Mycobacterium tuberculosis} and the host response

Stefan H.E. Kaufmann, Stewart T. Cole, Valerie Mizrahi, Eric Rubin, and Carl Nathan

Mycobacterium tuberculosis} remains a leading cause of morbidity and mortality worldwide. Advances reported at a recent international meeting highlight insights and controversies in the genetics of {M. tuberculosis} and the infected host, the nature of protective immune responses, adaptation of the bacillus to host-imposed stresses, animal models, and new techniques.

Some hold that research should focus on a “security council” of “model organisms” whose sole bacterial representative should be {Escherichia coli}. Not so, according to 559 attendees who traveled from around the world to the 5th Biennial Keystone Meeting on Tuberculosis (TB) at Whistler, British Columbia from April 2–6, 2005, under the chairmanship of Gilla Kaplan, Stewart Cole, and Clifton Barry III. Mycobacterium tuberculosis} (MtB) poses extraordinary intellectual and medical challenges, as ~40% of its genes are of unknown function and it has infected ~30% of the world’s population. These challenges attract scientists of diverse disciplines who surprise each other with examples of biology and biochemistry that the so-called model organisms lack. Despite the difficulties of working with a slow-growing, highly infectious pathogen to which genetic tools came late and remain incomplete, MtB has become an uninvited guest at the model organisms’ table. Below, some highlights of the meeting are related, with emphasis on work not yet published or only recently reported.

**Mycobacterial genomics and genetics**

Genomics has catalyzed MtB research and the latest genome sequence, that of the vaccine strain {Mycobacterium bovis} (BCG), reveals that duplication and deletion of genes shape genome plasticity (Stewart Cole, Paris, France). One gene family of particular interest encodes Esx proteins, immunodominant T cell antigens that are secreted by a dedicated apparatus (2). Attenuation of BCG is mainly due to loss of the {esx} locus, which encodes two family members, CFP-10 and ESAT-6. Although their role in pathogenesis is unclear, the NMR structure of the CFP-10/ESAT-6 complex (Kirsty Lightbody and co-workers, Leicester, UK) eliminates one mechanism. Each protein contains two α-helices that interact to form a heterodimeric four-helix bundle (Fig. 1). Despite reports of association with and damage to host cell membranes, the complex presents no hydrophobic faces, suggesting that these two proteins alone are unlikely to form a transmembrane structure. Secretion of CFP-10 and ESAT-6 requires other genes, not all of which are closely linked to the {esx} locus in Mycobacterium marinum} (Bryant McLaughlin, San Francisco, CA) or MtB (Eric Rubin, Boston, MA). ESAT-6, CFP-10, and a protein encoded outside the locus are mutually dependent for secretion.

Another gene family, occupying 2% of the genome, is required for synthesis of the complex lipids that play both structural and immunomodulatory roles in MtB. Christophe Guilhot (Toulouse, France) delineated the synthesis pathway of the cell wall–associated lipids phosphatidyl dimycocerosate (PDIM) and phenolic glycolipid (PGL), and the soluble p-hydroxybenzoic acid derivative (p-HBAD), and identified the relevant glycosyltransferases and methyltransferases that help build these lipids. All MtB strains make PDIM and p-HBAD, whereas PGL is only associated with some clinical isolates and enhances their virulence by modulating the host immune response (Gilla Kaplan, Newark, NJ) (3). Similarly, Rajesh Gokhale (New Delhi, India) has decoded the synthesis of PDIM, with final verification furnished by an elegant retro-biosynthetic approach (4). Studies combining biochemistry with purified bacterial components, structural predictions, and site-directed mutagenesis have defined protein domains involved in each of the catalytic steps of PDIM synthesis. Both the Gokhale and Guilhot groups now have MtB strains in hand that synthesize modified lipids, which should enable characterization of their biological roles.

