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IMMUNOTHERAPY WITH MYCOBACTERIUM VACCAE IN THE TREATMENT OF TUBERCULOSIS

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1. ABSTRACT

All the trials of immunotherapy of tuberculosis with killed Mycobacterium vaccae, published or not, that are known to the authors are reviewed here.

Following an introduction giving a brief account of some earlier immunotherapies for tuberculosis, the origins of the concept of immunotherapy with M.vaccae are considered. Progress is traced from the early work with irradiation-killed organisms in leprosy to the study in London of modulation of tuberculin skin-test responses, and the first comparative trials in The Gambia and Kuwait. In the last of these studies, dosages and different preparations were compared. As a result of this subsequent studies have used 10^9 heat-killed organisms, equivalent to 1mg wet-weight of bacilli, as a standard dose.

A series of small trials in Argentina, India, Nigeria, Romania, South Africa and Vietnam have pioneered the way forward, disclosing geographic variability, with South Africa as the only country where almost no effects were recorded. Together the studies have shown that a single dose may not be sufficient. These studies have confirmed the mode of action of M.vaccae to be regulation of cell-mediated immunity with enhancement of Th1 and down-regulation of Th2, and they have shown benefits in faster bacteriological conversion, reduction in ESR, recovery of body weight and resolution of radiological opacities, leading to better recovery from the disease even when given to patients receiving directly observed therapy, short-course (DOTS).

Three major randomised, placebo-controlled and partly blinded trials have been carried out in Africa. The first, in South Africa showed no M.vaccae-related effects. The second trial, in Uganda, confirmed the observations made in the earlier studies of faster sputum conversion and

better radiological clearance. The third trial, in Zambia and Malawi, showed a trend towards benefits in the treatment of HIV seronegative patients but failed to show beneficial effects in HIV seropositive patients.

Studies in patients with multi-drug-resistant tuberculosis have shown that multiple doses of immunotherapy are required in most cases, and that these markedly improve cure-rates for these patients. This is especially so when they are also treated with chemotherapy tailored to the resistance pattern of their infecting organisms. A small study has just commenced in which repeated doses of M.vaccae are being administered to a group of patients who have failed treatment with DOTS-Plus (directly observed therapy with drugs selected on the basis of drug susceptibility profiles).

Late in the investigation came publications from China supporting and confirming the data in both drug-sensitive and drug-resistant disease, by the use of multiple injections of their own different preparation of M.vaccae.

The trial that is now almost complete in Vietnam of 3 doses of M.vaccae in the treatment of newly diagnosed pulmonary tuberculosis, is accompanied by a chemotherapeutic regimen with a shortened continuation phase. If this important study is successful, immunotherapy with killed M.vaccae should be introduced into the treatment regimens for tuberculosis worldwide.

2. INTRODUCTION

Modern short course chemotherapy for tuberculosis is among the most effective and cost-effective interventions for any chronic infectious disease. Yet tuberculosis remains one of the major public health threats in today's world. Numbers of cases continue to rise and only a quarter of patients have access to adequate therapy, as defined by the World Health Organization, even though declared a Global Emergency in 1993. In addition, resistance to standard therapeutic regimens poses an increasing problem (1). Alternative modes of treatment are urgently required, and immunotherapy is one that merits very serious consideration.

Immunotherapy for tuberculosis is not a new concept. Indeed, it forms the central theme of George Bernard Shaw's play of 1906 The Doctor's Dilemma (2). The first immunotherapy used in patients with tuberculosis was developed by Robert Koch in 1891, following his observation that the immune response of tuberculous guinea pigs could be modified beneficially by injections of an extract of tubercle bacilli, his 'brown fluid' (3). After his pre-clinical experiments in guinea pigs and in a healthy volunteer, a 17 year old art student who subsequently became his wife, Koch went on to treat patients with repeated injections into the skin of their backs until he was able to give quite large amounts without inducing a severe local reaction (4). In his carefully selected patients, including his colleague Paul Ehrlich who developed the acid-fast staining method for tubercle bacilli and then found them in his own sputum, Koch achieved remarkable success. With repeated injections of his 'brown fluid', subsequently identified as 'old tuberculin' (OT), Koch at first enhanced necrosis and then induced tolerance to the injection which was accompanied by similar changes around the tubercle bacilli in lesions. This might have been expected to result in uncontrolled growth of the bacilli and to have precipitated death, but in Koch's hands, it did not do so. Its use by others, however, was not always so successful, the intervention could be fatal (5), and the treatment fell into disrepute. Others attempted to develop safer and more regularly successful methods.

