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MINIREVIEW

Leprosy, Tuberculosis, and the New Genetics

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INTRODUCTION

During the first half of this century, tuberculosis was one of the most intensively researched infectious diseases, engaging the attention of the most able physicians and microbiologists. With the introduction of streptomycin in 1945 and of isoniazid (INH) in 1952, the imperative for tuberculosis research decreased. Together with improved living standards in developed countries, chemotherapy seemed to hold the key to tuberculosis control, suggesting that efficient implementation of existing measures could lead to elimination of the disease. However, although for many years the incidence of tuberculosis dropped steadily in developed countries, it continued to exact a high mortality in the developing world (39), and the last few years have witnessed a revival of tuberculosis in the United States (3, 47). Infection with human immunodeficiency virus is associated with increased susceptibility to tuberculosis and with accelerated progression of disease and mortality (2), prompting fears of a new era of uncontrolled increase in tuberculosis worldwide. There is an urgent need for new tools to assist in the fight against tuberculosis. In contrast, the situation is more encouraging with respect to leprosy, which has always attracted the attention of a small number of dedicated enthusiasts, as there is real hope that the disease can be eliminated as a public health problem before the end of the millenium.

The challenge for basic researchers is to apply modern molecular approaches to readdress the questions of mycobacterial pathogenesis left unsolved at the start of the chemotherapy era. The age of active tuberculosis research preceded the development of modern bacterial genetics, and the present minireview focuses on the scope for applying molecular genetics in understanding fundamental aspects of mycobacterial pathogens and their interactions with their mammalian hosts. Paradoxically, it was leprosy research which initiated the application of molecular genetics to mycobacteria. *Mycobacterium leprae*—the leprosy bacillus—has yet to be grown in laboratory culture, but the availability of bacteria isolated from infected armadillos in the early 1980s stimulated an active research effort coordinated by the World Health Organization Programme for the Immunology of Leprosy. Attempts to characterize defined antigenic components of *M. leprae* led to cloning and expression of mycobacterial genes in *Escherichia coli* (7, 62) and initiated the era of mycobacterial molecular genetics. Subsequently, the goal of constructing a new generation of recombinant vaccines based on *Mycobacterium bovis* BCG (bacillus Calmette-Guérin, an attenuated strain of bovine tuberculosis) promoted development of techniques for introducing and expressing genes in mycobacteria, opening up the possibilities for genetic manipulation of mycobacteria described below.

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MYCOBACTERIAL GENES AND GENOMES

The first genes to be cloned from mycobacteria were those encoding proteins recognized by monoclonal antibodies and by T lymphocytes involved in the immune response to infection (13, 61, 62). More than 50 such antigen-encoding genes have now been characterized, and in several cases, sequence analysis has allowed identification of the functional role of the encoded protein (58, 60). Notably, a number of prominent antigens have been identified as members of conserved heat shock protein families (33, 59). Interest has moved on to the characterization of the genome structures of mycobacterial pathogens, with the construction of contig maps of *M. leprae* (12) and *Mycobacterium tuberculosis* chromosomes (Table 1). Genome sequencing projects are being actively pursued, and several cosmids from *M. leprae* have already been completely sequenced (22, 44a). In addition to providing information about chromosomal organization, information from the sequencing projects will provide invaluable support to efforts to characterize biochemical and immunogenic features of mycobacteria by identifying genes encoding relevant antigens and enzymes.

FINGERPRINTS AND EPIDEMIOLOGY

An immediate application for research on genome structure has resulted from the discovery of repetitive DNA elements, which allows identification of strain-specific fingerprint patterns. While it has long been recognized that there are differences among individual clinical isolates of *M. tuberculosis*, as evidenced by their growth patterns in guinea pigs and by a limited diversity in phage sensitivity, DNA fingerprinting has provided the first epidemiologically useful tool for monitoring the spread of individual strains of *M. tuberculosis*. Outbreaks of disease can be traced to a single source in some instances, for example, and the question of whether renewed onset of disease in a previously treated patient results from reactivation of the original infection or from reinfection with a new strain can now be addressed (9, 52).

