is to define the pathogenesis of leprosy. Also, we would like to study *M. leprae* from the viewpoint of the physiology and the metabolism of the organism, given that it is an obligate intracellular parasite, and this might lead to new drug targets, an area of development that may have a future. We mould like to identify the elements necessary for the survival of the bacterium within the host organism, tissue, and cells, and identify the mechanisms by which it induces its particular pathogenesis and virulence.

There are other reasons. M. *leprae* is an obligate, intracellular parasite, and it is derived from a living organism. We harvest it from the armadillo; it is not derived from a culture medium and the false situation that induces, but the organism is harvested from the living cell. Few other bacterial pathogens, including *Mycobacterium tuberculosis,* can be readily obtained from living tissue.

The External Components of *M. leprae*

What are the materials that *M. leprae* excretes into that intracellular environment? What effect do they have on the organism itself and on the disease process? Very early electron micrographs demonstrated extraordinary globules on the face of M. *leprae.* They were assumed to be excreted lipid, and there was much evidence to support this claim. Some of the first work we did was on the definition of these globules associated with *M. leprae* within the tissue. The chemical characterization of these globules led to the identification of phenolic glycolipid I (PGL-I), which turned out to be a major development in terms of the molecular definition of *M. leprae.* PGL-I is a long-chain fatty alcohol with a phenol ring and with fatty acids linked to the alcohol function, and a specific trisaccharide bound to the phenol. Within the globules that are outside the bacillus, within the host cell and tissue itself,

there are other versions of this molecule. There is the version in which the lipid itself is found. It is now accepted that this type of derivative and its relatives are responsible for much of the environment in which *M. leprae* is found and hence for much of the protection and pathogenesis of *M. leprae.* This phase of research led to a new approach to the serodiagnosis of leprosy based on this specific glycolipid and its specific sugars. Synthesis of semi-synthetic relatives of phenolic glycolipid was undertaken, particularly those which contained the native trisaccharides, and these were hooked onto bovine serum albumin. These neo-phenolic glycolipids were used extensively in the hope that new, sensitive assays for leprosy would be produced. This led to the development of several kits, the first of which was the *M. leprae* particle agglutination (MLPA) kit, which was produced by a Japanese. company and based on some of the molecules which we and some of our Japanese colleagues synthesized, leading to an era in which this kit was used extensively for the serodiagnosis of leprosy. However, there was a problem with sensitivity, in that anti-PGL antibodies were not generally seen in the case of tuberculoid leprosy, which, in general, is a greater clinical problem area. These types of assays diagnosed lepromatous leprosy, but this was not a real issue at the time. Subsequently, much of this work fell into abeyance. Nevertheless, phenolic glycolipid, given the fact that it is so copious and it does induce a strong humoral antibody response, continues to be the focal point of new tests. A graduate student from Dr. Samos lab, Samira Bührer, along with Dr. Paul Klatser from Amsterdam, have developed a simple, colorimetric assay, an IgM dipstick assay based on a disaccharide surrogate of phenolic glycolipid-I. Disaccharide-BSA is coated on the dipstick as also is anti-human IgM antibody in a separate band as an internal control. The dipstick is immersed into 200111 of solution containing 4µl of serum (or whole blood) and the detection reagent which is a monoclonal anti-human IgM antibody. It is incubated, rinsed in tap water, and read visually. This has been used in Brazil and in the Philippines, and is similar in terms of sensitivity and specificity to the MLPA assay and the ELISA assay. The IgM dipstick is effective in the case of only lepromatous leprosy (only 16% positivity rate in tuberculoid leprosy). Nevertheless, these types of assays continue to be developed and are useful in case management and for confirming the diagnosis of lepromatous leprosy.

Phenolic glycolipid, being so copious, is also the focus of assays to detect the antigen itself, and several new, sensitive assays have been developed. Dr. Sang-Nae Cho has developed quantitative assays for PGL-I in both serum and in urine. He applies 100 µl of serum to a filter-disk, dries it, and extracts the phenolic glycolipid from this with chloroform, dissolves these lipids in hexane, adds some of this to polystyrene plates, and runs them in ELISA with anti-PGL MAbs. The sensitivity obtained is remarkable. Using plate ELISA (versus dot-ELISA, which he used previously), he obtained a sensitivity rate of 1 nanogram of PGL-I per ml of serum. He has shown similar rates of sensitivity using urine.