Host genetics and genomics

Adrian Hill (Oxford, UK) reviewed evidence that susceptibility to TB in humans is a polygenic trait, including increased concordance of disease in monozygotic compared with dizygotic twins, increased susceptibility among inbred populations, and identification of numerous genes each of which contributes to susceptibility on a minor extent that varies in different populations. Genes encoding HLA-DRB1, vitamin D receptor, NRAMP-1, and interferon γ (IFN-γ) have each been implicated in independent studies. Cathepsin Z (expressed in early phagosomes), SP110 (human homologue of Ipr1; see below), the adaptor of Toll-like receptor signaling MAL (TIRAP), and complement receptor 1 (CR1, or CD35) (5) have been implicated in single studies. An Oxford-Gambia collaboration on a genome-wide association study has...
been launched to enrol up to 2,000 individuals with TB and 2,000 controls that will survey 500,000 SNPs in each.

A poster by Mauricio Rojas-López et al. in Igor Kramnik’s group (Boston, MA) described a candidate transcriptional regulator, *intracellular pathogen resistance 1* (*Ipr1*), that is expressed in resistant macrophages after *Mtb* infection but is not expressed in susceptible phagocytes. *Ipr1* appears to foster macrophage apoptosis and confers resistance not only against *Mtb* but also against *Listeria monocytogenes* (6).

**Immune responses**

Stefan Kaufmann (Berlin, Germany) reported on a mechanism that contributes to apoptosis in *Mtb*-infected macrophages. Transcriptome analyses of *Mtb* from lung specimens obtained from TB patients revealed marked up-regulation of the genes *Rv0634* and *Rv2581c*, which both encode putative glyoxylases. Glyoxylases can detoxify keto-aldehydes such as methylglyoxal. It was shown that *Mtb*-infected cells produce methylglyoxal while also impeding cross-priming.

Hill described phase I clinical trials of a vaccine consisting of modified vaccinia virus Ankara encoding an *Mtb* mycolyl transferase, antigen 85A (MVA85A). Profound increases in antigen-specific, IFN-γ–producing CD4 T cells were observed in blood from both MVA85A-vaccinated and BCG-primed, MVA85A-boosted volunteers (8). These appear to be the strongest effector T cell responses yet described in any human vaccine clinical trial. Peter Andersen (Copenhagen, Denmark) described a planned clinical trial using a fusion protein of Ag85B and ESAT-6 with different adjuvants for intramuscular and oral vaccinations. Animal studies using adenovirus as a carrier for the fusion protein resulted in strong CD8 T cell responses and high IFN-γ titers. However, these responses were not paralleled by marked protection against *Mtb* replication, although protein–adjuvant formulations of the same fusion protein, which induced CD4 IFN-γ–secreting T cells, were protective. Mark Alderson (Corixa) reported that Corixa has conducted a phase I trial with a subunit vaccine comprised of a fusion protein of Rv1196 (a PPE family protein) and Rv0125 (a putative serine protease) and an adjuvant. However, he focused on preclinical studies, which again demonstrated that IFN-γ production generated by CD8 T cells induced by an adenoaviral vector were not protective. Although protein–adjuvant vaccines using the GSK Biologicals adjuvants AS02A or AS01B were protective, neither of these subunit vaccines afforded better protection in mice than BCG.

Kaufmann’s group engineered recombinant BCG by deleting urease and introducing the *L. monocytogenes* pore-forming protein listeriolysin to enhance presentation of BCG antigens by MHC class I. The recombinant BCG induced better protection against *Mtb* in mice than native BCG. Although the em-
phasis was originally placed on antigen translocation into the cytosol as a route to enhanced recognition of infected host cells by CD8 T cells, this strain has now been found to induce apoptosis of infected host cells, leading to cross presentation (Fig. 2).