Amongst Koch's successors was Henry Spahlinger who developed two methods, one using what he thought were dead tubercle bacilli cultured on media containing human sera (6), but which today might be considered bacilli in a stationary phase (7). Spahlinger's other therapy was based on injections of sera raised in horses immunised with his dead tubercle bacilli. With both of these therapies Spahlinger apparently achieved remarkable success, treating patients in London, including President Nehru of India, with his method until the early 1970s (personal communication).

Friedrich Franz Friedmann, a German physician believed that immunization of the tuberculosis patient with a less pathogenic mycobacterial species might be as efficacious as Koch's therapy and less dangerous. He had himself described a new species isolated from a diseased turtle in the Berlin Zoological Gardens (8), now known as Mycobacterium chelonei, and this was the organism that he developed as an immunotherapeutic agent. As "Anningzochin", named after Friedmann's companion, Anna Maria Zoch (9), this material is still obtainable from its manufacturers in Germany though we know of no publications on its use in recent years. It was originally used as a living suspension since it was thought necessary to induce a 'limited tuberculous process' for success to be achieved. Injection of M. chelonae often induced discharging abscesses but patients welcomed this as a sign that the therapy was working (10).

The next logical step forward, the search for mycobacteria able to induce protective immune responses even when killed was taken in the early 1970s.

In 1971 strains of Mycobacterium vaccae (11) were isolated from the environment in Uganda where a successful trial of BCG against leprosy had been carried out (12, 13). Subsequent work in London showed that animals primed by exposure to M.vaccae and then vaccinated with BCG, produced enhanced protection from challenge with several species of mycobacterial pathogens (14). A suspension of killed M.vaccae was first investigated as an adjunct to BCG as a vaccine against leprosy (15,16), and later as an immunotherapeutic agent for the treatment of multibacillary leprosy and erythema nodosum leprosum (17). Subsequently, with appreciation of responses to common mycobacterial antigens being an essential part of the protective immune response to tuberculosis, preparations of M.vaccae were investigated for the treatment of tuberculosis. This paper is a review of the results achieved.

3. THE THEORETICAL BASIS OF IMMUNOTHERAPY WITH MYCOBACTERIUM VACCAE

3.1. The effects on Immunopathology

The lesions of active tuberculosis are principally due to the tissue destruction caused by the immune response to the antigens of tubercle bacilli, including their heat shock proteins which are highly cross-reactive with the stress proteins of mitochondrial origin in host tissues. The death of tissue in tuberculosis is due to a combination of immune reaction to the invading bacilli with an auto-immune response to locally produced stress proteins. The mechanism of tissue death is thought to involve the necrotising toxicity of tumour necrosis factor (TNF) in the presence of the type 2 cytokines interleukins 4 and 5 (IL-4, IL-5) and immunoglobulin-E (IgE) (18, 19).

There appear to be at least two mechanisms by which immunotherapeutic strategies against tuberculosis could operate. That of Koch was aimed at increasing necrosis of the tuberculous lesions so that superficial ones sloughed off and deep ones were converted into caseous abscesses with thick fibrous walls, putting the bacilli at a disadvantage through reducing the availability of oxygen. Koch's method then progressed to induction of immune tolerance to the bacillary antigens and so long as this is maintained the thick-walled abscesses still containing live bacilli lie dormant and the disease process stops, sometimes permanently.

The second mechanism for immunotherapy is completely different. It depends on suppressing immune necrosis and replacing it with a cellular immune response that can actively destroy tubercle bacilli. The therapies of Spahlinger, and Friedmann may have acted through this mechanism. With the use of Mycobacterium vaccae, the mode of action is through cellular immune regulation. Th1 responses to antigens shared between bacilli and host tissues are increased and the Th2 mechanisms to the specific antigens of the bacilli are down-regulated (20,21).

Except for our own largely unpublished work in mice (14,22) and guinea pigs (23), animal investigations of immunotherapy with M.vaccae have only been done and reported after the first clinical studies were performed. These have demonstrated the way in which injection of the killed organism can strengthen and modulate the immune response relevant to protection from and treatment of tuberculosis (20,24,25). As mentioned above, treatment with M.vaccae has been shown to enhance Th1 and depress Th2 mechanisms. In addition, investigations in animal models of allergy (26,27) show that M.vaccae enhances immunoregulation through the induction of T-regulatory (Tr) cells (28,29).

