

infections and cancer<sup>3</sup>. Similarly, immune cells collected from the lungs of marijuana smokers produce lower than normal amounts of several cytokines and fail to produce nitric oxide (another intermediary in the immune system), severely limiting their ability to kill bacteria<sup>4</sup>.

Steffens and colleagues<sup>2</sup> set the stage for their work by demonstrating that immune cells expressing CB2 receptors infiltrate atherosclerotic plaques in humans and in a strain of mice that is used to study atherosclerosis (ApoE<sup>-/-</sup> mice). In this mouse model, the animals develop progressive narrowing of their arteries as lipids and inflammatory cells called macrophages enter the walls of their blood vessels and produce plaques<sup>5</sup>. When low doses of THC (1 mg per kg body weight per day) were added to their diet, the progression of atherosclerosis was markedly slowed. Mice that were fed THC still had elevated levels of serum lipids but had fewer plaque-infiltrating macrophages when compared with controls, suggesting an effect on immune function.

The authors go on to substantiate the immunosuppressive properties of THC on the migration, infiltration and function of immune cells in this model. Spleen cells collected from THC-treated mice responded poorly to stimulation *in vitro*, showing limited proliferation and impaired production of interferon- $\gamma$ , a cytokine involved in atherosclerosis. Macrophages harvested from THC-treated animals expressed little of the messenger RNA encoding the CCR2 receptor protein, and were poorly responsive to the CCR2 ligand, MCP-1. Both CCR2 and MCP-1 are involved in the migration of macrophages and have roles in atherosclerosis<sup>6</sup>. These effects were blocked when mice were pre-treated with a selective antagonist to the CB2 receptor or when macrophages were collected from mice that lack functional CB2 receptors.

Using live vascular microscopy, the authors observed that adhesion of immune cells to the internal surface of blood vessels was considerably reduced in mice that were fed THC. Again, this effect was blocked by pre-treating the animals with a CB2 antagonist. The protective effects of THC occurred at very low doses, producing blood THC levels well below the range usually associated with activation of CB1 receptors in the brain. The authors conclude that low doses of THC, or perhaps selective CB2 ligands, should be further investigated for their possible use in treating human atherosclerosis.

The findings by Steffens *et al.* are striking, but they should not be taken to mean that smoking marijuana is beneficial for the heart. The dose-response curve to THC in this study was very narrow and U-shaped, with higher and lower concentrations failing to produce protective effects. It would be difficult to achieve such specific concentrations

in the blood by smoking marijuana. Also, no studies have been performed in humans to evaluate the effects of THC on atherosclerosis. As the authors note, the ApoE<sup>-/-</sup> mice develop extremely high levels of serum lipids, and THC, which is very fat-soluble, is likely to be stored at high local concentrations within atherosclerotic lesions<sup>7</sup>. Whether this local storage occurs, and whether the same effect will occur in human atherosclerotic lesions, remains to be determined.

Finally, THC binds to and activates CB1 and CB2 receptors with similar affinity. Marijuana smoking, acting through its effect on CB1 receptors in the brain, increases the pulse rate, produces an acute rise in blood pressure and then results in sudden falls in blood pressure upon standing or walking (Fig. 1). These effects lower the exercise threshold for angina, and are an independent risk factor for heart attack and stroke<sup>8,9</sup>. When inhaled, marijuana smoke also increases the concentration of carboxy-

haemoglobin in the blood, impairing oxygen delivery. Ultimately, to take advantage of the positive effects reported by Steffens *et al.* will probably mean developing cannabinoids that target CB2 receptors, rather than using marijuana or oral THC as medicines. ■

Michael D. Roth is in the Division of Pulmonary and Critical Care, Department of Medicine, CHS 37-131, David Geffen School of Medicine, University of California, Los Angeles, California 90095-1690, USA.  
e-mail: mroth@mednet.ucla.edu

1. Di Marzo, V., Bifulco, M. & De Petrocellis, L. *Nature Rev. Drug Discov.* **3**, 771–784 (2004).
2. Steffens, S. *et al.* *Nature* **434**, 782–786 (2005).
3. Klein, T. W. *et al.* *J. Leukoc. Biol.* **74**, 486–496 (2003).
4. Shay, A. H. *et al.* *J. Infect. Dis.* **187**, 700–704 (2003).
5. Meir, K. S. & Leitersdorf, E. *Arterioscler. Thromb. Vasc. Biol.* **24**, 1006–1014 (2004).
6. Charo, I. F. & Taubman, M. B. *Circ. Res.* **95**, 858–866 (2004).
7. Nahas, G. G., Erick, H. C., Lattimer, J. K., Latour, C. & Harvey, D. *Hum. Psychopharmacol.* **17**, 103–113 (2002).
8. Mittleman, M. A., Lewis, R. A., Maclure, M., Sherwood, J. B. & Muller, J. E. *Circulation* **103**, 2805–2809 (2001).
9. Geller, T., Loftis, L. & Brink, D. S. *Pediatrics* **113**, e365–e370 (2004).

