

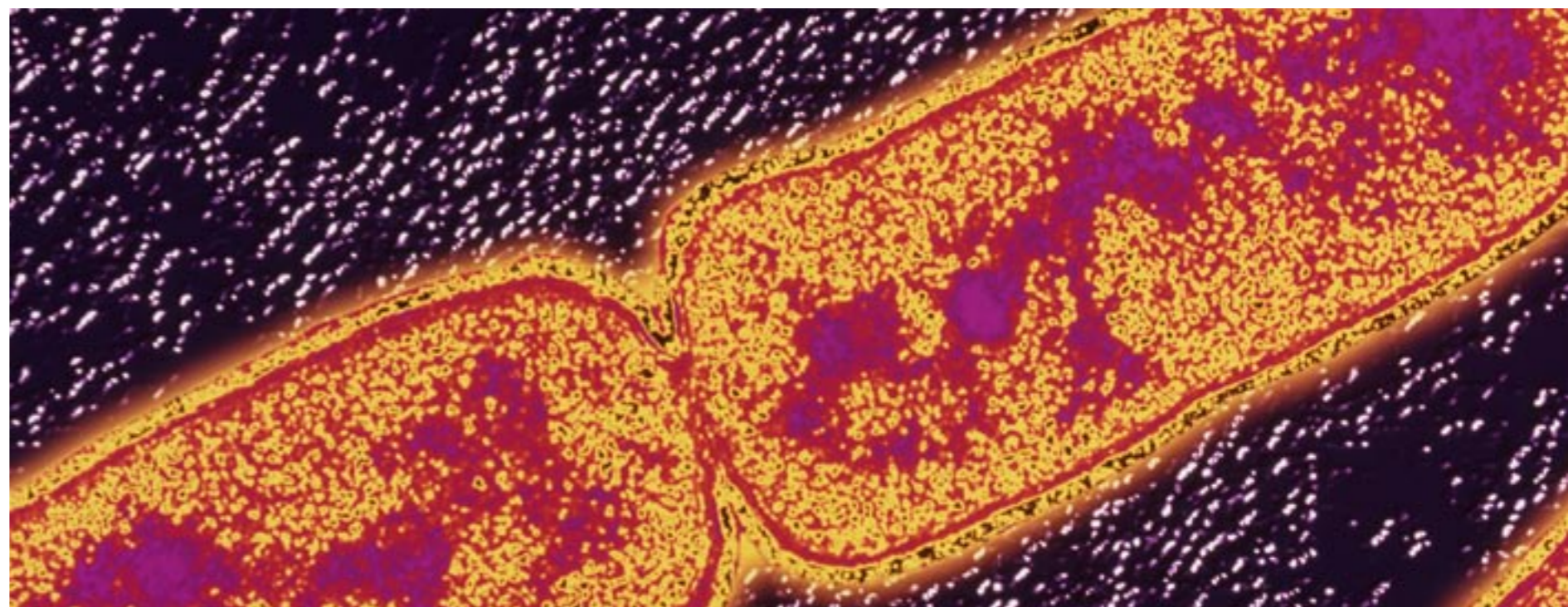
▲ Computer-generated model showing the proposed structure of gamma interferon. J.C. Revy / MGH J. Novotny – Boston / Roussel Uclaf / Biosym. / Science Photo Library

▶ Transmission electron micrograph (TEM) of *Mycobacterium tuberculosis* bacteria. Alfred Pasiaka / Science Photo Library

M*ycobacterium tuberculosis* is an intracellular pathogen, choosing to live within macrophages, where it inhibits antibacterial processes such as phagosome-lysosome fusion. It also expresses haemolysin-like molecules that might, like *Listeria*, enable its escape into the cytoplasm, although confirmed evidence of this is still lacking. It induces granuloma formation within the lungs, which can progress to caseating necrosis, enabling its spread by coughing, and resulting in the destruction of lung tissue. The classic test for infection, the Mantoux skin test, measures recruitment and activation of antigen-specific T cells in a delayed-type hypersensitivity test. This focus on cell-mediated immunity has led to a major interest in the role of gamma interferon (IFN- γ).

Mice – and humans – that are unable to make or to respond to IFN- γ are highly susceptible to mycobacterial disease

Some of the earliest experiments in which cytokine genes were knocked out clearly showed that mice unable to make IFN- γ become highly susceptible to infection with *M. tuberculosis*. This susceptibility has been exploited to provide a



Gamma interferon – key, but not sufficient for protection against TB?