At least two strain-specific repetitive DNA markers, IS*6110* (or IS*986*) and IS*1081*, have been identified in the tuberculosis complex (8, 32, 51), and one, RLEP, has been found in *M. leprae* (6, 17, 56). A third repetitive sequence, the major polymorphic tandem repeat, has also been described in *M. tuberculosis* but is present in several other

TABLE 1. Characteristics of mycobacterial genomes

Mycobacterial species	Estimated genome size (Mb)	No. of contigs	No. of loci mapped	Amt (Kb) of DNA sequenced	Repetitive sequence(s)	dG+dC content (%)
M. leprae	≈2.8[a]	4	75	>220	RLEP	56
M. tuberculosis	≈4.2[b]	40	82	<20	IS*6110* (IS*986*), IS*1081*, MPTR[c]	66

[a] Estimated from contig sizes.
[b] Based on pulsed-field gel electrophoresis analysis.
[c] MPTR, major polymorphic tandem repeat.

mycobacteria (20). Analysis of *M. tuberculosis* elements IS*6110* and IS*1081* revealed characteristics typical of bacterial insertion sequences, and indirect evidence for transposition of IS*6110* has been obtained from the sequences of several copies from a clinical isolate, as the element was flanked, in each case, by 3- or 4-bp duplications of the target sequences (29a). It is thus most likely that the strain diversity revealed by IS*6110*-mediated restriction fragment length polymorphisms does in fact reflect the ability of these sequences to move around the mycobacterial chromosome, although movement appears to be a relatively infrequent event (52). A family of 29 dispersed repeats, RLEP, which vary in size from 545 to 732 bp, has been found in *M. leprae*, but these repeats do not seem to correspond to insertion sequences and no positional polymorphisms have been observed in isolates of different geographical origins (56; also unpublished data).

Repetitive elements have also provided an important target in the development of tests for detection of mycobacteria in clinical specimens based on the polymerase chain reaction. Diagnosis of infection currently relies on detection of acid-fast mycobacteria in microscopic smears or by more-sensitive, though time-consuming, bacterial cultures. Culture of *M. tuberculosis* can take from 4 to 6 weeks, and the goal of developing rapid tests based on DNA amplification has important clinical applications (50). Similar RLEP-based polymerase chain reaction tests have been developed for the detection of *M. leprae* in human biopsy material (55).

MYCOBACTERIA AND THE IMMUNE RESPONSE

A complex and intimate interaction with the host immune system is a characteristic feature of mycobacterial infection. The clinical features of leprosy vary widely, depending on the immune response of the patient, for example, and unregulated immunological reactions contribute significantly to the pathological manifestations of tuberculosis. Consequently, attempts to understand and eventually to manipulate the immune response to mycobacteria hold a prominent position in mycobacterial research.

Recombinant BCG. In the 1920s, Calmette and Guérin attenuated a virulent strain of *M. bovis* by serial passage in laboratory medium and found that the resulting bacillus could vaccinate against subsequent exposure to virulent *M. tuberculosis* in animal models. Clinical trials demonstrated that their vaccine, BCG, could also protect against tuberculosis in some human populations (British schoolchildren, for example), although trials in other areas where tuberculosis is highly endemic failed to show protection against the predominant adult forms of the disease (14). Variable, though significant protection against leprosy has also been reported as a result of BCG vaccination (14). Widespread use of BCG has demonstrated its safety, and its potent immunogenicity has led to a suggested use as a carrier to vaccinate against other diseases (25). Appropriate vectors utilizing the replication origin from a mycobacterial plasmid (42, 45, 46) or the chromosomal integration system from a mycobacterial phage have been developed and used to introduce foreign antigens into BCG (28, 49). Such recombinant BCG vaccines have been shown to induce a broad spectrum of immune responses in experimental animals and hold exciting promise as a new generation of human vaccines (1, 49, 54). The addition of appropriate mycobacterial antigens might improve the effectiveness of BCG as an antituberculosis vaccine, and as noted above, considerable effort has been directed to the characterization of mycobacterial proteins involved in immune recognition.

Mycobacterial antigens. A broad spectrum of mycobacterial antigens has been defined, including conserved and species-specific proteins found in the cytoplasm and cell wall and in extracellular growth medium (for a recent survey, see reference 60). Some of these antigens have been made available in large amounts by biochemical fractionation or by application of recombinant DNA expression technology and are under active immunological investigation. In addition to their possible application as subunit vaccines, the use of defined antigens as probes for analysis of the immune status of different individuals is an area of active research interest. Measurement of immune responses to whole mycobacteria or to complex mycobacterial extracts, such as purified protein derivative, fails to distinguish individuals with active disease from those who have been exposed to infection but have no clinical symptoms. It is possible that detailed study of responses to defined antigens will identify immunological parameters which correlate with protective immunity and may subsequently allow the development of tests for detection of individuals at risk of developing clinical disease. Sequence analysis of antigen-encoding genes in concert with synthetic peptide chemistry has a major role in such detailed immunological characterization (19, 27, 34, 53).