Robert North (Saranac Lake, NY) gave an impressive overview of what the mouse model has taught us about host immunity against Mtb that appears pertinent in humans, including preferential persistence in the lung, the critical role of CD4 T cells, the supportive role of CD8 T cells, the lack of evidence for a role of γ/δ and NKT cells, and the dependence on tumor necrosis factor (TNF). Mice have also taught us the importance of IFN-γ and nitric oxide synthase 2 (NOS2) in protection against Mtb. However, a nonredundant role of IFN-γ in defending humans against Mtb is not as clear as is its nonredundant role in defense against other mycobacteria. Although NOS2 is expressed in human TB (9, 10), there is no genetic or pharmacological evidence addressing its contribution to the control of Mtb infection in humans.

North’s findings in mice (11) highlight a critical point in vaccine design. Increasing the number of antigen-specific memory T cells before challenge did not afford sterilizing immunity. Mice were infected with Mtb and then cured pharmacologically. These mice responded to a second Mtb infection by mounting an adaptive, T cell–dependent immune response 5 days earlier than naive mice. The anamnestic response reduced bacterial viability only 10-fold, which was insufficient to prevent lethal pathology. North argued that the limiting feature of the immune response to Mtb may be a defect in macrophage effector function, not an inadequate number of antigen-specific T cells. In this view, vaccination may be futile if it does no more than induce a naive host to form Mtb-specific memory T cells earlier than it would upon infection. Others were optimistic that subunit vaccines inducing T helper 1–type CD4 T cell responses will reduce death and disease in TB, as they are doing in mice with other infections.

Although the mechanisms that account for insufficient T cell–dependent protection in response to BCG vaccination and Mtb infection remain unclear, regulatory T (T reg) cells might be involved. Willem Hanekom (Cape Town, South Africa) described the emergence of T reg cells in children vaccinated with BCG as newborns. Hill described induction of the T reg cell specific transcription factor (FoxP3) in MVAAg85 vaccinees. A poster from Simone Joosten et al. from Michel Klein’s and Tom Ottenhoff’s groups (Leiden, Netherlands) described activation of T reg cells after in vitro stimulation with BCG of lymphocytes from purified protein derivative (PPD)–positive donors. A poster from Kevin Urdaal et al. in Michael Bevan’s lab (Seattle, WA) reported the emergence of T reg cells in the lungs of Mtb-infected mice. Should it turn out that T reg cells suppress optimal immune responses to Mtb or BCG, vaccination strategies may have to include ways to reduce development of T reg cells.

Immunodeficiency states are the major known predisposing factors for active TB. As reaffirmed by a poster from Blanca Restrepo et al. (Brownsville, TX), diabetes mellitus also constitutes a predisposing factor, but it has never been clear why. The finding reported in a poster from Gregory Martens et al. in Hardy Kornfeld’s lab (Worcester, MA) that hypercholesterolemia reversibly predisposes mice to severe TB suggests that dysregulated lipid metabolism, or the systemic inflammation sometimes associated therewith, may represent another category of predisposition that is potentially relevant to the diabetic state (Fig. 3). Given that diabetes and dysregulated lipid metabolism are reaching epidemic status, it is important to be alert to a possible intersection of these disorders with the TB pandemic.

