

Despite being preventable and curable, tuberculosis continues to be one of the biggest threats to human health worldwide. Recently, however, research into an immunomodulatory approach to the treatment of the disease has produced exciting results, as Susan Pearson explains.

Tuberculosis

Getting around the problem of drug resistance

At the end of January, Microsoft chairman Bill Gates pledged \$600 million towards an ambitious new global plan to fight tuberculosis (TB) worldwide. Unveiled at the World Economic Forum in Davos, Switzerland, the Stop TB Partnership, a global initiative led by the World Health Organization (WHO) and supported by more than 400 organisations worldwide, made an appeal for \$56 billion to halve TB prevalence and death rates by 2015 – one of the objectives of the United Nations Millennium Development Goals.

Tuberculosis kills around two million people each year and infects a further nine million, making the disease one of the biggest threats to human health alongside human immunodeficiency virus (HIV) and malaria. The incidence of TB has increased so significantly in recent years that up to a third of the world's population is now infected with *Mycobacterium tuberculosis*, and in 1993 WHO declared the disease a global emergency.

While one of the key obstacles to reducing TB prevalence is drug resistance to existing medications, promising new data from research at the MRC National Institute for Medical Research in London is now pointing the way to a totally new approach to TB treatment that could by-pass the problems of drug resistance altogether.

TB worldwide

Around 80% of TB cases occur in the developing world. South East Asia accounts for the largest number of TB cases (35%), while sub-Saharan Africa has the highest rate of infection (350 cases per 100,000 people). In contrast, Western Europe accounts for only 5% of cases. In the UK, around 7000 new cases are reported each year, representing an infected population of approximately 12.5 per 100,000, most of whom live in the major cities.

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Left untreated, each person with active TB will infect on average 10–15 people every year, yet TB is both curable and preventable. In 1991, WHO set targets that aimed to detect 70% of new infectious TB cases and to cure 85% of those detected by 2005. At the same time, DOTS, an internationally recommended approach to TB control, was launched. This consists of five key elements: government commitment to sustained TB control; detection of TB cases through sputum smear microscopy among people with symptoms; regular and uninterrupted supply of high-quality anti-TB drugs; six to eight months of regularly supervised treatment; and reporting systems to monitor treatment progress and programme performance.

More than 17 million patients have now received treatment under the DOTS strategy, which by the end of 2002 had been adopted by all 22 of the countries with the highest number of TB cases. By the end of 2003, 182

countries were implementing DOTS, treating 45% – four times the amount reported in 1995 – of the estimated infectious cases worldwide. Clearly, DOTS has had a significant impact on global TB incidence but this is by no means the end of the story. Numbers of infections are still rising, especially in developing countries.

M. tuberculosis is a remarkably adaptable pathogen with strains resistant to all major anti-TB drugs and a multi-drug resistant (MDR) form, caused by TB bacilli resistant to isoniazid and rifampicin, currently the two most powerful drugs in the anti-TB battery. Drug-resistant strains emerge when treatment regimes are not completed, when incorrect medication is prescribed or when drug supplies are unreliable. From a public health perspective, poorly supervised or incomplete TB treatment is worse than no treatment at all.

However, increasing population, migration from rural to urban areas (with associated poverty), deterioration of the public health structures needed to control tuberculosis, and the HIV epidemic all mean that halting the emergence of drug-resistant TB and the spread of the disease is a massive challenge. The close supervision of treatment required to make it effective, as put forward by programmes such as DOTS, remains at a cost beyond the reach of the world's most affected. The development of new, shorter treatments for TB has never been more urgent.

Immunomodulatory approach

Led by Dr Stephen Jolles of the University of Wales, the MRC research explored the

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possibility of using an immunomodulatory approach in the fight against TB.¹ Intravenous immunoglobulin (IVIg) is used regularly to treat patients with primary antibody deficiencies, and at high doses (hdIVIg) to treat a range of autoimmune and inflammatory disorders (Table 1). Recently, the use of IVIg as an anti-infectious agent in viral and bacterial infections has been reviewed,² and IVIg given in combination with ampicillin has been shown to be protective against pneumococcal pneumonia.³ Dr Jolles's study investigated the capacity of hdIVIg to influence the course of TB infection in a mouse model.

Prior to beginning the study, there were concerns that this approach might actually reactivate latent or undiagnosed TB, given that hdIVIg is used as a steroid-sparing agent. Of the many people who have been exposed to TB, only around 10% actually develop the full disease. Many will reach a compromise with the organism, which is walled off in granulomas in the lungs where viable bacilli can persist for years.

The results, however, were the opposite of these expectations, to the extent that the team even considered the possibility that the groups of mice had been mixed up. The procedures were repeated again and again, each time yielding the same results.