Attenuated vaccines? An alternative approach to development of an improved BCG vaccine is to generate further attenuated mycobacterial strains. Pathogenic mycobacteria resemble certain *Salmonella* species in being able to survive within host phagocytic cells and it can be proposed that as with *Salmonella* species, different routes of genetic attenuation may have dramatic differences with regard to vaccine potential. For example, while auxotrophs of *Salmonella typhimurium* generated by lesions in the aromatic biosynthesis pathway are effective as vaccines (21), the corresponding purine auxotrophs fail to induce appropriate immunization (40), suggesting that the precise mode of bacterial death may be critical in delivery of the correct immunological stimulus. The goal of generating attenuated mutants of *M. tuberculosis* has yet to be achieved, however. Transposons that are functional in mycobacteria have been demonstrated, but the frequency of transposition obtained so far has been insufficient to permit efficient generation of mutant strains (30).

Introduction of mutations by gene replacement has been achieved in a rapidly growing mycobacterium, *Mycobacterium smegmatis* (24), but attempts to apply a similar strategy for inactivation of *aro* genes in *M. tuberculosis* have been unsuccessful (14a). Random insertion of DNA into the chromosome of *M. tuberculosis* has been reported, leading to the suggestion that homologous recombination may be a relatively inefficient process in slowly growing mycobacteria (26). It may be relevant that the *M. tuberculosis recA* gene has a highly unusual structure (10). The lack of efficient methods for generation of defined mutations in *M. tuberculosis* currently imposes a major constraint in the development of mycobacterial genetics.

MYCOBACTERIAL VIRULENCE

Mycobacterial pathogens induce chronic disease evolving over a period of years, with pathological damage resulting largely from aberrant activities of the host immune system rather than from release of potent toxins by the bacteria. Mycobacterial virulence, therefore, is related more to their ability to persist in tissues and to activate the immune system than to the direct damage of host cells. Resistance to killing by host immune functions is in part a reflection of the structure of the mycobacterial cell. Mycobacteria synthesize a characteristically lipid-rich cell wall and often an extracellular lipid, or glycolipid, capsule which undoubtedly confers some protection against the damaging effects of complement components and phagocyte-derived free radicals (5). In addition to such inherent resistance mechanisms, other factors are involved in mycobacterial virulence. Attenuated variants of originally virulent isolates (such as BCG and *M. tuberculosis* H37Ra) retain their characteristic ultrastructural features but show considerable reduction in their pathogenicity. More subtle differences in virulence are found in some clinical isolates of *M. tuberculosis* which lose their ability to cause disseminated disease in guinea pigs (38). Strains which acquire resistance to INH, for example, often display reduced virulence in guinea pigs (35, 36, 48). In 1955, Dubos suggested that "virulence is not a qualitative, all-or-none characteristic, but an attribute which varies quantitatively from one strain of mycobacterium to another" (11). Establishment of a molecular basis for such observations presents a formidable challenge for the new mycobacterial genetics.

Adaptation and transcriptional regulation. In the same article in 1955 (11), Dubos suggested that virulence could be considered as an "ecological concept" reflecting the ability of the organism to adapt to changing environmental conditions (11). This is clearly in line with current ideas of bacterial pathogenesis (37), and a search for environmentally induced changes in mycobacterial gene expression may provide clues to virulence mechanisms. Little is known about gene regulation in mycobacteria. The major heat shock genes have been characterized and increased transcription has been demonstrated in response to heat shock (41, 49). It has yet to be shown whether as for *S. typhimurium* (4), these genes are also induced on entry into host phagocytic cells. By analogy with *E. coli*, it seems likely that the mycobacterial heat shock response will be regulated by altered levels of particular RNA polymerase sigma subunits (15), but this regulation has yet to be demonstrated in mycobacteria, and preliminary transcriptional data indicate a difference at least in consensus promoter sequences (27a, 49). A further striking difference from *E. coli* is evidenced by the fact that as in *Streptomyces* species (18), mycobacteria have two genes encoding members of the GroEL heat shock family (43). Characterization of transcriptional changes associated with entry into host cells could assist in understanding mycobacterial virulence and may also identify novel antigens important in the immune response to live infection.

Persistence and the stationary phase. The major adult form of tuberculosis is often the result of reactivation of an old lesion, in which bacteria had apparently remained in a dormant form for many years following initial control of primary infection. The ability to persist in tissues for long periods is an important aspect of mycobacterial virulence and underlies the need for prolonged chemotherapy to completely eliminate the disease.