M. tuberculosis is a classic intracellular pathogen – so macrophage activation by gamma interferon should be key to protection. But the picture may be more complicated, as **Hazel Dockrell** discusses.

model system in which the activity of anti-TB drugs can be tested in 8 days rather than the usual 1–1.5 months.

Mutations in the human IFN- γ receptor also result in susceptibility to mycobacterial disease. Although very rare, a number of individuals with mutations in the IFN- γ R1 or IFN- γ R2 chains of the IFN- γ receptor have been identified, and these individuals are more susceptible to infection with mycobacteria. Supporting the role of IFN- γ and its signalling pathways in immunity, similar Mendelian inheritance of Stat1, IL-12RB1 and IL-12p40 mutations have also been identified in susceptible individuals. More minor variations in the ability to make or respond to IFN- γ may also exist, as two variants in the IFN- γ promoter region, and a variant in the IFN- γ R1 promoter have been shown to be associated with pulmonary tuberculosis in a West African population.

IFN- γ during active disease and following treatment

During active clinical tuberculosis, peripheral blood IFN- γ responses to

mycobacterial antigens are present in cultures stimulated for 5–6 days, but are suppressed relative to those in healthy contacts. This depression is associated with the extent of disease, increasing from mild to moderate to advanced, and is particularly marked in patients with the disseminated miliary forms of disease. The ratio of IFN- γ to IL-10 can also be used as a marker of disease severity. With successful treatment, there is a recovery of the responses, one surprise being the rapidity with which such changes start after the initiation of treatment. A common interpretation of these depressed IFN- γ responses in active tuberculosis is that the cells capable of making IFN- γ are attracted to the site of disease in the lungs, and so are being missed in the assays normally performed on blood samples. The cells in bronchoalveolar lavage are able to produce better IFN- γ responses than those in the blood – and the presence of IFN- γ in pleural effusions can be used to help diagnose tuberculous pleurisy. However, recent findings that IFN- γ signalling processes are also

depressed in peripheral blood cells suggest that something more fundamental than just compartmentalization of antigen-specific cells is occurring and that *M. tuberculosis* may be actively inhibiting such beneficial T cell immunity.

If IFN- γ is key to protection, can it be used as an adjunct to treatment?

IFN- γ , given as an aerosol, has been used as a treatment for patients with refractory multidrug-resistant tuberculosis, or *M. avium/intracellulare* infection, with beneficial although temporary effects on bacterial loads. In mice, IFN- γ given intranasally extends the passive protection given by IgA antibody to the α -crystallin antigen.

Use of antigen-specific IFN- γ secretion as a marker of tuberculosis infection

Two commercial assays that measure IFN- γ production in response to stimulation with peptides from antigens called ESAT-6 and CFP10 (with an additional TB7.7 peptide in one case) are now available. These determine either the release of IFN- γ in a simple overnight whole-blood assay that uses a very sensitive ELISA (the QuantiFERON-Gold test) or the number of IFN- γ -producing cells in an overnight ELISPOT assay (T-SPOT.TB). Due to the relative specificity of the peptides used, BCG vaccination does not induce a false-positive result in these tests. These IFN- γ release assays were introduced to diagnose latent TB infection, but both latent and active tuberculosis infection can give positive results. Certainly, recent work has shown that latent tuberculosis is not a situation in which the bacilli are in a deep and silent sleep, but rather that during latency particular families of proteins are expressed, and that both maintenance of latency and the resuscitation of bacilli back into active growth are active processes. If an IFN- γ response is related to bacterial load, then such assays may also be able to monitor disease progression.

These overnight assays require the presence of cells that are capable of making IFN- γ quickly, and it is assumed that this reflects an ongoing immune response in the individual. Some studies, using an overnight ELISPOT assay, have shown that the numbers of IFN- γ -

Although IFN- γ is a good indicator of TB vaccine immunogenicity, it is unlikely that it will prove to be a correlate of protection on its own.

secreting T cells falls with a decline in bacterial load during treatment. However, in a group of household contacts in Uganda, IFN- γ production in a 5-day assay was greater in those that subsequently became Mantoux-test-positive than in those who remained test-negative. So the relationship between the shorter and longer term IFN- γ assays may not be as simple as 'short=ongoing immune response' and 'longer term=memory response resulting from prior exposure'.