Mice were infected then treated and

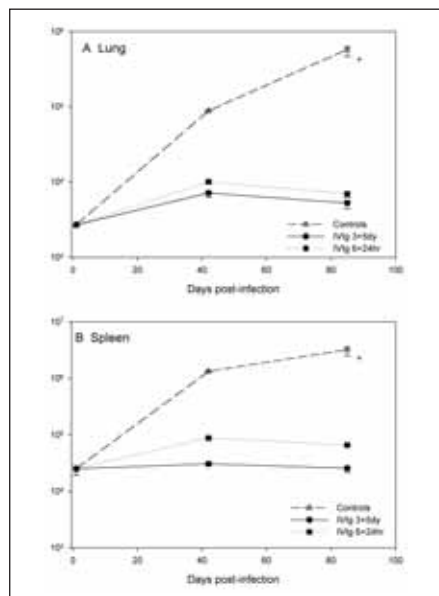


Fig 1. Effect of IVIg on the growth of *M. tuberculosis* in mice. C57BL/6 mice were infected with H37Rv and then received two identical intraperitoneal (ip) injections of IVIg at either six and 24 hours or three and five days post-infection. Viable counts were conducted on spleen and lungs on days 0, 42 and 85 post-infection. Results are expressed as \pm standard error of the mean (SEM) of five mice per group and are representative of at least four independent experiments ($*P < 0.01$ as measured by Student's *t*-test).

control mice received two identical-volume injections of IVIg or saline at either six and 24 hours or at three and five days post-infection – basically the equivalent of a single cycle split into two doses. At 40 days post-infection, the organism count in the lungs and spleen was 10-fold less than at infection. At 80 days, the count had been reduced by almost 100-fold and this effect persisted to the last count at 130 days. While the treated mice could not be considered 'cured', they had persistently 100-fold lower counts in terms of the numbers of viable organisms present in the lungs and spleen (Fig 1).

There was some concern that the lower counts were due to the organisms being mopped up in the blood by the polyclonal IVIg. However, the half-life of the antibody is only 17–21 days, so the persistence of the effect would not occur in the way it did if that were the case. In addition, TB is an intracellular organism that invades cells within hours of infection and so it is not normally accessible to antibodies. In fact, the six hour and 24 hour doses were slightly less effective than the three and five day cycles – a further indication that the bacilli are affected not in the bloodstream but in an intracellular location.

Another concern for Dr Jolles's team was that the introduction of a xenogeneic protein (foreign human protein into a mouse) could be stimulating an inflammatory response that was attacking the TB bacilli. However, testing the mice with equimolar concentrations of both human albumin and the IVIg carrier molecule (maltose) had no statistical effect on the results.

The next stage of the study was to compare different doses of IVIg. Saline was compared to doses of 0.1 g, 0.5 g, 1 g and 2g/kg of IVIg, the highest of these doses being equivalent to the concentration that would be used for immunomodulatory treatment in humans. In clinical practice, it is impossible to know how many days a patient has been infected and this was reflected experimentally by treating the mice late in the disease process (at over 100 days). Three weeks later, the organism counts were shown to drop, indicating that it really is possible to alter an ongoing infection.

Promising breakthrough

These results are exciting because until now long courses of multiple antibiotics have been the mainstay of TB treatment, while immunomodulation is still an emerging field. Using immunomodulation to treat TB is a new approach, and this research is the first time it has been demonstrated using hdIVIg.

The study also looked at some of the possible mechanisms involved. For example, were the results a direct effect on the macrophages that the TB bacilli typically infect? To test this possibility, the team treated TB-infected macrophages grown from bone marrow with IVIg and saline. No statistical difference between the IVIg-treated cells and the controls was found, suggesting that the success of the antibody treatment in

controlling TB infection is not due to direct inhibition of infection or replication in the macrophages (Fig 2).

As T cells are believed to be the conventional effectors that control TB, the protection experiments were repeated using nude (athymic) mice, which lack conventional CD4+ and CD8+ T cells. The results showed no difference between the control mice and those treated with IVIg, suggesting that this immunomodulatory mechanism is likely to involve a conventional T-cell response (Fig 3).

According to Dr Jolles: "The probable involvement of T cells fits in with the duration of the effect we saw with the hdIVIg treatment. It may be that what we are seeing is the development or enhancement of a T-cell memory response that has been set up in the interaction between the organism and the host."

"In a simplistic way, we wondered whether or not the TB organisms that have evolved with us for thousands of years are sufficiently clever to establish a suboptimal immune response that permits their persistence inside granulomas, and so the immune system is not able to cause sterility and eradication of the organism. What we seem to be doing here is improving on that and tipping the balance towards the host being able to win the fight."

Looking ahead

"Now we need to study the mechanisms involved. The next phase of research might, for example, look at which part of the antibody produces the effect. As antibodies have two regions, the variable F(ab')₂ region, responsible for the huge repertoire of antigen binding sites, and the constant Fc region, which interacts with the cellular Fc receptors, potential effects could depend on either the repertoire (F(ab')₂) or the constant Fc region. If an experiment were to be conducted with

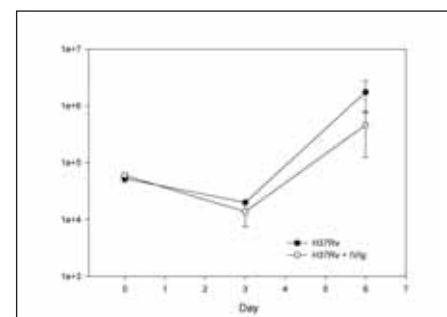


Fig 2. Effect of IVIg on growth of *M. tuberculosis* in macrophages. Murine C57BL/6 cells were infected with H37Rv at a multiplicity of infection of 2:1 for six hours and maintained in either control medium or medium containing 25 mg/mL IVIg. Viable counts were conducted on days 0, 3 and 6 post-infection. Results are expressed as \pm SEM of triplicate wells and represent three independent experiments.

human Fc alone, this might help to determine if the effect is due to Fc interactions or the antigen binding repertoire F(ab')₂."