Mycobacterial stress and adaptation

Advances in understanding how Mtb resists and adapts to stresses encountered during infection are paving the way toward new interventions. Trehalose, the major intracellular sugar of mycobacteria, protects against cellular stress, is a component of glycolipids, and is involved in the transport of mycolic acids during cell wall biogenesis. As mammalian cells do not make trehalose, its biosynthesis may provide targets in Mtb, perhaps both in replicating and nonreplicating organisms. Brian Robertson (London, UK) reported that, of the three biosynthetic routes, only the OtsAB pathway is essential, thus prompting the development of a high-throughput screen for inhibitors against OtsB2, a trehalose 6-phosphate phosphatase. However, the late-stage attenuation of a mutant in an alternate pathway (treS) implicates the alternate pathway in persistent infection, either through synthesis of additional trehalose or via its breakdown to glucose (12). Carl Nathan (New York, NY) described an approach to TB drug discovery predicated on sensitizing Mtb to immune attack by reactive nitrogen intermediates (RNs) through targeting enzymatic components of Mtb’s RNI defense systems. The approach was illustrated with examples of Mtb enzymes involved in macromolecule repair and degradation—UvrB (13), mycobacterial proteasomal ATPase (Mpa) (14) (Fig. 4), and the proteasomal protease. The attenuation of mpa and wrvB mutants in wild-type mice, which was partially reversible in NOS2-deficient mice, supports this approach. Valerie Mizrahi (Johannesburg, South Africa) illustrated how Mtb uses stress to its advantage through the induction of specialized DNA poly-
sigma factor, SigC (17), switches to a mice by an Mtb mutant in the alternate North pointed out that mice deficient advanced TB in humans. However, of the cavities characteristic of ad-
veloping symptomatic, necrotizing TB lesions may have immunologic features similar to mice whose genetics predis-
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Mtb deficient in the transcription factor whiB described in a poster by John Trombley et al. in Adrie Steyn’s lab (Birm-
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ally different phenotypes in mice and guinea pigs. Thus, it is possible that the animal species that best models human TB may vary not just with the aspect of TB being considered but also with the genetic makeup of the infecting strain.

JoAnne Flynn (Pittsburgh, PA) reported that macaques infected with Mtb by the pulmonary route sorted spontaneously into latently infected (60%) and clinically diseased (40%) subsets, enabling many important analyses. However, the high proportion of animals that develop active disease and the lack of a demonstrable delayed type hypersensitivity response to PPD in the macaque represent distinct differences from the human situation.

Lalita Ramakrishnan (Seattle, WA) reported that Mtb deficient in DosR, a master regulator of hy-
poxic responses, had no phenotype in mice. Stefan Ehlers (Borstel, Germany) speculated that this might reflect lack of severe hypoxia in tuberculous lesions in mice. However, it is not yet clear whether animal models differ amongst themselves with regard to the extent of oxygenation in tuberculous lesions as much as the diverse lesional microenvi-
ronments may differ in a given host. Nathan noted that the prevalence of nitrotyrosine within human tuberculous lesions (9, 10) and the reported $K_{on}$ of NOS2 for O$_2^-$ (reported values range from 6–135 $\mu$M) (20) suggest that human TB lesions support functionally relevant O$_2^-$-dependent biochemistry. At the same time, hypoxia may mark-
edly limit the rate of NO generation, contributing to escape of Mtb from immune control. It remains an important challenge to quantify O$_2^-$ and NO concentrations in the microenvironments of tuberculous lesions in humans and experimental animals.

New technologies and research resources

Target validation by conditional gene silencing has been hampered by limited knowledge of tightly regulated promoters for use in mycobacteria (21). An important step has been taken to address this need through the development of a range of tetracycline-regu-
lated gene expression systems by Sabine Ehrt and Dirk Schnappinger (New York, NY), Robertson, Tanya Parish (London, UK), and Bishai and their colleagues (22–24). The successful application of these systems to condi-
tional gene silencing in vitro, coupled with the accessibility of intracellular bacteria to the tetracycline inducer, suggests that the elusive goal of being able to silence Mtb genes at specific stages of infection may be attainable.

Finally, a major contributor to the growing sense of Mtb as a model or-
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Animal models