Accordingly, I regard these developments as important in the context of leprosy control and should be recognized as such by the major practitioners of leprosy control programs, and a place should be found for these forms of serodiagnosis in control programs.

There are other biological functions associated with PGL-I: (i) it is a unique antigen of *M. leprae* and it stimulates a strong antibody response at the lepromatous end; (ii) it scavenges reactive oxygen radicals and in this way it lends to the survival of *M. leprae* within the host cell; (iii) it is one of the ligands responsible for the phagocytosis of *M. leprae,* in that it fixes C3 which binds to the C3 receptor on the phagocytic cell. Dr. Pessolani has shown that PGL-I has other immuno-suppressive effects. For instance, in the context of whole *M. leprae,* it induces the production of α-TNF by human monocytes. It also inhibits the IL-2-dependent activation of T- cells and the expression of CD69, suggesting a suppression of the normal immune response and adding to the immunological anergy associated with lepromatous leprosy.

Another major excreted component of *M. leprae* is the antigen 85 complex. It is a major excreted protein of *M. leprae* and is found in sizable amounts in the environment surrounding *M. leprae* within the whole cell. It is a

major target of the humoral response in lepromatous leprosy patients. A number of diagnostic assays are based on the antigen 85 complex for the diagnosis of leprosy. It evokes a lympho-proliferative response and gamma-interferon production from PBMCs from lepromatous and tuberculoid patients, and those features have been exploited in simple whole blood assays for leprosy or exposure to it. Dr. Pessolani isolated this particular antigen 85 complex from *M. leprae* and from its surroundings. She purified the antigen 85 A, B, and C complex using classical biochemical procedures. This work of hers generated our interest in antigen 85, which we have returned to recently in a different context. We have become interested in the concept of how the cell walls of M. *leprae* and *M. tuberculosis* are put together. We wanted to determine what enzyme was responsible for the addition of mycolic acid into the cell wall of mycobacteria in general. We started purifying a mycolyl transferase, the enzyme that is responsible for taking mycolic acid and inserting it into the end products in the mycobacterial cell wall assembly process. We purified mycolic transferase to near-homogeneity by 2-D polyacrylamide gel electrophoresis, and we saw a characteristic pattern which was reminiscent of the pattern that Dr. Pessolani had shown years ago with the antigen 85 complex of *M. leprae.* We were then able to show that the mycolyl transferase and the antigen 85 complex were synonymous. We then purified the individual components of the antigen 85 complex, a triad of proteins encoded by three different genes that are similar in terms of their sequence; the three of them were shown to react to the antibodies generated by Dr. Pessolani. All three Antigen 85A, Antigen 85B, and Antigen 85C had this mycolyl transferase activity. The genes encoding these antigens were examined for carboxyl esterase consensus sequence which was found in all three antigens. These were compared with the antigen 85 complex from a *Corynebacterium;* there was also a sequence with a genuine carboxyl esterase motif. The consensus sequences are similar. We then generated a mutant enzyme in which we replaced within the consensus sequence a serine residue with an alanine residue. We were then able to express in *E. coli* bot the native antigen 85 and the mutant antigen 85 in which the serine was converted into alanine and then to purify these enzymes. We showed that the antigen 85 had lost its mycolyl esterase activity. Our conclusions are that antigen 85, which is a major excreted protein of *M. leprae* and *M. tuberculosis,* has several functions. It is a protective antigen and a humoral antigen. It also has fibronectin binding capacity, which may be important in the pathogenesis of leprosy. And, finally, it has this mycolic acid transferase activity, where it takes newly synthesized mycolic acid, transfers it to a carrier, which is carried through the membrane of the bacterial cell, and then it catalyzes the transfer of mycolic acid into the various components of the mature cell wall of mycobacteria in general.