While the results from this work are certainly promising, Dr Jolles remains cautious: "What we have to bear in mind is that we have achieved these results in mice, providing us with a model of what could happen in humans, but we cannot make a direct translation from a mouse to a human. That is why establishing the mechanism involved is so important. However, what we have on our side in terms of applying this treatment in a human setting is that the use of IVIg is already established as a safe treatment – it's a medicine that we can take off the shelf and will not need to go through the same clinical trials as a new drug.

"We are keen to do a pilot study and are in the process of putting together a grant proposal for a study in patients with pulmonary TB. Such patients would be given the optimal conventional treatment as well as the hdIVIg and would be studied in great detail during the early phase of their treatment. As our research showed no effect in athymic mice, it would be important at this stage to choose patients who have a functioning immune system (ie do not also have HIV or other T-cell defects) so that in the first instance the proof of principle study is as straightforward as possible.

"It would be fantastic if by using a tried and tested antibody treatment such as IVIg as an adjunct to conventional TB therapy we could shorten treatment times, enhance the immune response and get around the problem of drug resistance."

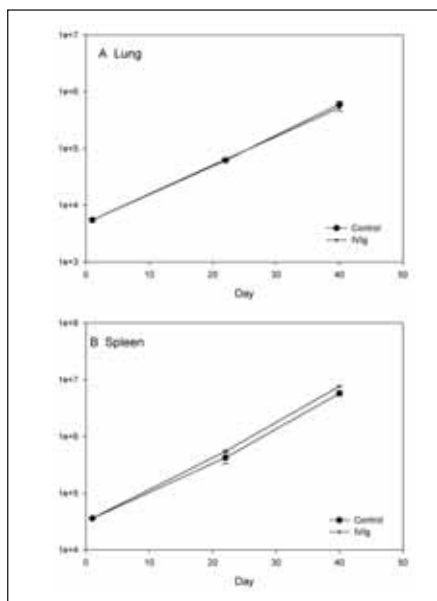


Fig 3. Effect of IVIg on the growth of *M. tuberculosis* in athymic mice. The mice (BALB/c nu-/nu- litter mates) were infected with H37Rv and then treated ip. Viable counts were conducted on days 0, 22 and 40 post-infection. Results are expressed as means \pm SEM of five mice per group.

Table 1. Frequent uses of intravenous immunoglobulin (IVIg).

SPECIALTY	CONDITION	
Neurology	Guillain Barre syndrome (RCT and CR)	
	Multifocal motor neuropathy (RCT)	
	Chronic inflammatory demyelinating polyneuropathy (RCT)	
	Dermatomyositis and inflammatory myopathies (RCT)	
	Myasthenia gravis (RCT)	
	Lambert-Eaton syndrome (RCT)	
Haematology	Immune thrombocytopenia (RCT)	
	Post bone marrow transplant (RCT)	
	Myeloma and chronic lymphatic leukaemia (RCT)	
	Parvovirus B19-associated aplasia	
	Immune neutropenia	
Immunology	Immune haemolytic anaemia	
	Primary antibody deficiencies (eg XLA, CVID, HIGM, WAS)	
	Secondary antibody deficiencies (eg myeloma, CLL (RCT), drugs)	
Dermatology	Kawasaki syndrome (RCT)	
	Dermatomyositis (RCT)	
	Toxic epidermic necrolysis	
	Blistering diseases*	
	Immune urticaria	
	Atopic dermatitis	
Others	Vasculitis (RCT)	
	Systemic lupus erythematosus	
	Streptococcal toxic shock syndrome	
	Birdshot retinochoroidopathy	
	Autoimmune uveitis	
	Mucous membrane pemphigoid	
	IVIg is used mainly at high dose (2 g/kg) for the indications listed in neurology, haematology, rheumatology, dermatology and others. In immunology, replacement doses (0.4 g/kg) are given. The uses listed are not exhaustive but cover most of the disorders for which IVIg is most frequently used, and whether a randomised controlled (RCT) study or Cochrane review (CR) is available.	
	CVID: common variable immunodeficiency; XLA: X-linked agammaglobulinaemia; HIGM: hyper-IgM syndrome; WAS: Wiskott Aldrich syndrome; CLL: chronic lymphocytic leukaemia.	
*Blistering diseases include pemphigus vulgaris, pemphigus foliaceus, bullous and nodular pemphigoid, mucous membrane pemphigoid, gestational pemphigoid, epidermolysis bullosa acquisita and linear IgA disease.		

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