Although pathogenic mycobacteria have extremely slow growth rates, they were among the first microbes to be identified as agents of disease. Obviously, such redoubtable microbiologists as Koch and Hansen were not troubled by this intriguing trait! *M. tuberculosis* has a doubling time of around 24 h, and *M. leprae*, which fails to grow in vitro, has an estimated generation time of around 2 weeks in infected tissues (29). One might anticipate that a more rapidly dividing variant would soon outgrow the parent strain, and the absence of such variants suggests that the evolutionary strategy of mycobacterial pathogens includes a strong selective advantage in favor of slow growth. Clearly, in order to survive, a successful parasite should not perturb its host unnecessarily. Mycobacterial pathogens obviously differ from *E. coli* in their approach to regulation of cell division, and exponentially growing *E. coli* may not, therefore, represent the most appropriate model system when studying gene regulation in mycobacteria. The normal physiological state of these bacteria may more closely resemble that of *E. coli* in the stationary phase of growth. On entry into the stationary phase, patterns of gene expression in *E. coli* alter, making the bacteria better able to resist starvation and environmental stress, with an accompanying reduction in bacterial multiplication (31, 44). Perhaps mycobacterial survival depends on the ability to remain in a condition resembling the stationary phase of *E. coli*, with cell division relegated to a priority secondary to that of cell survival. Recent progress in elucidating stationary phase-related changes in *E. coli* gene expression (44) may provide insights into mechanisms relevant for mycobacterial survival and virulence. The dormant forms of mycobacteria thought to be involved in long-term persistence may represent an extreme form of stationary phase, in which the entire efforts of the organism are directed toward survival, with the temporary loss of replicative ability. Molecular analysis of dormant mycobacteria will be of particular interest, and there is a need to develop reproducible animal and in vitro models for generating such forms.

DRUGS AND DRUG RESISTANCE

Although chemotherapy has been the mainstay of mycobacterial control programs for 40 years, we now find ourselves in a paradoxical situation. In the 1950s, chemotherapy had a massive impact on tuberculosis, reducing both its incidence and transmission. In contrast, initial efforts to control leprosy involved dapsone monotherapy and were only modestly successful as problems of drug resistance soon emerged. The 1980s have witnessed a dramatic turnaround in the situation, as the implementation of multidrug therapy (clofazamine, dapsone, and rifampin) by the World Health Organization has resulted in striking improvements (16, 57). The rationale behind multidrug therapy, evolved on

the basis of experience with development of antituberculosis regimens, is that the few mutants that emerge which are resistant to a single drug will be killed by the other two components. There is now reasonable hope that the incidence of leprosy in the main countries in which leprosy is endemic can be reduced to less than 1 in 10,000 by the year 2000. Ironically, at the same time, there have been alarming increases in the prevalence of tuberculosis, mainly resulting from the impact of the AIDS epidemic, although declining social conditions have also contributed (3). The most worrying feature of what has been referred to as the new tuberculosis (47) has been highlighted by recent outbreaks of multiple-drug-resistant isolates of *M. tuberculosis* in the United States, exposing an urgent need for the development of new drugs and for rapid tests for drug resistance (3, 47). It is of particular concern that drug-resistant strains frequently fail to respond to rifampin and INH, the two key front-line antituberculosis drugs.

There has been relatively little progress in understanding molecular mechanisms of drug action in mycobacteria in the last 40 years or in elucidating pathways of drug resistance. The absence of such information is regrettable, as it would enable rapid screening for resistance to be developed in addition to providing new leads for the design of alternative chemotherapeutic agents. Rifampin inhibits RNA polymerase, and in *M. leprae*, drug resistance stems from missense mutations in the *rpoB* gene encoding the β subunit of the enzyme (23). The corresponding gene has been cloned from *M. tuberculosis*, and sequence analysis of rifampin-resistant strains should reveal whether the same mechanism is involved or whether differences in drug permeability also play a role in resistance. INH is a much more selective drug, active only against mycobacteria, with *M. tuberculosis* being particularly susceptible. It has long been known that INH-resistant isolates have reduced levels of catalase activity, but the nature of the link between catalase and INH has remained obscure. A recent genetic approach has shown that transformation with the *M. tuberculosis* catalase gene, which is deleted from the chromosome of some INH-resistant isolates, restores susceptibility to INH in resistant mutants of *M. smegmatis* and *M. tuberculosis* (63, 64) and even confers susceptibility to high concentrations of INH in certain strains of *E. coli*. The catalase enzyme has associated peroxidase activity, and it is possible that this activity is responsible for conversion of the drug into a toxic derivative within the bacterium. Application of molecular genetic techniques to the study of drug action will point the way to future development of new drugs and of tests for detection of resistant strains.