In the initial animal investigations it was found that giving too high a dose of M.vaccae to mice was prejudicial, and for beneficial effects it was necessary to give them doses 10 to100 times smaller than the effective dose in humans (20,30). It was these observations that delayed the introduction of repeated doses of M.vaccae for the treatment of human disease. Neither are all animals suitable for the assessment of immunotherapy with M.vaccae. Detailed studies in experimentally infected rabbits failed to show any benefit from the treatment (31).

3.2. The evidence from skin-testing

The first immunotherapeutic agent for tuberculosis was Koch's 'old tuberculin' (OT), the forerunner of the modern tuberculin skin test reagent. The principle of the tuberculin skin test is to inject a very small quantity of antigenic material from M.tuberculosis very superficially into the skin. This triggers a cascade of responses from rapid binding of circulating antibody producing a wheal and flare within a few minutes to the accumulation of macrophages and lymphocytes at the site and their subsequent release of cytokines maximal between 2 and 3 days after injection. This reaction is thought to be representative of what occurs around tuberculous lesions, or around sites of challenge with live tubercle bacilli. Depending on the antibodies bound, the T-lymphocytes attracted and the cytokines released, different responses to Tuberculin are observed after 72 hours when the test is read (32,33).

Koch described the necrotising immunological response to tubercle bacilli in his early work on tuberculous guinea pigs overcoming superficial re-challenge with tubercle bacilli. This occurs by rapid necrosis and sloughing off of the tissues around the second challenge site, with subsequent healing. Despite this, the animal proceeds to die of the initial tuberculous infection. This is the Koch phenomenon (34,35). The classical 'Koch-type' response to skin tests with tuberculin is tender on palpation, sharply demarcated with an irregular craggy edge and of brick-red colour. Such reactions, which are thought to result from a combination of Th1 and Th2 mechanisms, tend to be quite large, depending on the amount of tuberculo-protein injected, and the more intense responses may show blistering and even ulceration as a result of immune-mediated necrosis. They occur in many patients with clinical tuberculosis and in a proportion of those with arrested disease or with live bacilli dormant in their tissues.

The positive response to the tuberculin skin test associated with protective immunity has been called the 'listeria-type' response (14,36), though this is a misnomer. It is mediated by Th1 cells without an additional Th2 component. Reactions of this type are not normally tender on palpation, they have a smooth and gradual edge and are pink and less well-demarcated than 'Koch-type' responses. They do not blister or ulcerate and there is no indication of necrosis.

Distinguishing reliably between these two pathways would add tremendously to the value of the test, but is quite difficult with the commonly used purified protein derivative (PPD) though much easier with the "New Tuberculins" prepared from ultrasonically disintegrated organisms (37). (The term New Tuberculin was introduced by Robert Koch to distinguish reagents prepared by liberation of cytoplasm – in Koch's work by grinding in a mortar - from viable tubercle bacilli from those, the Old

Tuberculins, prepared from filtrates of old cultures containing dying and autolysing bacilli.) A recently described *in vitro* test is more specific for infection by M. tuberculosis but, like the tuberculin test, fails to characterise the precise nature of the immune response (38).Qualitative differentiation between the two types of response can be used to recognise patients with likely clinical lesions and to assess the efficacy of BCG vaccination (39), as well as to assess the efficacy of immunotherapy (40,41).

Other studies have confirmed and extended these observations but the relationship between tissue necrotising hypersensitivity and protective immunity remained enigmatic. While some workers considered them to be separate and distinct reactions, others held that they merely differed in degree (42). One problem is that both were associated with tuberculin reactivity and were mediated by T cells. In 1975 Lefford (43) suggested that the two reactions were mediated by different subpopulations of T cells or by these cells at different stages of maturation. Later studies show how accurate Lefford's postulate was (28,29).

The use of quadruple skin testing with reagents prepared from different species has been used to differentiate between responses to species-specific antigens and to common mycobacterial antigens, including those cross-reacting with antigens of the mitochondria of human tissues. Patients with active tuberculous disease, and a whole series of other diseases with similar dysregulated immunity, show a diminished or absent response to common mycobacterial antigens (44,45).

3.3. The evidence from mixtures of new tuberculins

Some new tuberculins rarely or never produce 'Koch-type' reactions. Amongst these are reagents prepared from some of the fast-growing mycobacterial species, including M.vaccae and, somewhat surprisingly, M.leprae. This may be attributable to the finding that these species lack both slow-grower associated and fast-grower associated groups of antigens (46). From these observations the question arose as to which type of response would be made to a mixture of Tuberculin with Vaccin (a new tuberculin prepared from M.vaccae) in a person making a Koch-type response to Tuberculin alone?

