

# Building a better tuberculosis vaccine

Restoration of genes lost during the original attenuation of the commonly used BCG tuberculosis vaccine enhances the ability of a recombinant strain to protect against *Mycobacterium tuberculosis*.

DOUGLAS B. YOUNG

The immune response to tuberculosis is often described as a 'double-edged sword', as it protects the host against disease but also assists the pathogen by causing the tissue damage required for effective transmission. The notion of a double-edged sword has attractive poetic resonance but is less than satisfactory as a conceptual platform for the rational design of improved vaccines and immunodiagnostic reagents. There is a need for greater precision in our understanding of the relationship between virulence and protective immunity in tuberculosis. In this issue, Pym *et al.*<sup>1</sup> begin to fill in the gaps.

In the early years of the twentieth century, Calmette and Guérin generated a tuberculosis vaccine by attenuating an initially virulent mycobacterial isolate. The bacille Calmette-Guérin (BCG) remains one of the world's most widely used vaccines. Delivered at or near birth, BCG reduces the incidence of certain childhood forms of tuberculosis by around 70% but has little or no effect on the predominant adult pulmonary disease responsible for the current global emergency. With 2 million deaths from tuberculosis every year, there is a pressing need for improved vaccines.

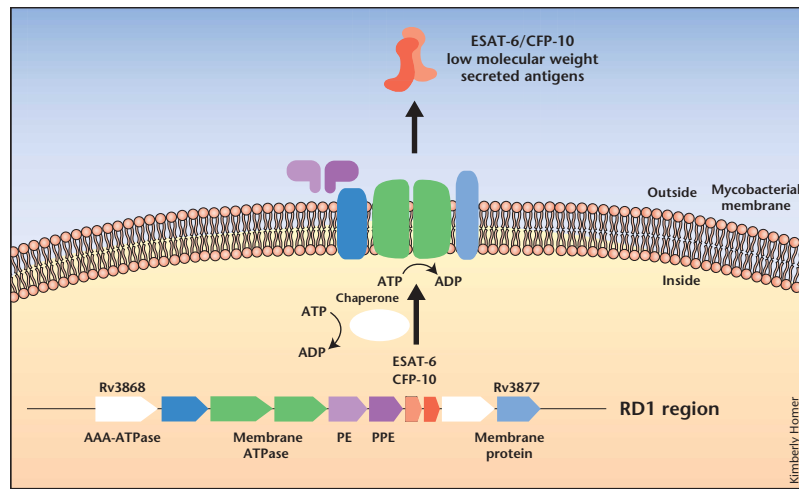
Gene deletion was an important part of the attenuation process that led to the BCG vaccine. A key event was the loss of a group of nine genes, referred to as region of deletion-1 (RD1). Targeted removal of RD1 genes reduces the virulence of *M. tuberculosis* in cell culture and mouse models<sup>2</sup>. Likewise, reintroduction of the RD1 region into BCG changes colony morphology and increases virulence in immunodeficient

mice<sup>3</sup>. But RD1 addition does not fully restore virulence, showing that additional genetic changes contribute to the attenuation of BCG.

level of a single antigen.

Pym *et al.* begin to clarify this thinking with two contributions. First, they show that several genes in the RD1 region are required for secretion of ESAT-6 (Fig. 1). By reintroducing different RD1 fragments into BCG, Pym *et al.* provide evidence that ESAT-6 and its binding partner CFP-10 (ref. 6) are exported from the bacteria by a specialized secretion system dependent on flanking genes in the RD1 locus. Secretion was essential for induction of an optimal T-cell response.

Second, Pym *et al.* observed that immunization with a recombinant BCG strain carrying the RD1 region improves protection against aerosol challenge with *M. tuberculosis* in mice and guinea pigs. In particular, the authors observed reduced bacterial load in the spleen, suggesting an enhanced ability to restrict spread of *M. tuberculosis* from the initial site of infection.



**Fig. 1** RD1: on the cusp of virulence and protective immunity. Genes in the RD1 region were deleted during attenuation of the BCG tuberculosis vaccine. These genes comprise 1 of 5 similar loci in *M. tuberculosis* that are predicted to encode specialized secretion systems<sup>8,9</sup>. Pym *et al.*<sup>1</sup> provide evidence that flanking genes are required for secretion of the low-molecular-weight antigens ESAT-6 and CFP-10, and of the products of the PE and PPE genes, which may act as variable surface antigens<sup>8</sup>. The presence of the RD1 secretion system contributes to the virulence of *M. tuberculosis* and also enhances the protective efficacy of the BCG vaccine.

The best-studied gene in the RD1 region encodes ESAT-6, a secreted protein of unknown function that stimulates a strong T-cell response. The absence of ESAT-6 from BCG has led to the exploitation of this antigen as a diagnostic tool to distinguish infection with *M. tuberculosis* from BCG vaccination.

ESAT-6-specific responses increase during progressive infection, so detection of ESAT-6-reactive T cells may be useful for identifying individuals harboring clinical or subclinical tuberculosis infection who would benefit from treatment<sup>4</sup>. The immune response to ESAT-6 is also an element of protective immunity, and subunit vaccines that induce ESAT-6-reactive T cells provide partial protection against tuberculosis in animal models<sup>5</sup>.

As a marker of protection as well as pathology, ESAT-6 illustrates the double-edged thinking that occurs even at the

Why does RD1 enhance the protective efficacy of BCG? Two models can be proposed. It may be that BCG suffers from a shortage of antigens, and that addition of ESAT-6 and CFP-10 results in an increase in the number of CD4<sup>+</sup> T cells primed by vaccination. Although the relationship between number of CD4<sup>+</sup> T cells and degree of protection is not clear, there is precedence for the notion that increased antigen expression enhances the effectiveness of BCG<sup>7</sup>. If this is the mechanism of the RD1 effect, then it may be possible to reproduce it by bolstering BCG through separate delivery of RD1 proteins in some suitable vaccine formulation.

Alternatively, RD1 may alter the biology of BCG infection so as to exert a secondary effect on the immune response. For example, if addition of RD1 allows viable BCG to persist in the host for a

longer period after vaccination, then one might expect an increase in the number of activated T cells present at the time of challenge. In planning how to build rationally and progressively on improvements to BCG, it would be useful to disentangle these different mechanisms.

The aim of 'classical' vaccines is to mimic natural infection as closely as possible without causing disease. There are reasons to be wary of this strategy in the case of tuberculosis. Rates of reinfection and reactivation of disease attest to the incomplete protection associated with the natural response to infection, and it is likely that *M. tuberculosis* manipulates particular elements of the immune response to benefit its own survival.

We should look to natural infection for both positive and negative lessons.

Pym *et al.* show that RD1 is a part of the biology of infection that we can usefully reproduce during vaccination.

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Centre for Molecular Microbiology and Infection  
Imperial College London, UK  
E-mail: d.young@imperial.ac.uk