CONCLUDING REMARKS

While effective control of leprosy may be a realistic prospect for the year 2000, tuberculosis, considered a disease of poverty and of the past, has returned with a vengeance. The problems of understanding the complex interactions between mycobacteria and their mammalian hosts which occupied previous generations of researchers must now be faced once more. Can we exploit the progress in molecular genetics over the intervening decades to provide new perspectives on these problems? Perhaps with the new techniques at our disposal we will be able to discover the mechanisms which allow *M. tuberculosis* to survive in the hostile environment of host macrophages and to uncover the molecular basis of the strong affinity between *M. leprae* and Schwann cells, which underlies the nerve damage characteristic of leprosy. Study of the genetics and physiology of mycobacterial pathogens represents a conceptually demanding and exciting challenge for modern bacteriology.

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REFERENCES

1. **Aldovini, A., and R. A. Young.** 1991. Humoral and cell-mediated immune responses to live recombinant BCG-HIV vaccines. Nature (London) **351**:479–482.
2. **Barnes, P., A. B. Bloch, P. T. Davidson, and D. E. Snider.** 1991. Tuberculosis in patients with immunodeficiency virus infection. N. Engl. J. Med. **320**:545–550.
3. **Bloom, B. R., and C. J. L. Murray.** 1992. Tuberculosis: commentary on a reemergent killer. Science **257**:1055–1064.
4. **Buchmeier, N. A., and F. Heffron.** 1990. Induction of *Salmonella* stress proteins upon infection of macrophages. Science **248**:730–732.
5. **Chan, J., T. Fujiwara, P. Brennan, M. McNeil, S. J. Turco, J.-C. Sibille, M. Snapper, P. Aisen, and B. R. Bloom.** 1989. Microbial glycolipids, possible virulence factors that scavenge oxygen radicals. Proc. Natl. Acad. Sci. USA **86**:2453–2457.
6. **Clark-Curtiss, J. E., and M. A. Docherty.** 1989. A species-specific repetitive sequence in *Mycobacterium leprae* DNA. J. Infect. Dis. **159**:7–15.
7. **Clark-Curtiss, J. E., W. R. Jacobs, M. A. Docherty, L. R. Ritchie, and R. Curtiss.** 1985. Molecular analysis of DNA and construction of genomic libraries of *Mycobacterium leprae*. J. Bacteriol. **161**:1093–1102.
8. **Collins, D. M., and D. M. Stephens.** 1991. Identification of an insertion sequence, IS1081, in *Mycobacterium bovis*. FEMS Microbiol. Lett. **83**:11–16.
9. **Daley, C. L., P. M. Small, G. F. Schecter, G. K. Schoolnik, R. A. McAdam, W. R. Jacobs, and P. C. Hopewell.** 1992. An outbreak of tuberculosis with accelerated progression among persons infected with the human immunodeficiency virus. An analysis using restriction-fragment-length-polymorphism. N. Engl. J. Med. **326**:231–235.
10. **Davis, E. O., S. J. Sedgwick, and M. J. Colston.** 1991. Novel structure of the *recA* locus of *Mycobacterium tuberculosis* implies processing of the gene product. J. Bacteriol. **173**:5653–5662.
11. **Dubos, R. J.** 1955. Properties and structures of tubercle bacilli concerned with their pathogenicity. Symp. Soc. Gen. Microbiol. **5**:103–125.
12. **Eiglmeier, K., N. Honoré, S. A. Woods, B. Caudron, and S. T. Cole.** Use of an ordered cosmid library to deduce genomic organisation of *Mycobacterium leprae*. Mol. Microbiol., in press.
13. **Engers, H. D., V. Houba, J. Bennedsen, T. M. Buchanan, S. D. Chaparas, G. Kadival, O. Closs, J. R. David, J. D. A. van Embden, T. Godal, S. A. Mustafa, J. Ivanyi, D. B. Young, S. H. E. Kaufmann, A. G. Khomenko, A. H. J. Kolk, M. Kubin, J. A. Louis, P. Minden, T. M. Shinnick, L. Trnka, and R. A. Young.** 1986. Results of a World Health Organization-sponsored workshop to characterize antigens recognized by mycobacterium-specific monoclonal antibodies. Infect. Immun. **51**:718–720.
14. **Fine, P. E. M., and L. C. Rodrigues.** 1990. Modern vaccines. Mycobacterial diseases. Lancet **335**:1016–1020.

14a. **Garbe, T. R., and D. B. Young.** Unpublished data.