Skin test reagents were prepared, and pooled, from a number of slow-growing species to which reactions are often of Koch-type. This 'slow-grower' reagent was tested on one arm of volunteer leprosy patients and healthy persons, and on the other arm the same mixture was tested after adding a reagent prepared from an individual fast-growing species (47-50). The results from these studies allowed fast-growing species to be clustered into those showing little effect on the response in either arm, those suppressing the local response to the complete slow-grower/fast-grower reagent, and those suppressing responses on both arms. In the latter case, of which M.vaccae was a notable example, the complete mixture on one arm regulated the response to the incomplete mixture on the other arm – a phenomenon that could be utilised for the immunotherapy for tuberculosis. Hence, if antigens of M.vaccae injected intradermally are able to regulate the reaction to other species on the opposite arm, why should they not alter the immune response going on around a tuberculous lesion in the lung?

4. THE FIRST PREPARATION USED FOR IMMUNOTHERAPY

In the mixed reagent experiments the immunoregulatory effect of soluble antigens of M.vaccae only lasted a few weeks, but when whole killed organisms were used in animal models, the effect lasted several months. In preliminary studies comparing BCG alone with BCG plus 10^7 irradiation-killed M.vaccae (see below) enhanced responsiveness to mycobacteria was shown to persist for at least 10 years in recipients of the mixture (15).

The initial reagent for immunotherapeutic use was prepared from a strain of M.vaccae originally isolated from the Ugandan environment (13). The original growth consisted principally of smooth colonies which gave rise to stable variants with rough colonial morphology. One of the latter variants was selected and checked for purity by repeated subculture of single colonies on solid media. The organism, termed R877R was grown on a simple medium containing no antigenic constituents (Sauton's medium), harvested after incubation at 32^oC for 28 days and suspended in M/15 Borate buffered (pH 8) physiological saline at a concentration of 10mg bacilli/ml. This suspension was sterilised by exposure to 2.5 megarads from a Co^{60} source (40,41).

In 1980 following pre-clinical studies, two healthy volunteers in London with 'Koch-type' responses to Tuberculin measured a few days earlier received an injection of 1mg irradiation killed M.vaccae (M.v. ir). When the Tuberculin test was repeated 3 months later, their reactions were well-controlled, soft reactions similar to those following BCG in British schoolchildren, and of 'listeria-type' rather than 'Koch-type'. This was the first direct evidence that the type of immune response of relevance to pathogenicity could be regulated and modified by a therapeutic intervention.

5. THE FIRST STUDIES IN MAN OF IMMUNOTHERAPY WITH M. VACCAE

5.1. Studies in Leprosy Patients

Patients with chronic lepromatous (multibacillary) leprosy who have been fully treated with chemotherapy are bacteriologically cured but still have most of the immunological manifestations of their disease (51), including persisting lack of cell-mediated immunity to the antigens of M.leprae demonstrable by skin testing. This potentially provides a 'test-bed' for immunotherapy, where correction of dysregulated immunity can be investigated in the absence of on-going infection or a bacterial load.

Studies were set up in Spain in 1983-85 amongst volunteers with long-treated lepromatous disease to determine the dose of M.v. ir. required to induce positive skin-test responses to Leprosin A. This reagent is prepared

from M.leprae extracted from the tissues of experimentally infected armadillos and thoroughly cleaned from host tissue antigens (52). It is a tuberculin-like reagent, with reactions read after 72 hours, and not comparable with Mitsuda Lepromin.

Single injections of 10^7, 10^8 or 10^9 M.v. ir. (equivalent to 0.01, 0.1 and 1mg wet weight of M.vaccae), or of buffered saline as a control, were given at yearly intervals in ascending order to randomised, Leprosin A negative patients (53). Just before injections of M.vaccae or buffer were administered, patients were tested with Leprosin, Tuberculin, Vaccin and Scrofulin (prepared from M.scrofulaceum). A year after the 10^9 dose about one third of patients produced positive responses to Leprosin for the first time. During the study it was found that many of the patients had quite large responses to Tuberculin, though generally not of 'Koch-type', and it was hypothesised that a low dose of Tuberculin, $1/10^{th}$ of the skin-test dose, added to 10^9 M.vaccae might achieve a better Leprosin response rate amongst its recipients. Some of the patients receiving 10^9 M.vaccae alone, but not responding to Leprosin, together with some additional volunteers with chronic lepromatous disease, received the M.vaccae plus Tuberculin (M.v. ir +T) mixture and were retested with Leprosin a year later (Tuberculin alone elicits no lasting immunological effect since it was amongst the skin-test antigens used each year on all patients without there being any evidence for an effect on the next years test results). The mixture was more effective than the M.vaccae alone (see table 1a) and, surprisingly, the local reactions to injection were less than those to M.vaccae alone. A group of volunteers amongst the staff of the leprosarium received injections of M.v. ir and were followed with repeat skin tests a year later (54). No increase in skin-test positivity was observed.