## Tuberculosis

# The genetics of vulnerability

Nada Jabado and Philippe Gros

Susceptibility to tuberculosis is known to be under complex genetic control in humans, but what are the genes involved? A mouse strain that is unusually prone to the disease shows the way.

It is estimated that as much as one-third of the world's population is infected with the tubercle bacillus *Mycobacterium tuberculosis*. Yet only one in ten infected people actually develops active tuberculosis (TB), suggesting that innate mechanisms of immune defence (among other factors) can often contain the infection<sup>1</sup>. Innate susceptibility to TB has been known for years to be genetically controlled, but this genetic component is very complex and has been difficult to unravel<sup>2</sup>. On page 767 of this issue, Pan *et al.*<sup>3</sup> report that they have identified a host gene that controls susceptibility to TB in mice. This gene is expressed in macrophages — the host cells in which *M. tuberculosis* replicates — and appears to determine the type of death that macrophages will suffer following infection. These studies not only provide insight into a novel aspect of TB pathogenesis, but, if validated in humans, may reveal new molecular targets for drug treatments.

Tuberculosis is endemic in many parts of the world and continues to be a major global health problem, resulting in 2 million deaths a year. Contributing factors include poor health services, widespread poverty and other socioeconomic problems, the HIV epidemic and the appearance of multidrug-resistant *M. tuberculosis*<sup>1,4</sup>. The bacterium (Fig. 1, overleaf) infects lung macrophages,

and is concentrated in granulomas — solid clumps of macrophages, lymphocytes (types of immune cell) and epithelial cells. These granulomas appear to contain the infection physically. TB can remain dormant in this form for years. But, in a proportion of individuals, the protective granulomas or other aspects of host immunity break down, leading to active pulmonary TB and the spread of the disease through the production of aerosols by the lungs.

The host factors that underlie protective immunity and that may fail in active TB are poorly understood, but they are at the centre of TB pathogenesis<sup>5</sup>. Studies of twins, and population studies in regions in which TB is endemic (as well as during 'first-contact' epidemics and local outbreaks), indicate that host genetics plays a part. But this component involves numerous genes, with other factors — such as genetic variability and gene-environment interactions — further complicating the identification and analysis of single-gene effects in humans<sup>2,6</sup>.

In their approach to the problem, Pan *et al.*<sup>3</sup> have studied the inbred C3HeB/FeJ mouse strain. In these mice, extreme susceptibility to TB is associated with uncontrolled replication of *M. tuberculosis* in the lungs, with animals rapidly succumbing to infection. This susceptibility is controlled in part by a chromosomal region, or locus, called



Figure 1 Agent of tuberculosis: the tubercle bacillus, *Mycobacterium tuberculosis*.

*sst1* (for 'supersusceptibility to tuberculosis 1') that the authors previously mapped<sup>7</sup> to chromosome 1. Now, Pan *et al.* have generated a mouse strain in which the *sst1* locus from the C3HeB/FeJ mice has been replaced with the same region from C57BL/6J mice, which are resistant to TB. The authors used this 'congenic' strain to study the contribution of *sst1* to the pathogenesis of TB.

Pan *et al.* first show that the acquisition of resistance in the congenic strain is associated with an increased capacity of bone-marrow-derived macrophages to restrict intracellular replication of *M. tuberculosis*. Interestingly, resistance is also linked to the induction of apoptosis (a strictly regulated form of cell suicide) in macrophages following infection. Macrophages in susceptible mice, by contrast, die by necrosis — an uncontrolled process that may be less likely to restrict bacterial spread.

Scrutiny of the *sst1* chromosomal region for candidate genes that are expressed in macrophages then led the authors to a gene that they call *Ipr1* (for 'intracellular pathogen resistance 1'). *Ipr1* is expressed in resistant macrophages and is induced upon *M. tuberculosis* infection, but is absent from susceptible cells. The authors' gene hunt was complicated by the fact that the *sst1* locus includes part of a 'homogeneously staining region' — a large, unstable region of repeated DNA that contains several rearranged copies of *Ipr1*-related sequences. This may explain the susceptibility of C3HeB/FeJ mice: the instability in this region may have decapitated the regulatory sequences of the *Ipr1* gene, preventing it from being expressed.

Pan *et al.* went on to validate their findings by showing that reintroducing full-length *Ipr1* into C3HeB/FeJ mice could partially suppress the replication of *M. tuberculosis* in the lungs *in vivo*, and in macrophages infected *in vitro*. A particularly

exciting finding is that *Ipr1* expression in such genetically altered C3HeB/FeJ macrophages could also restrict the replication of another bacterium, *Listeria monocytogenes* — suggesting a general role for *Ipr1* in innate macrophage defences against intracellular infections.