15. **Gross, C. A., D. B. Strauss, J. W. Erikson, and T. Yura.** 1990. The function and regulation of heat shock proteins in *Escherichia coli*, p. 167–189. *In* R. I. Morimoto, A. Tissieres, and C. Georgopoulos (ed.), Stress proteins in biology and medicine.

Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
16. **Grosset, J. H., and B. Ji.** 1990. Recent advances in the chemotherapy of leprosy. Lepr. Rev. **61:**313–329.
17. **Grosskinsky, C. M., W. R. Jacobs, Jr., J. E. Clark-Curtiss, and B. R. Bloom.** 1989. Genetic relationships among *Mycobacterium leprae*, *Mycobacterium tuberculosis*, and candidate leprosy vaccine strains determined by DNA hybridization: identification of an *M. leprae*-specific repetitive sequence. Infect. Immun. **57:**1535–1541.
18. **Guglielmi, G., P. Mazodier, C. J. Thompson, and J. Davies.** 1991. A survey of the heat shock response in four *Streptomyces* species reveals two *groEL*-like genes and three GroEL-like proteins in *Streptomyces albus*. J. Bacteriol. **173:**7374–7381.
19. **Harris, D. P., H. M. Vordermeier, E. Roman, R. Lathigra, S. J. Brett, C. Moreno, and J. Ivanyi.** 1991. Murine T cell-stimulatory peptides from the 19-kDa antigen of *Mycobacterium tuberculosis*. J. Immunol. **147:**2706–2712.
20. **Hermans, P. W. M., D. van Soolingen, and J. D. A. van Embden.** 1992. Characterization of a major polymorphic tandem repeat in *Mycobacterium tuberculosis* and its potential use in the epidemiology of *Mycobacterium kansasii* and *Mycobacterium gordonae*. J. Bacteriol. **174:**4157–4165.
21. **Hoiseth, S. K., and B. A. D. Stocker.** 1981. Aromatic-dependent *Salmonella typhimurium* are nonvirulent and are effective live vaccines. Nature (London) **241:**238–239.
22. **Honoré, N., S. Chanteau, F. Doucet-Populaire, K. Eiglmeier, T. Garnier, C. Georges, P. Launois, P. Limpaiboon, S. Newton, K. Nyang, P. del Portillo, G. K. Ramesh, T. Reddy, J. P. Riedel, N. Sittisombut, S. Wu-Hunter, and S. T. Cole.** Nucleotide sequence of the first cosmid from the *Mycobacterium leprae* Genome Project: structure and function of the Rif-Str regions. Mol. Microbiol., in press.
23. **Honoré, N., and S. T. Cole.** The molecular basis of rifampin resistance in *Mycobacterium leprae*. Submitted for publication.
24. **Husson, R. N., B. E. James, and R. A. Young.** 1990. Gene replacement and expression of foreign DNA in mycobacteria. J. Bacteriol. **172:**519–524.
25. **Jacobs, W. R., R. Tuckman, and B. R. Bloom.** 1987. Introduction of foreign DNA into mycobacteria using a shuttle phasmid. Nature (London) **327:**532–535.
26. **Kalpana, G. V., B. R. Bloom, and W. R. Jacobs.** 1991. Insertional mutagenesis and illegitimate recombination in mycobacteria. Proc. Natl. Acad. Sci. USA **88:**5433–5437.
27. **Lamb, J. R., J. Ivanyi, A. D. M. Rees, J. B. Rothbard, K. Howland, R. A. Young, and D. B. Young.** 1987. Mapping of T cell epitopes using recombinant antigens and synthetic peptides. EMBO J. **5:**1245–1249.
27a. **Lathigra, R. B., C. K. Stover, and D. B. Young.** Unpublished data.
28. **Lee, M. H., L. Pascopella, W. R. Jacobs, and G. F. Hatfull.** 1991. Site-specific integration of mycobacteriophage L5: integration-proficient vectors for *Mycobacterium smegmatis*, *Mycobacterium tuberculosis*, and bacille Calmette-Guérin. Proc. Natl. Acad. Sci. USA **88:**3111–3115.
29. **Lévy, L.** 1976. Studies of the mouse footpad technique for cultivation of *Mycobacterium leprae*. III. Doubling time during logarithmic multiplication. Lepr. Rev. **47:**103–106.
29a. **Martin, C., and B. Gicquel.** Personal communication.
30. **Martin, C., J. Timm, J. Rauzier, R. Gomez-Lus, J. Davies, and B. Gicquel.** 1990. Transposition of an antibiotic resistance element in mycobacteria. Nature (London) **345:**739–743.
31. **Matin, A., E. A. Auger, P. H. Blum, and J. E. Schultz.** 1989. Genetic basis of starvation survival in nondifferentiating bacteria. Annu. Rev. Microbiol. **43:**293–316.
32. **McAdam, R. A., P. W. M. Hermans, D. van Soolingen, Z. F. Zainuddin, D. Catty, J. D. A. van Embden, and J. W. Dale.** 1990. Characterization of a *Mycobacterium tuberculosis* insertion sequence belonging to the IS3 family. Mol. Microbiol. **4:**1607–1613.