5.2. The first study in tuberculosis patients

This study was carried out in 1985 in 10 volunteers with active pulmonary tuberculosis under treatment with standard short-course chemotherapy 2RHZ/4RH (This indicates an intensive phase of 2 months treatment with daily rifampicin [R], isoniazid [H] and pyrazinamide [Z], followed by a continuation phase of 4 months of daily rifampicin and isoniazid) in the Middlesex Hospital, London (55). The study was based on skin-testing with the same reagents used for the leprosy study, viz. Tuberculin, Leprosin A, Scrofulin and Vaccin. Quantitatively, results were read as two diameters of induration in millimetres, one measured in the vertical axis and the other in the transverse axis. The recorded value is the mean of the two readings, fractions of a mm being increased to the higher value. Qualitatively, the type of response after 72 hours was recorded as of "Koch-type" (K), or otherwise. Eight patients received an intradermal injection of 1mg M.v. ir. Two patients received similar injections but of buffered saline alone as controls. One month later skin-testing was repeated and significant differences were found in the results for Tuberculin but not for the other reagents (see table 1b).

Both these studies depending on changes in skin test response confirmed the principles upon which immunotherapy with M.vaccae was based, and led to trials of efficacy and patient benefit.

6. THE FIRST TRIALS OF EFFICACY

Two studies were set up almost at the same time in 1985, one in Kuwait and the other in The Gambia.

6.1. The Kuwaiti Studies

These studies were carried out in volunteers starting treatment for pulmonary tuberculosis (56-58). They were designed to investigate which of a series of preparations of M.vaccae had the greatest effect on *in vitro* cell-mediated immune recognition of common mycobacterial antigens, or lymphocyte transformation response in the presence of added interleukin-2 (IL-2), and on antibody production. Irradiation-killed preparations of M.vaccae (M.v. ir) tested were at doses of 0.1mg, 1mg and 2mg and an autoclaved preparation (M.v. hk) was tested at 1mg per dose. Additionally, M.v. ir at 1mg per dose was enriched with $1/10^{th}$ skin-test dose of Tuberculin (M.v. ir + T), as selected in the studies of leprosy in Spain, or with the adjuvant, Murabutide, added at 50μg per dose to M.v. ir alone (M.v. ir + M) or to M.v. ir + T (M.v. ir +T +M). (Murabutide is a commercially available preparation of the muramyl dipeptide of bacterial cell walls and has been extensively investigated as an immunological adjuvant in various vaccine studies (59). Cell-mediated responses induced to common mycobacterial antigens and lymphocyte transformation in the presence of IL-2, measured 2 months after administration of the different M.vaccae preparations are shown in table 2a. Total IgG antibody responses to Tuberculin, Scrofulin, Vaccin, Nonchromogenicin (new tuberculin prepared from M.nonchromogenicum), and a streptococcal antigen, were measured immediately before and 2 months after some of the immunotherapeutic injections. The IgG subclasses IgG1, IgG2, IgG3 and IgG4 were tested in relation to some of the antigens and immunotherapeutic agents. The paired t-test data for the IgG and its subclasses is shown in table 2b.

The Kuwait study was also important in that it was the first to test the immunotherapeutic agents for toxicity with a wide range of biochemical and haematological tests. No evidence of toxicity was found. It was also the first study to seek evidence of efficacy. The numbers were few and no significant changes were found between the placebo and test groups in sputum smear or culture and although small differences in x-ray changes were observed, they did not reach statistical significance. Regain of body weight marginally improved, and fall in erythrocyte sedimentation rate (ESR) was similar in all the groups. The very first observations of efficacy of immunotherapy in multi-drug-resistant-tuberculosis (MDRTB) were made during the course of this study.

Unfortunately the Gulf War put an end to the studies before the intended randomised, placebo-controlled trial could be carried out.