IFN- γ as an indicator of immunogenicity in vaccine trials

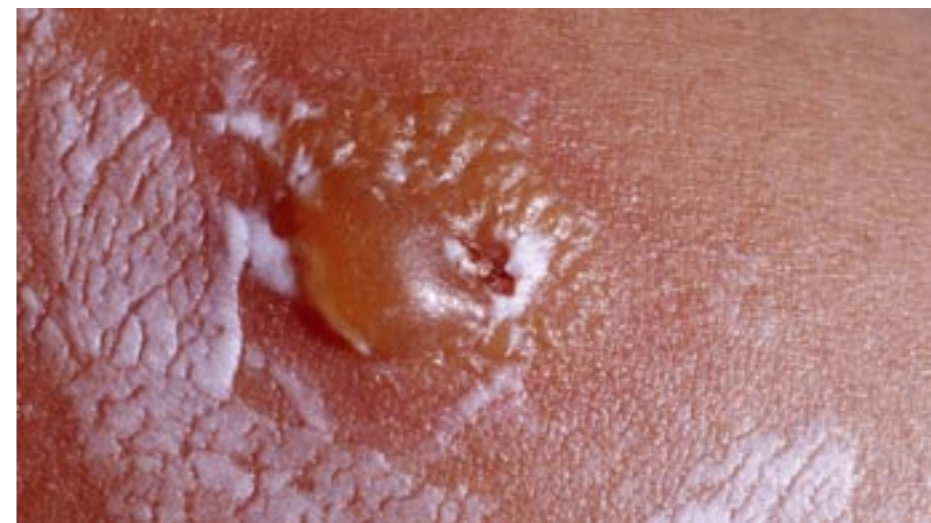
There are early trials of several new TB vaccines either ongoing, or starting in the near future, and most are using the *ex vivo* IFN- γ ELISPOT as the main read-out of immunogenicity, with a recommendation from a WHO TB vaccine working group that a longer term IFN- γ assay, such as the 6- to 7-day diluted whole-blood assay, be used as well. In studies of BCG vaccination in infants or adolescents, vaccination induces a marked increase in IFN- γ production to mycobacterial antigens in most immunologically naïve vaccinees. So far, in trials of novel TB vaccines, IFN- γ seems to be a good measure of vaccine immunogenicity.

IFN- γ may not be a correlate of protection

So can IFN- γ be used as a correlate of protection? It is a well behaved cytokine that is produced in excess in culture and that is stable in culture supernatants or on storage. Its production, by antigen-specific T cells, is increased by BCG or TB vaccines. And it seems to be important in immunity. Yet when asked about its value as a correlate of protection, the consensus among immunologists seems to be 'IFN- γ is necessary, but not sufficient'. So what is the problem?

The problem comes from three separate issues. First, although IFN- γ may be required for protection, it is a marker of disease as well as immunity. The finding that IFN- γ secretion is associated with Mantoux skin test induration in most, if

not all, subjects also raises questions about exactly what IFN- γ production reflects. Second, when IFN- γ production is present, there are situations where more is not better – more IFN- γ production may mean



◀ Arm of a person who has tested positive for tuberculosis (TB) in a Mantoux skin test. Martin M. Rotker / Science Photo Library

more pathology or less protection, for example in bovine tuberculosis in calves. Another recent study showed a poor correlation between antigen-specific IFN- γ production by CD4 T cells and protection in BCG-vaccinated mice. And finally there really is no hard evidence in man to show that those with greater IFN- γ production are better protected against development of TB than those with less or no detectable IFN- γ production.

A complex future...

So what is needed? First, trials that will measure IFN- γ production prior to development of disease, to assess whether those who do make more or less IFN- γ (in shorter term or longer term assays) will have better or worse protection against tuberculosis. One such study is underway in South Africa, where production of IFN- γ (as well as other cytokines) in overnight assays following stimulation with BCG is measured 10 weeks after BCG vaccination and then the infants were followed for 2 years, so that immune responses

in infants that did, or did not, develop tuberculosis could be compared. Other large multicentre studies are comparing immune responses in HIV-negative or -positive known TB contacts over time so that biomarker profiles can be compared in those who do or do not develop TB.

So although IFN- γ is a good indicator of TB vaccine immunogenicity, it is unlikely that it will prove to be a correlate of protection on its own. Additional assays that will measure more cytokines and chemokines using multiplex methods, that will assess whether IFN- γ is made by particular T cell subsets that simultaneously make one or more other cytokines, or that assess gene activation on the grand scale using microarrays, will be used in these studies. This is very sensible, because if IFN- γ induces expression of about 1,000 genes in a macrophage, true protective immunity may require a particular group of genes to be switched on (or alternatively, some genes to be switched on while others are switched off). Given the heterogeneity

of real people, and the variability in the clinical features of tuberculosis, there is unlikely to be a quick or easy answer here. But what is discovered may give us further insights into immunity to intracellular infections, and to the complex interplay between *M. tuberculosis* and its host.

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Further reading

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