33. **Mehra, V., B. R. Bloom, A. C. Bajardi, C. L. Grisso, P. A. Sieling, D. Alland, J. Convit, X. Fan, S. W. Hunter, P. J. Brennan, T. H. Rea, and R. L. Modlin.** 1992. A major T cell antigen of *Mycobacterium leprae* is a 10-kD heat-shock cognate protein. J. Exp. Med. **175:**275–284.
34. **Mehra, V., D. Sweetser, and R. A. Young.** 1986. Efficient mapping of protein antigenic determinants. Proc. Natl. Acad. Sci. USA **83:**7013–7017.
35. **Middlebrook, G.** 1954. Isoniazid-resistance and catalase activity of tubercle bacilli. Am. Rev. Tuberc. **69:**471–472.
36. **Middlebrook, G., M. L. Cohn, and W. B. Schaefer.** 1954. Studies on isoniazid and tubercle bacilli. III. The isolation, drug-susceptibility and catalase-testing of tubercle bacilli from isoniazid-treated patients. Am. Rev. Tuberc. **70:**852–872.
37. **Miller, J. F., J. J. Mekalanos, and S. Falkow.** 1989. Co-ordinate regulation and sensory transduction in the control of bacterial virulence. Science **243:**916–922.
38. **Mitchison, D. A., J. B. Selkon, and J. Lloyd.** 1963. Virulence in the guinea pig, susceptibility to hydrogen peroxide, and catalase activity of isoniazid-sensitive tubercle bacilli from South Indian and British patients. J. Pathol. Bacteriol. **86:**377–386.
39. **Murray, C. J. L., K. Styblo, and A. Rouillon.** 1990. Tuberculosis in developing countries: burden, intervention and cost. Bull. Int. Union Tuberc. **65:**6–24.
40. **O'Callaghan, D., D. Maskell, F. Y. Liew, C. S. F. Easmon, and G. Dougan.** 1988. Characterization of aromatic- and purine-dependent *Salmonella typhimurium*: attenuation, persistence, and ability to induce protective immunity in BALB/c mice. Infect. Immun. **56:**419–423.
41. **Patel, B. K. R., D. K. Banerjee, and P. D. Butcher.** 1991. Characterization of the heat shock response in *Mycobacterium bovis* BCG. J. Bacteriol. **173:**7982–7987.
42. **Ranes, M. G., J. Rauzier, M. Lagranderie, M. Gheorghiu, and B. Gicquel.** 1990. Functional analysis of pAL5000, a plasmid from *Mycobacterium fortuitum*: construction of a "mini" Mycobacterium-*Escherichia coli* shuttle vector. J. Bacteriol. **172:**2793–2797.
43. **Rinke de Wit, T., S. Bekelie, A. Osland, T. L. Miko, P. W. Hermans, D. van Soolingen, J.-W. Drijfhout, R. Schoningh, A. A. M. Janson, and J. E. R. Thole.** 1992. Mycobacteria contain two *groEL* genes: the second *Mycobacterium leprae groEL* gene is arranged in an operon with *groES*. Mol. Microbiol. **6:**1995–2007.
44. **Siegele, D. A., and R. Kolter.** 1992. Life after log. J. Bacteriol. **174:**345–348.
44a. **Smith, D. R.** Personal communication.
45. **Snapper, S. B., L. Lugosi, A. Jekkel, R. E. Melton, T. Kieser, B. R. Bloom, and W. R. Jacobs.** 1988. Lysogeny and transformation in mycobacteria: stable expression of foreign genes. Proc. Natl. Acad. Sci. USA **85:**6987–6991.
46. **Snapper, S. B., R. E. Melton, S. Mustafa, T. Kieser, and W. R. Jacobs.** 1990. Isolation and characterization of efficient plasmid transformation mutants of *Mycobacterium smegmatis*. Mol. Microbiol. **4:**1911–1919.
47. **Snider, D. E., and W. L. Roper.** 1992. The new tuberculosis. N. Engl. J. Med. **326:**703–705.
48. **Steenken, D. E., and E. Wolinsky.** 1953. Virulence of tubercle bacilli recovered from patients treated with isoniazid. Am. Rev. Tuberc. **68:**548–556.
49. **Stover, C. K., V. F. De la Cruz, T. R. Fuerst, J. E. Burlein, L. A. Benson, L. T. Bennett, G. P. Bansal, J. F. Young, M. H. Lee, G. F. Hatfull, S. B. Snapper, R. G. Barletta, W. R. Jacobs, and B. R. Bloom.** 1991. New use of BCG for recombinant vaccines. Nature (London) **351:**456–460.
50. **Thierry, D., A. Brisson-Noël, V. Vincent-Lévy-Frébault, S. Nguyen, J. Guesdon, and B. Gicquel.** 1990. Characterization of a *Mycobacterium tuberculosis* insertion sequence, IS*6110*, and its application in diagnosis. J. Clin. Microbiol. **28:**2668–2673.
51. **Thierry, D., M. D. Cave, K. D. Eisenach, J. T. Crawford, J. H. Bates, B. Gicquel, and J. L. Guesdon.** 1990. IS*6110*, an IS-like element of *Mycobacterium tuberculosis* complex. Nucleic Acids Res. **18:**188.
52. **van Soolingen, D., P. W. M. Hermans, P. E. W. de Haas, D. R. Soll, and J. D. A. van Embden.** 1991. Occurence and stability of insertion sequences in *Mycobacterium tuberculosis* complex strains: evaluation of an insertion sequence-dependent DNA