6.2. The Gambian Study

Run by the MRC Laboratories at Fajara in 1986-8, and supported by the British Council, the results of this trial have still not been formally published, or fully analysed. This is the only trial in tuberculosis exclusively

Table 1. Effect of M. vaccae immunotherapy on skin test reactivity

A. Results of studies based on skin-test conversion		
Group	Numbers +ve (%) To Leprosin A	Fisher's exact test
Placebo	1/29 (3.4%)	
10^7 M.v. ir	2/28 (7.1%)	n.s.*
10^8 M.v. ir	0/4	
10^9 M.v. ir	10/29 (34.5%)	p<0.003
10^9 M.v. ir + T	10/21 (47.6%)	p<0.0003
Conversion to Leprosin A positivity in patients with fully treated lepromatous leprosy one year after different doses and preparations of irradiation-killed M.vaccae. All were negative (<2mm) at the start. This study was carried out at Sanatorio Fontilles, Spain between 1983 and 1987 (53). Note: Only doses of 10^9 M.v.. preferably with added Tuberculin, show significant conversion of lepromatous leprosy patients to Leprosin A positivity. * n.s. = not significant, M.v. ir Irradiation-killed M.vaccae in the doses shown. M.v. ir + T 10^9 irradiation-killed M.vaccae plus 0.1 skin-test doses of Tuberculin.		

B. The mean sizes in mm of Tuberculin responses in patients (numbers in parenthesis) undergoing short-course chemotherapy for pulmonary tuberculosis at the Middlesex Hospital, London in 1985 (55).			
	Immunotherapy	t-test	Placebo
Time 0	19.2±5.6 K (6)	n.s.	25±0 K (2)
t-test	p<0.005		n.s.
1 month	9.8±2.5 (8)	p<0.001	24.5±4.9 K (2)
Tests were carried out immediately before and 1 month after an injection of irradiation-killed M.vaccae (immunotherapy), or of buffered saline (placebo). Note: The letter K indicates that reactions were qualitatively of "Koch-type", showing incipient necrosis. This type of reaction to Tuberculin is the result of combined Th1 and Th2 responsiveness. Switching off of Koch-type responses was the first evidence of down-regulation of Th2 following immunotherapy. Comment:These early studies using skin tests as indicators of immune regulation herald the potential value of immunotherapy with M.vaccae as a modulator of immune response in the treatment of mycobacterial disease.			

Table 2. Effect of M. vaccae immunotherapy on immunological parameters

A. Results obtained in Kuwait (57,58)		
	Numbers responding to group i antigens	
Placebo	5/49 (11%)	
10^8 M.v. ir	2/8 (25%)	n.s.*
10^9 M.v. ir	11/38 (29%)	p<0.03
$2x10^9$ M.v. ir	9/12 (75%)	p<0.0001
10^9 M.v. ir + T	5/19 (26%)	n.s.
10^9 M.v. ir + M	1/18 (6%)	n.s.
10^9 M.v. ir + T + M	9/25 (36%)	p<0.02
10^9 M.v. hk	10/22 (45%)	p<0.002
	Increased responses to added IL-2	
Placebo	n.s.	
10^8, 10^9, + T, or + M	n.s.	
10^9 M.v. + T + M	p<0.005	
10^9 M.v. autoclaved	p<0.0005	
Changes in lymphocyte transformation in the presence of common mycobacterial (group i) antigens (46) or interleukin-2, two months after receiving a single dose of immunotherapy with M.vaccae killed by irradiation, with or without, added Tuberculin (T) or Murabutide (M) or killed by autoclaving. Probability assessed by Fisher's exact test. Note: In these studies response to common mycobacterial antigens was determined by measuring lymphocyte transformation in the presence of sonicated extracts of at least 4 different mycobacterial species of which 2 were slow-growing species and 2 were fast-growing species. Those making positive responses to all 4 species were taken as responders to group i antigens. Comment. Doses of 10^9 or more M.vaccae, especially when heat-killed, are associated with improved cellular responses to common mycobacterial (group i) antigens and to interleukin-2. * n.s. = not significant, M.v. ir = Irradiation-killed M.vaccae in the doses shown, M.v. ir + T = 10^9 M.v. ir plus 0.1 skin-test doses of Tuberculin, M.v. ir + M = 10^9M.v. ir plus 50microgram murabutide, M.v. ir + T + M.=.10^9 M.v. ir + T plus 50 microgram murabutide, M.v. hk = 10^9 heat-killed M.vaccae.		

