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 (Mt) 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, Mt 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 Ex proteins, immunodominant T cell antigens that are secreted by a dedicated apparatus (2). Attenuation of BCG is mainly due to loss of the esx1 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 esx1 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 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 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

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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 adenosiral 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...
emphasis 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 γδ 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 Urbahl 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.

Immune deficiency 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 Kornfield’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, 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, 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, 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, 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, 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, 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, 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, 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, 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, or the systemic inflammation sometimes associated therewith, may represent another category of predisposition that is potentially relevant to the diabetic state (Fig. 3)

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 Kornfield (Worcester, MA).
sigma factor, SigC (17), switches to a
mice by an Mtb mutant in the alternate
in IFN-
advanced TB in humans. However,
of the cavities characteristic of ad-
develop necrotic lesions, the precursors
 Eventually succumb to Mtb infection,
res of mice as a model are that all mice
the latter view, two of the shortcom-
models human disease. According to
the larger the animal, the better it
controversial variant of this view is that
caques infected with Mtb model
There is a consensus that mice, guinea
Animal models
relate to those in another.
how conditions in one animal model
which the organism must adapt, and
underscores that the phenotype of a
type when assessed in guinea pigs. This
giv
MD) reported that the “immunopa-
increased use of fluoroquinolones in
drug resistance in Mtb in light of the
mutant implications for the evolution of
lesions may have immunologic features
veloping symptomatic, necrotizing TB
lesions from 6–135
man 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 im-
une control. It remains an important
challenge to quantify O$_2$ and NO con-
centrations 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 pro-
moters for use in mycobacteria (21).
An important step has been taken to
address this need through the develop-
ment 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 ap-
lication 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-
ganism was the emergence of special
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 in-
fected, 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-
pose to a necrotic response.

Mtb deficient in the transcription
factor whiB described in a poster by John
Trombley et al. in Adrie Steyn’s lab (Bir-
mingham, AL) and the sigC-deficient
Mtb studied by Bishai displayed strik-
ingly 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) re-
ported that macaques infected with
Mtb by the pulmonary route sorted
spontaneously into latently infected
(60%) and clinically diseased (40%)
subsets, enabling many important analy-
ses. 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) sug-
gested that in macaques tuberculous le-
sions are hypoxic, with such profound
impact on Mtb’s metabolism that the
study of host species with predomin-
antly nonhypoxic lesions can be fun-
damentally misleading. David Sherman
(Seattle, WA) reported that Mtb defi-
cient 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-
roments may differ in a given host.
Nathan noted that the prevalence of ni-
totyrosine within human tuberculous
lesions (9, 10) and the reported $K_m$
 of NOS2 for O$_2$ (reported values range
from 6–135 $\mu$M) (20) suggest that hu-
man TB lesions support functionally
relevant O$_2$-dependent biochemistry.
At the same time, hypoxia may mark-
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une control. It remains an important
challenge to quantify O$_2$ and NO con-
centrations in the microenvironments of
tuberculous lesions in humans and
experimental animals.
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