Mycobacterium tuberculosis and the host response

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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 (1). 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 Eox proteins, immunodominant T cell antigens that are secreted by a dedicated apparatus (2). Attenuation of BCG is mainly due to loss of the ess1 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 ess1 locus in Mycobacterium marinum (Bryant McLoughlin, 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 phthiocerol 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 to 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 Iprt; 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, a tuberculostatic compound that participates in mycobacteria-induced host cell apoptosis. Cross priming of T cells in TB (7) involves apoptosis via a methylglyoxal-dependent mechanism. Glyoxylase may thus help defend Mtb against host-derived 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 adenoviral 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 Urdahl 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 (RNIs) 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 uvrB 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-

**Figure 3. Hypercholesterolemia predisposes to severe TB.** ApoE–deficient mice fed on a high cholesterol diet develop giant lung inflammatory lesions when infected with M. tuberculosis. Image provided by Hardy Kornfeld (Worcester, MA).
necrotic lesions. To this list can be added mice lacking lpr1 (6) and those of the I/St strain (18). That some inbred strains of mice respond to Mt�� with necrotic lung lesions but most do not, could be considered to mimic the distribution of responses to Mtﬁ in infected, outbred humans. That is, the small minority of HIV-negative people who respond to Mtﬁ infection by developing symptomatic, necrotizing TB lesions may have immunologic features similar to mice whose genetics predispose to a necrotic response.

Mtﬁ deﬁcient in the transcription factor whiB described in a poster by John Trombley et al. in Adrie Steyn’s lab (Birmingham, AL) and the sigC-deﬁcient Mtﬁ studied by Bishai displayed strikingly 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 Mtﬁ 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) showed that zebrafish develop caseating granulomas upon infection with M. marinum (19). Thus, necrotic responses to mycobacteria are not conﬁned to large hosts.

Clifton Barry (Rockville, MD) suggested that in macaques tuberculous lesions are hypoxic, with such profound impact on Mtﬁ’s metabolism that the study of host species with predominantly nonhypoxic lesions can be fundamentally misleading. David Sherman (Seattle, WA) reported that Mtﬁ deﬁcient in DosR, a master regulator of hypoxic 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 microenvironments may differ in a given host.

Nathan noted that the prevalence of nitrotyrosine within human tuberculous lesions (9, 10) and the reported $K_m$ of NOS2 for O$_2$ (reported values range from 6–135 μM) (20) suggest that human TB lesions support functionally relevant O$_2$-dependent biochemistry. At the same time, hypoxia may markedly limit the rate of NO generation, contributing to escape of Mtﬁ 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-regulated 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 conditional 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 Mtﬁ genes at speciﬁc stages of infection may be attainable.

Finally, a major contributor to the growing sense of Mtﬁ as a model organism 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