There is a consensus that mice, guinea pigs, rabbits, and cynomologous macaques infected with Mtb model overlapping features of human TB. A controversial variant of this view is that the larger the animal, the better it models human disease. According to the latter view, two of the shortcomings of mice as a model are that all mice eventually succumb to Mtb infection, as opposed to 5–10% of immunocom-
petent humans; and that mice fail to develop necrotic lesions, the precursors of the cavities characteristic of advanced TB in humans. However, North pointed out that mice deficient in IFN-γ, NOS2, or TNF do develop necrotic lesions. To this list can be added mice lacking lpr1 (6) and those of the I/St strain (18). That some in-
bred strains of mice respond to Mtb with necrotic lung lesions but most do not, could be considered to mimic the distribution of responses to Mtb in infected, outbred humans. That is, the small minority of HIV-negative people who respond to Mtb infection by de-
veloping symptomatic, necrotizing TB lesions may have immunologic features similar to mice whose genetics predis-
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Finally, a major contributor to the growing sense of Mtb as a model or-
anism was the emergence of special
resources for the Mtb research community. Table S1 offers a compendium of resources, including some announced at the meeting, and is available online at http://www.jem.org/cgi/content/full/jem.20050842/DC1.

REFERENCES

1. Field, S., and M. Johnston. 2005. Cell biology. Whither model organism research? Science. 307:1885–1886.
2. Pym, A.S., P. Brodin, L. Majlesi, R. Brosch, C. Demangel, A. Williams, K.E. Griffiths, G. Marchal, C. Leclerc, and S.T. Cole. 2003. Recombinant BCG exporting ESAT-6 confers enhanced protection against tuberculosis. Nat. Med. 9:533–539.
3. Reed, M.B., P. Domenech, C. Manca, H. Su, A.K. Barczak, B.N. Kreuswirth, G. Kaplan, and C.E. Barry III. 2004. A glycolipid of hypervirulent tuberculosis strains that inhibits the innate immune response. Nature. 431:84–87.
4. Trivedi, O.A., P. Arora, A. Vats, M.Z. Ansari, R. Tickoo, V. Sridharan, D. Mohanty, and R.S. Gokhale. 2003. Dissecting the mechanism and assembly of a complex virulence mycobacterial lipid. Mol. Cell. 17:631–643.
5. Fitness, J., S. Floyd, D.K. Warndorff, L. Sichali, S. Malema, A.C. Crampin, P.E. Fine, and A.V. Hill. 2004. Large-scale candidate gene study of tuberculosis susceptibility in the Karonga district of northern Malawi. Am. J. Trop. Med. Hyg. 71:341–349.
6. Pan, H., B.S. Yan, M. Rojas, Y.V. Shchukin, H. Zhou, L. Kobliz, D.E. Higgins, M.J. Daly, B.R. Bloom, and I. Kramnik. 2005. Ipr1 gene mediates innate immunity to tuberculosis. Nature. 434:767–772.
7. Schable, U.E., F. Winau, P.A. Sieling, K. Fischer, H.L. Collins, K. Hogens, R.L. Modlin, V. Brinkmann, and S.H. Kaufmann. 2003. Apoptosis facilitates antigen presentation to T lymphocytes through MHC-I and CD1 in tuberculosis. Nat. Med. 9:1039–1046.
8. McShane, H., A.A. Pathan, C.R. Sander, S.M. Keating, S.C. Gilbert, K. Huygen, H.A. Fletcher, and A.V. Hill. 2004. Recombinant modified vaccinia virus Ankara expressing antigens 85A boosts BCG-primed and naturally acquired anticytobacterial immunity in humans. Nat. Med. 10:1240–1244.
9. Choi, H.S., P.R. Rai, H.W. Chu, C. Cool, and E.D. Chan. 2002. Analysis of nitric oxide synthase and nitrotyrosine expression in human pulmonary tuberculosis. Am. J. Respir. Crit. Care Med. 166:178–186.