Also, in the context of excreted products of *M. leprae,* I would like to talk briefly about what I regard as the "Molecular Highlight of 1997." There has been speculation that antigen 85, with its fibronectin-binding properties, is one of the key ligands involved in the phagocytosis of *M. leprae,* in that fibronectin is found on the surface of the host phagocytic cell, and, in this way, *M. leprae* is phagocytosed. However, some of this work has been disputed. The recent elegant work conducted by Dr. Rambukkana in Dr. Tuomanen's laboratory at Rockefeller University adds a new perspective to this topic. *M. leprae* has a particular propensity for neural Schwann cells. Schwann cells are surrounded by a substance called lamina, and this distinguishes Schwann cells from other fibroblasts. In fact, Schwann cells are devoid of any fibronectin. Lamina is an interesting conglomeration of molecules, composed of laminan, type-4 collagen, intactin, heparin sulfate, proteoglycans, and laminan, particularly laminan α-2 and very little of laminan α-1. In an elegant series of experiments, these investigators demonstrated the α-chain of laminan (laminan consists of an α-chain, a β-chain, and a y-chain; various laminans consist of an assortment of these chains). In the case of laminan 1 and laminan 2, the a-chain is characterized by a special domain called the α-2-G chain, which is found on the a-chain but not on the other two chains. Rambukkana demonstrated that *M. leprae* specifically binds to the G chain of laminan 2 using recombinant laminan 2 with a 2 G chain

and also laminan 2 without the 2 G chain. From this work, we now have an idea of the molecular basis of the specificity of *M. leprae* for Schwann cells. It is the fact that they contain laminan 2, particularly the α 2 G chain to which M. *leprae* binds specifically and hence this very unusual tropism of *M. leprae* for nerve cells and for Schwann cells.

The other major excreted product of *M. leprae* is LAM. It was discovered in M. *leprae,* and the structure of it was worked out by us years ago. It consists of phosphatidylinositol, a long, linear mannan chain, attached to which is arabinan, which is capped by a number of short mannose oligosaccharides. In *M. leprae,* there are few of these mannose oligosaccharides, and this has led us to hypothesize that the mannose receptor is not a major conduit by which *M. leprae* is phagocytosed. Recent work by Rambukkana supports this; it indicated that in Schwann cells, the laminan α -2 chain is probably the major conduit. In *M. tuberculosis,* these mannose caps, which are much more copious, are one of the key mechanisms by which M. *tuberculosis* is ingested and phagocytosed. *M. leprae* LAM has been highlighted recently in a different context.

There are two major groupings of T cells. You are familiar with the CD-4, MHC-II, CD-8, and MHC-I restricted T cells and with their roles, the effector role, protection, the issue of whether they are cytotoxic, etc. Unconventional T cells also exist, and some are capable of recognizing both lipids and carbohydrates. The first of these was the double negative CD8-CD4 Sy T cells, which have the *Sy* heterodimer T-cell receptor, not the a". We now recognize that such cells are fairly common in leprosy and tuberculosis infections, and these are capable of recognizing a few exotic ligands. All of these ligands share the feature that they are phosphorylated; sometimes they are nucleotide phosphates, sometimes sugar phosphates, sometimes aliphatic phosphates. A second group of unusual and unconventional T-cells recognized more recently by Mike Brenner and Robert Modlin are CD-1 restricted, not MHCrestricted; they are also double negative CD8CD4-, and also have the αβ T-cell receptor. These particular groups of T cells, which are also fairly prominent in leprosy lesions, recognize two major molecules from *M. leprae,* mycolic acids and LAM; the CD1 has recently been crystallized and mycolic acids fit nicely into the hydrophobic pocket. Recently, Robert Modlin, who cloned some of these CD-1 restricted double negative cells from a lepromatous leprosy lesion, was able to show that these CD1-restricted cells can recognize LAM and the elemental portion of LAM, i.e., the lipid portion, the phosphatidylinositol mannoside. This work shows that this other, very unusual, relatively minor group of T cells, compared to the more conventional ones, now recognize some of the major excreted products of M. *leprae.* Robert Modlin has hypothesized that *M. leprae,* either itself or its LAM, is taken up into the early endosomes, using CD14 and perhaps the mannose receptor to a small extent, and this enters early endosomes where, in late endosomes, it undergoes a processing and an association with CD1 and is then presented on the surface of these specialized antigen-presenting cells and is recognized by the T-cell receptor. It fits into the same hydrophobic group on account of the lipid part of LAM. Thus, there is the situation in which the copious, major excreted glycolipids of *M. leprae,* whether lipoarabinomannan or phenolic glycolipid, now are known to have important roles in the modulation of the immune response in leprosy.