B.Paired t-test results expressed as probability values between initial and 2-month serum samples for IgG and its subclasses to a range of antigens

	Placebo	M.v.ir	M.v.ir+T	M.v.ir+M	M.v.ir+T+M	M.v. hk
IgG	n.s.*	<0.001	n.s.	<0.001	<0.007	<0.001
IgG_1	n.s.	n.s.	<0.01	<0.001	<0.05	<0.005
IgG_2	n.s.	n.s.	n.s.	n.s.	<0.03	<0.02
IgG_3	n.s.	n.s.	n.s.	<0.04	n.s.	<0.03
IgG_4	n.s.	n.s.	n.s.	<0.02	<0.04	<0.03

Changes in antibody titres to streptococcal antigen were not significant throughout. Comment. Increased antibody production, particularly of IgG_1, occurs with the addition of Tuberculin or Murabutide to M.v.ir, or after heat-killing. The studies shown in Tables 1 and 2 identify immunological parameters following injection of M.vaccae, and help establish the theoretical basis for its immunotherapeutic effect.

Table 3. Studies on M. vaccae immunotherapy in the Gambia

A. Results of the first randomised, placebo-controlled and blinded trial of irradiation-killed M.vaccae plus Tuberculin (M.v. ir + T) against pulmonary tuberculosis. Study of Dr P.T. Corrah, MRC The Gambia, 1990 (PhD thesis)

	Cured			Died		
	BCG –ve		BCG +ve	BCG –ve		BCG +ve
Placebo	61/101 (60%)	p<0.002	38/44 (86%)	16/101 (16%)	p<0.002	0/44
	p<0.01		n.s.*	(p<0.08)		n.s.
M.v. ir + T	61/78 (78%)	n.s.	35/41 (85%)	6/78 (8%)	n.s.	0/41

Results according to BCG scar. These are shown separately for those cured of their tuberculosis (AFB culture negative, bodyweight improved and chest x-ray improved, maintained for at least 6 months after treatment was stopped) or for those who died. Probabilities are assessed by Fisher's exact test. Note: Patients with a scar of past BCG vaccination are indicated as BCG +ve, and those without such a scar as BCG –ve. Comment. Past BCG vaccination had a remarkable influence on survival. The improved cure-rate and reduced death-rate after immunotherapy with M.vaccae was seen in those without BCG scars. * n.s. = not significant.

B. Results, as numbers cured or numbers dying, according to whether the chemotherapeutic regimen was of short-course ($2HREZ_3/4HR_3$) or long-course (2SHTh/16HTh)

B

	Cured			Died		
	Short-course		Long-course	Short-course		Long-course
Placebo	49/63 (79%)	p<0.03	50/82 (61%)	4/63 (6%)	(p<0.1)	12/82 (15%)
	(p<0.1)		(p<0.1)			
M.v. ir + T	53/60 (88%)	p<0.03	43/59 (78%)	2/60 (3%)	n.s.	4/59 (7%)

Note: There is a trend for an improved cure-rate after immunotherapy in patients receiving either short-course or long-course chemotherapy. Comment. Immunotherapy with a single dose of M.v.ir+T increased the number of patients successfully cured and reduced the numbers dying in a double-blind, placebo-controlled, randomised trial in The Gambia, whichever chemotherapeutic regimen was used.

using irradiation-killed M.vaccae to which a low dose of Tuberculin was added (M.v. ir + T), as selected in the leprosy studies in Spain. This trial had the disadvantage of spanning the period when treatment for tuberculosis changed from the old streptomycin-based treatment to rifampicin-based short-course chemotherapy. Nonetheless, the results were illuminating as they showed a trend towards improved cure rate and reduced mortality and were the subject of a PhD thesis (Corrah, P.T. Studies of tuberculosis in The Gambia. The Open University 1994). The immunotherapeutic injection was given after 6 weeks of chemotherapy. Results are summarised in table 3.

7. INTERMEDIATE PLACEBO-CONTROLLED TRIALS OF HEAT-KILLED M.VACCAE

A series of randomised, placebo-controlled, partially blinded studies/trials in newly diagnosed pulmonary tuberculosis patients were carried out in Argentina, India, Nigeria, Romania, South Africa and Vietnam. These investigated the activity of single doses of M.vaccae immunotherapy against tuberculosis in different geographic situations. A degree of efficacy was observed in each country, and the value of a variety of parameters for measuring the effects were confirmed in these studies.