polymorphism as a tool in the epidemiology of tuberculosis. J. Clin. Microbiol. **29:**2578–2586.

53. **Vordermeier, H. M., D. P. Harris, P. K. Mehrotra, E. Roman, A. Elsaghier, C. Moreno, and J. Ivanyi.** 1992. *M. tuberculosis*-complex specific T-cell stimulation and DTH reactions induced with a peptide from the 38-kDa protein. Scand. J. Immunol. **35:**711–718.
54. **Winter, N., M. Lagranderie, J. Rauzier, J. Timm, C. Leclerc, B. Guy, M. P. Kieny, M. Gheorghiu, and B. Gicquel.** 1991. Expression of heterologous genes in *Mycobacterium bovis* BCG: induction of a cellular response against HIV-1 Nef protein. Gene **109:**47–54.
55. **Woods, S. A., and S. T. Cole.** 1989. A rapid method for the detection of potentially viable *Mycobacterium leprae* in human biopsies: a novel application of PCR. FEMS Microbiol. Lett. **65:**305–310.
56. **Woods, S. A., and S. T. Cole.** 1990. A family of dispersed repeats in *Mycobacterium leprae*. Mol. Microbiol. **4:**1745–1751.
57. **World Health Organization Scientific Working Group.** 1982. Chemotherapy of leprosy for control programmes. Technical Report Series no. 675. World Health Organization, Geneva.
58. **Young, D., T. Garbe, R. Lathigra, and C. Abou-Zeid.** 1990. Protein antigens: structure, function and regulation, p. 1–35. *In* J. McFadden (ed.), Molecular biology of mycobacteria. Academic Press Ltd., London.
59. **Young, D., R. Lathigra, R. Hendrix, D. Sweetser, and R. A. Young.** 1988. Stress proteins are immune targets in leprosy and tuberculosis. Proc. Natl. Acad. Sci. USA **85:**4267–4270.
60. **Young, D. B., S. H. E. Kaufmann, P. W. M. Hermans, and J. E. R. Thole.** 1992. Mycobacterial protein antigens: a compilation. Mol. Microbiol. **6:**133–145.
61. **Young, R. A., B. R. Bloom, C. M. Grosskinsky, J. Ivanyi, and R. W. Davis.** 1985. Dissecting *Mycobacterium tuberculosis* antigens using recombinant DNA. Proc. Natl. Acad. Sci. USA **82:**2583–2587.
62. **Young, R. A., V. Mehra, D. Sweetser, T. Buchanan, J. Clark-Curtiss, R. W. Davis, and B. R. Bloom.** 1985. Genes for the major protein antigens of the leprosy parasite *Mycobacterium leprae*. Nature (London) **316:**450–452.
63. **Zhang, Y., B. Heym, B. Allen, D. Young, and S. T. Cole.** 1992. The catalase-peroxidase gene and isoniazid resistance of *Mycobacterium tuberculosis*. Nature (London) **358:**591–593.
64. **Zhang, Y., and D. B. Young.** Unpublished data.