The Bacillus Itself

Regarding the bacillus itself, methods have been developed for the isolation of *M. leprae* from armadillo livers and spleens with harvests of 95%-96% pure *M. leprae* without much contaminating tissue. We have begun to carefully analyze the bacillus itself. One of the researchers working with me is Angela Marques, who comes from the Fiocruz laboratory in Rio de Janeiro from Dr. Cristina Pessolani's lab. She has developed a subcellular purification/fractionation of *M. leprae.* She takes purified *M. leprae,* sonicates it, and subjects it to differential centrifugation to remove whole cells and tissue contamination. Whole cells are treated with RNAse and DNAse, centrifuged, and from this we get the sediment, which represents the partially purified cell walls which are then purified on a sucrose gradient. From that we obtain the highly purified cell walls distributed over different bands. The supernatant from this fractionation

contains the membrane and the cytosol, and these can be separated by centrifugation to give the pure cytosol and the pure membranes. This approach has allowed us to examine the cell wall and other subcellular fractions of *M. leprae.* We have been able to do this in great detail, which hadn't been done previously. We have concluded that the cell wall of *M. leprae* is much like that of *M. tuberculosis* and other mycobacteria, in terms of the basic structure. It consists of the basic peptidoglycan, and attached to that is a special linker region attached to a galactan, and this, in turn, is attached to an arabinan, and attached to these are mycolic acids. These mycolic acids are complemented by an assortment of lipids. Where *M. leprae* distinguishes itself from other mycobacteria is in the nature of these lipids that are complementing the mycolic acids. In the case of M. *leprae,* these are mostly phenolic glycolipid **I** and the diacylphthiocerols, with some specialized phospholipids, the phosphatidylinositol mannosides. We have a good concept of what that core structure cell wall is in *M. leprae,* largely through comparison with other mycobacteria. The detailed structure of the core of *M. leprae* cell wall is very similar to that of *M. tuberculosis.* It has peptidoglycan with a special linker region, rhamnose, and acetyl glucosamine phosphate. The biosynthesis of this could provide information on new drug targets and the development of new drugs which could be effective against leprosy and tuberculosis. Extending from this linker region is a linear galactan, and attached to this are three chains of the arabinan, and at the ends of the arabinans are the mycolic acids. We have recently demonstrated the biosynthesis of this, and this information has given us the means by which we can start to use the biosynthesis of the cell wall core as an assay and begin to test potential new drugs which might be effective against mycobacterial infections. The synthesis of this core takes place on the carrier lipid through the addition of the individual sugars of the linker region (N-acetylglucosamine-P and rhamnose) donated by the respective nucleotide sugars. This is followed by the addition of galactose donated by its nucleotide sugar, to result in the long, linear galactan chain. This all happens while this molecule is still attached to a carrier lipid, which allows the transport of the newly synthesized cell wall across the plasma membrane of *M. leprae.* In the final steps, the arabinose is donated by a separate carrier lipid, resulting in growth of the arabinan, and then somehow or other this is transferred from the carrier lipid to the peptidoglycan and there is addition of mycolic acids. We know little about these steps, except we know that the antigen 85 complex catalyzes the transfer of the mycolic acid from a mycolyl carrier lipid into the final cell wall. Accordingly, we now have a good idea of the basic structure and the biosynthesis of the cell wall of *M. leprae* and other mycobacteria. In the case of *M. leprae,* the basic cell membrane, in terms of its phospholipid composition, is very similar to *M. tuberculosis.* Structural work on the peptidoglycan has revealed that it contains a glycine residue instead of L-alanine, the consequence of which is unknown. In summary, the cell wall of *M. leprae* contains a special linker region which is a very good drug target in the case of all mycobacterial infections. Attached to that is a linear galactan; attached to that is the arabinan and then the mycolic acid. Where *M. leprae* distinguishes itself is in the nature of the complementing glycolipids. In *M. leprae,* these glycolipids are very much dominated by the phenolic glycolipids and their relatives.

The Proteome of M. leprae.