10. Schon, T., G. Erlenberger, Y. Negesse, R.H. Pando, T. Sundqvist, and S. Brutton. 2004. Local production of nitric oxide in patients with tuberculosis. Int. J. Tuberc. Lung Dis. 8:1134–1137.
11. Jung, Y.-J., L. Ryan, R. LaCourse, and R.J. North. 2005. Ipr1 gene mediates innate immunity to tuberculosis. Mol. Cell. 17:1885–1886.
12. Murphy, H.N., G.R. Stewart, V.V. Mishchenko, A.S. Apt, R. Harris, M.S. McAlister, P.C. Driscoll, D.B. Young, and B.D. Robertson. 2005. The OreAB pathway is essential for trehalose biosynthesis in Mycobacterium tuberculosis. J. Biol. Chem. 280:14524–14529.
13. Darwin, K.H., and C.F. Nathan. 2005. Role for nucleotide excision repair in virulence of Mycobacterium tuberculosis. Infect. Immun. In press.
14. Darwin, K.H., G. Lin, Z. Chen, H. Li, and C.F. Nathan. 2005. Characterization of a Mycobacterium tuberculosis proteasomal ATP-ase homologue. Mol. Microbiol. 55:561–571.
15. Boshoff, H.H., M.B. Reed, C.E. Barry III, and V. Mizrahi. 2003. DnaE2 polymerase contributes to in vivo survival and the emergence of drug resistance in Mycobacterium tuberculosis. Cell. 113:183–193.
16. Boshoff, H.H., T.G. Myers, B.R. Copp, M.R. McNeil, M.A. Wilson, and C.E. Barry III. 2004. The transcriptional responses of Mycobacterium tuberculosis to inhibitors of metabolism: novel insights into drug mechanisms of action. J. Biol. Chem. 279:40174–40184.
17. Sun, R., P.J. Converse, C. Ko, S. Tyagi, N.E. Morrison, and W.R. Bishai. 2004. Mycobacterium tuberculosis ECF sigma factor SigC is required for lethality in mice and for the conditional expression of a defined gene set. Mol. Microbiol. 52:25–38.
18. Erulajnov, E.B., I.V. Lyadova, T.K. Kondrat’eva, K.B. Majorov, I.V. Scheglov, M.O. Orlova, and A.S. Apt. 2005. Neutrophil responses to Mycobacterium tuberculosis infection in genetically susceptible and resistant mice. Infect. Immun. 73:1744–1753.
19. Cosma, C.L., O. Humbert, and L. Ramakrishnan. 2004. Superinfecting mycobacteria home to established tuberculous granulomas. Nat. Immunol. 5:826–835.
20. Dweik, R.A., D. Laskowski, H.M. Abou-Soud, F. Kaneko, R. Htte, D.J. Snehr, and S.C. Erurzum. 1998. Nitric oxide synthase in the lung. Regulation by oxygen through a kinetic mechanism. J. Clin. Invest. 101:660–666.
21. Warner, D.F., and V. Mizrahi. 2004. Mycobacterial genetics in target validation. Drug Discovery Today. Technologies. 2:93–98.
22. Ehr, S., X.V. Guo, C.M. Hickey, M. Ryou, M. Monteleone, I.W. Riley, and D. Schnappinger. 2005. Controlling gene expression in mycobacteria with anhydrotetracycline and Tet repressor. Nucleic Acids Res. 33:e21.
23. Blokpoel, M.C., H.N. Murphy, R. O’Toole, S. Wiles, E.S. Runn, G.R. Stewart, D.B. Young, and B.D. Robertson. 2005. Tetra-cycline-inducible gene regulation in mycobacteria. Nucleic Acids Res. 33:e22.
24. Carroll, P., and D.G.N. Muttucumar. 2005. Conditional expression using a tetracycline-inducible expression system in Mycobacterium tuberculosis and Mycobacterium smegmatis. Appl. Environ. Microbiol. In press.
25. Groze, L., J. Hess, P. Seiler, V. Brinkmann, S. Baumann, A. Nasser Eddine, P. Mann, C. Goossmann, D. Smith, G.J. Bancroft, J.-M. Reytat, D. van Soolingen, B. Raupach, and S.H.E. Kaufmann. 2005. Increased vaccine efficacy against tuberculosis of recombinant Mycobacterium hofv Bacille Calmette-Guerin mutants secreting leonidycin. J. Clin. Invest. In press.