So what exactly does *Ipr1* do? The gene encodes a protein known as Ifi75, which contains several sequence motifs that indicate that it is localized to the cell nucleus and has a role in regulating gene expression. In support of that idea, Ifi75 is a relative of the human protein SP110 (ref. 8) — a proposed regulator of gene transcription. Like *Ipr1*, SP110 is regulated by interferons, signalling molecules involved in immunity. SP110 also interacts with certain viral proteins, including proteins from hepatitis C virus and Epstein–Barr virus<sup>9</sup>. All of this places *Ipr1* at the interface of host–pathogen interactions, possibly participating in transcriptional activation in macrophages in response to intracellular pathogens. (See also the supplementary information in ref. 3.)

It will be interesting to see how the presence of products from bacteria as different as *Listeria* and *Mycobacterium* can activate *Ipr1*, and what other host proteins may be involved in this signalling. Other questions are: which genes are in turn activated by *Ipr1*, and how do they contribute to the resistance of macrophages to bacterial replication in general, and to the induction of apoptosis in particular? Might this response involve the activation of 'inflammatory' or 'apoptotic' caspase enzymes? And does this pathway run parallel to, or intersect with, other pathogen-sensing pathways, such as those triggered by extracellular pathogens through the so-called Toll-like receptors or other proteins, including those of the NBS-LRR (nucleotide-binding site leucine-rich repeat) or NOD (nucleotide oligomerization domain) families<sup>10</sup>?

Suffice it to say that, once again, careful genetic studies in the laboratory mouse have delivered an unexpected gift. More exciting biology in an area of immense interest for global health is sure to follow. ■

Nada Jabado is in the Department of Pediatrics, Montreal Children's Hospital, McGill University Health Center, Montreal, H3Z 2Z3, Canada.

Philippe Gros is in the Department of Biochemistry, McGill University, Montreal, H3G 1Y6, Canada.

e-mail: philippe.gros@staff.mcgill.ca

- Ravignone, M. C. *Tuberculosis* **83**, 4–14 (2003).
- Bellamy, R. *Genes Immun.* **4**, 4–11 (2003).
- Pan, H. *et al. Nature* **434**, 767–772 (2005).
- Bloom, B. R. *N. Engl. J. Med.* **346**, 143–145 (2002).
- Smith, I. *Clin. Microbiol. Rev.* **16**, 463–496 (2003).
- Casanova, J. L. & Abel, L. *Annu. Rev. Immunol.* **20**, 581–620 (2002).
- Kramnik, I. *et al. Proc. Natl Acad. Sci. USA* **97**, 8560–8565 (2000).
- Kaderit, S. *et al. J. Biol. Chem.* **268**, 24432–24441 (1993).
- Nicewonger, J. *et al. J. Virol.* **78**, 9412–9422 (2004).
- Inohara, N. & Nunez, G. *Nature Rev. Immunol.* **3**, 371–382 (2003).



#### 100 YEARS AGO

**A Study of Recent Earthquakes.** A subject attractive to the general reader... is an account of signs which have given warning of a coming earthquake. Underground sounds have been heard, springs have varied in their flow, horses, birds, dogs, and even human beings have been restless for some time before great earthquakes. In his reference to the Riviera earthquake in 1887, Mr. Davison remarks that as premonitions were noted at 130 different places within the central area, "there can be little doubt that they were caused by microseismic movements for the most part insensible to man." In these days of psychical research we think that the author has lost an opportunity for romantic speculation... It is pointed out that the area over which earthquake sounds are heard is variable in different countries. One reason for this is that the limits of audibility vary with different races. From illustrations given it would appear that for certain sounds the Anglo-Saxon ear is more acute than that of the Neapolitan, and very much more than that of the Japanese. From *Nature* 6 April 1905.

#### 50 YEARS AGO

A new instrument called the 'maser' (microwave amplification by stimulated emission of radiation) has been invented by Prof. C. H. Townes, of the Physics Department, Columbia University, for which the claim is made that it enables time to be measured with an accuracy of one part in 10<sup>11</sup>. The 'clock' used is an ammonia molecule which radiates an electric dipole spectrum as a set of lines of about 6 mm. wavelength and, as used in the instrument, maintains its frequency to the above order of magnitude. This would be sufficient to enable it to measure variation in the rate of rotation of the earth... the instrument consists of a molecular beam of ammonia molecules which are excited in an electric field and then pass into a tuned cavity-resonator, where they induce each other to radiate by negative absorption... It seems that the method of use is to extract by means of wave-guides the radiation emitted by two 'masers', tuned to different but adjacent frequencies by the ammonia spectrum. When mixed, the beat frequency can then be counted electronically. The 'maser' is also claimed by its inventor to be very effective as an amplifier. From *Nature* 9 April 1955.