The proteins, or proteomics or protein mapping, of *M. leprae* is an ongoing area of research with Cristina Pessolani and Angela Marques. Our former approach was to fractionate *M. leprae* and from this obtain crude cell wall, cytosol and membrane fractions. Earlier work involved the application of poly-acrylamide gel electrophoresis and subsequent biochemistry to cytosol. From this, we were able to recognize the three , four , or now five major cytosolic proteins of *M. leprae.* Major cytosolic protein 1 is a truncated protein; major cytosolic protein 2 is an 18 kDa protein; major cytosolic protein 3 is a 28 kDa protein. We were also able to examine the membranes and using simple Triton were able to separate the LAM from the major membrane proteins. We were able to identify two major membrane proteins, which we called major membrane protein I

(MMP-I) and major membrane protein II (MMP-II). Cristina Pessolani, while she was in my lab, identified MMP-ll and purified it by classical biochemical means. She did partial amino acid analysis on the protein and recognized the gene within the data bank as a bacterioferritin by comparison with the bacterioferritins of other organisms. She then did elegant biochemistry on this molecule and established that bacterioferritin existed in an aggregate multi-molecular weight of 390 kDa and the subunit molecular weight was 18.2 kDa. It existed as a 20 mer; 20 of the subunits aggregated to give the final native molecule. We also established that this contained 1000-4000 iron atoms per aggregate, i.e., it was a bacterioferritin, and therefore was a sequester of iron. Why should there be so much of that particular protein in *M. leprae?* Is it because the obligate nature of *M. leprae* is to have a very effective bacterioferritin which then is capable of sequestering the majority of iron leading to iron deprivation in the host? We continue to work on the answers to these questions.

The second, more classical development conducted by Cristina and Angela was to take *M. leprae* subfractions and then do 2-D gel electrophoresis and transfer the spots onto sequencing membranes, do partial amino acid sequence on these spots and find the gene within the gene bank. We did immunoblotting of these gels with known antibodies, building up maps of the protein profile of *M. leprae.* From this approach, we identified MMP-I; 65 kDa GroEL; 71 kDa and 8 kDa proteins; 28 kDa superoxide dismutase; etc., and we were able to identify many new proteins expressed in *M. leprae* in this way. Because *M. leprae* is an obligate intracellular organism, we believe that these are all important in terms of the pathogenesis of leprosy and the survival of *M. leprae.*

n extension of this approach was used by Angela Marques in which 2D PAGE protein profiles of highly purified cell wall were generated, the individual proteins removed, N-group analysis conducted, and then the genes recognized within the databank. From this approach, we have recognized several new proteins in *M. leprae,* e.g., the L7/L12 ribosomal protein, a hydroperoxide reductase, thiosulfate sulfurtransferase, all of which are strongly expressed in relatively large amounts in *M. leprae.* The significance of all of these is unknown, but they may have something to do as antidotes to the oxidative effects of other enzymes. Some may be reductants; more may preserve the sulfur regions within the membrane proteins involved in electrontransport.

The latest of these proteins is very interesting. It is called the 34 kDa protein. It shows considerable homology to the 34 kDa of *M. paratuberculosis.* The intriguing feature about this protein is the presence of cysteine and the presence of a signal sequence. This protein is found in *M. leprae* without the signal sequence, in which case the cysteine residue is the first amino acid. We have been able to compare the region adjacent to this cysteine for other bacteria. Cysteine is indicative of a lipoprotein consensus sequence such as the consensus sequence of Braun's lipoprotein, very similar to the lipoprotein consensus sequence of 38 kDa antigen of *M. leprae* and *M. tuberculosis* and the 19 kDa protein of *M. tuberculosis.* However, in the case of the *M. leprae* sequence, there is a histidine instead of a glycine, and, in the minus two position, there is a leucine instead of a small amino acid, such as an alanine or a serine. The result of this is that *M. leprae* express a non-lipidated 34 kDa with an aborted lipoprotein consensus signal. There is something about the upstream part of the signal region that deters it from being lipidated, and this is quite intriguing as to why or how or what are the consequences of this.

We have now mapped about 30 or 40 different proteins of *M. leprae,* so we are moving toward a full, complete understanding of the entire protein profile of *M. leprae* as it is obtained from the infected tissue. Based on the identity and function of these proteins, we know something about their physiological role and their role in infectivity. There is an extraordinary amount of the chaperonin or the usher proteins or the heat-shock proteins in *M. leprae.* Considerable amounts of these are found in the cell wall. We have identified the secreted proteins, and these may possibly be involved in fibronectin binding, but they have a key role as mycolyl transferases, i.e., in synthesis of the cell wall. We have identified several of the proteins involved in iron metabolism, bacterioferritin, nine repressible protein, 28 kDa pro-

tein, the function of which we don't know, and ribosomal proteins. Why they are expressed so strongly is not obvious. Some intriguing proteins, the sulfate sulfurtransferase, and the major membrane proteins, particularly the 35 kDa protein, do not show any homology to any other proteins in *M. tuberculosis.*

Future

Given this really impressive work on the cell wall of *M. leprae,* the protein profile of *M. leprae,* on LAM and other glycolipids and their roles in unconventional T-cell response, where do we go, what do we do, what about the future? Is there a future at all for the basic type of research such as we practice and we advocate? Given the global leprosy situation, do we have a need or a disease to deal with? We cannot do our work without research money, and we cannot get research money without a disease. This issue has affected the global status of leprosy research and is having a profound effect on funding and the import of young people into this rather exciting area.

At a recent meeting in Thailand, we set research priorities in the context of fundamental research and also in the context of the disease. Some of the priorities listed dealt with prevention and treatment of reactions and nerve damage, detection of subclinical infection by *M. leprae,* sequencing of the *M. leprae* genome, identifying predictors of relapse and the epidemiology of relapse, strategies for monitoring nerve damage, methods for early assessment of paucibacillary disease, optimal organization for early diagnosis and prompt treatment, common chemotherapy regimens for MB and PB leprosy, an effective vaccine, and case definition.

I have come up with my own research priorities. Foremost is definition of the genome and the phenotype, not necessarily for reasons of disease but for reasons of the sheer scientific challenge of defining an obligate intracellular organism derived from host tissue. Tests are important and relevant to all of this work tests of a disease that has a high incidence and that is desperately in need of sensitive assays. Vaccine development is important, using leprosy as a target for a vaccine although it may be derived from our much - funded tuberculosis research.

What tests do we have? We have those based on detection of bacterial components such as PGL-I (future work should address greater sensitivity), those based on host response to bacterial proteins, humoral immune response, PGL-I, LAM, antigen 85. These tests are becoming more facile, and, with the dipstick IgM assay, they are becoming more sensitive. Tests based on cell-mediated immune response must be relied upon to detect single-lesion leprosy and to detect tuberculoid leprosy. For that reason, we have begun a project on new skin test antigens. PPD is a very useful diagnosis/monitoring tool in the context of tuberculosis, and the prime immune response in leprosy is the cell-mediated immune response. We have a strategy for how to proceed: develop products suitable for human use, capable of getting FDA approval, start with complex mixtures and then take the reductionist approach down to individual protein antigens that have just been described. There are two new, complex leprosy skin test antigens in the pipeline. We hope to get permission to test these in human volunteers within Colorado before they are tried anywhere else.

The final great challenge is the *M. leprae* genome. Quoting Douglas Young, "I do think that knowledge of the whole genome is going to break open basic leprosy research." **I** think that sequencing and interpreting the whole *M. leprae* genome will galvanize the whole area of leprosy research. I think it will be one of the most exciting developments, far surpassing the isolation of *M. leprae,* the recognition of *M. leprae* in armadillos, far ahead of PGL-I; the potential is enormous.

Where do we stand with the *M. leprae* genome? One of the most important and exciting things about it is its size, only about 2.8 megabases *vs.* 4.2 for *M. tuberculosis.* So the key question is not what is there, but what is missing. About 1.7 megabases have been sequenced, i.e., about 60% of the sequencing has been completed; about 1.1 megabases need to be completed. The Heiser Foundation has donated about $700,000 to the Sanger Center to complete the project. Dr. Barrel has predicted that the project will be completed by the end of this year. Potential applications are profound.

A closing thought: with a communicable disease, the closer a program gets to eliminating a disease, the more likely it is that the program is eliminated rather than the disease.

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