7.1. Studies in Vietnam

Carried out between 1990 and 1995, these compared the results achieved in patients receiving the locally used 9 month $3SHZ/6S_2H_2$ (in which streptomycin [S] is used and the drugs are given twice weekly in the continuation phase) or 8 month 2SHRZ/6HE (in which ethambutol [E] is used during the continuation phase) courses of chemotherapy, with injections of heat-killed M.vaccae (M.v. hk) or placebo. A number of indications suggestive of benefit from the immunotherapy were found. These included reduction in the proportion of agalactosyl IgG (60) (a non-specific accompaniment of certain chronic inflammations) and clinically observed symptomatic improvement, better recovery of body-weight and improved clearance of radiological abnormalities, though these did not reach statistical significance. For the first time a comparison was made between patients who had received placebo, one dose of M.v. hk given either at the beginning, or after 2 months of chemotherapy, and a group given two doses, one at the beginning and the other after 2 months of chemotherapy (see table 4a).

Of special interest to the Vietnamese was the possibility that immunotherapy might be used to reduce the period of chemotherapy, and two small pilot studies were carried out comparing the results of patients receiving a single dose of M.v. hk given at the beginning of treatment with courses of the 9-month regimen truncated to 6 months, and 4 months (see table 4b). Studies were also carried out to assess the potential use of single doses of M.vaccae in the treatment of relapses and treatment failures, though the numbers were too few to enable conclusions to be drawn.

7.2. The study in India

Carried out in 1994 around the city of Calicut, a trial was performed of two doses of heat-killed M.vaccae (M.v. hk) one given on day 1 of treatment and the second given 2 months after commencing a course of 2RHEZ/4RH. Like the Gambian study, this was never formally published except as an MD thesis (A.H. Mohammed Faizy. Effect of immunotherapy with M.vaccae in pulmonary tuberculosis. Calicut Medical

Table 4. Studies on M. vaccae immunotherapy in Vietnam

A. Results of an unpublished series of studies with heat-killed M.vaccae (M.v. hk) on patients with newly diagnosed pulmonary tuberculosis.		
Immunotherapy	Cure rate at the end of chemotherapy	Difference from placebo group. Fisher's exact test
Placebo	151/169 (89.3%)	
M.v. hk <2/52	144/152 (94.7%)	p<0.06
M.v. hk 8/52	19/19 (100%)	p<0.2
M.v. hk <2+8/52	66/67 (98.5%)	p<0.02
All immunotherapies	229/238 (96.2%)	p<0.006
These studies were carried out by the National Institute of Tuberculosis and Respiratory Diseases, Hanoi, and the Pham Ngoc Thach TB and Lung Disease Centre, Ho Chi Minh City, Vietnam. Full courses of 3SHZ/6S_2H_2 or 2SHRZ/6HE with, or without the addition of one or two doses of immunotherapy given either in the first 2 weeks of treatment, or after 8 weeks of treatment, or on both occasionsNote: By "Cure rate" is meant achieving sputum negativity for acid-fast bacilli by smear examination and culture, together with evidence of clinical improvement. Comment. Imunotherapy with M.v.hk significantly improved the numbers of patients cured in Vietnam, especially when given on two occasions, early in chemotherapy and after 8 weeks. The value of two doses of immunotherapy was demonstrated for the first time.		
B. Results of immunotherapy with M.v.hk given on day 1 of abbreviated courses of chemotherapy with 3SHZ/3S_2H_2 or 3SHZ/1S_2H_2 in comparison with results obtained with a full course of 9 months of chemotherapy without immunotherapy (3SHZ/6S_2H_2)		
Treatment	Cure rate at the end of chemotherapy	Difference from placebo group Fisher's exact test
3SHZ/6S_2H_2 + Placebo	151/169 (89.3%)	
3SHZ/3S_2H_2	42/46 (91%)	n.s.*
3SHZ/1S_2H_2	43/50 (86%)	n.s.
The abbreviated courses plus immunotherapy had 18 month relapse rates of 3/42 (7.1%) and 6/43 (14%) respectively, which are not significantly different from the relapse rate in those taking a full course of chemotherapy without immunotherapy. Comment. Immunotherapy with M.vaccae improved the cure rate of treatment in Vietnam and allowed a reduction in the length of chemotherapy without a reduction in efficacy. * Not significant.		

College, 1996). In this randomised study 70 patients received immunotherapy and 66 received placebo. All 70 patients receiving M.vaccae were cured at the end of treatment, compared with 63/66 in the placebo group. Faster and better clearance of x-ray changes were recorded and better weight regain and faster falls in ESR were associated with immunotherapy, though these did not reach statistical significance.

7.3. The first South African study

This randomised, double-blind, placebo-controlled study between a single dose of heat-killed M.vaccae or tetanus toxoid as placebo, given early in a course of chemotherapy was carried out between 1991 and 1997 in two hospitals in Kwa Zulu (61). Slightly different chemotherapy regimens were used in the two centres, but they were basically 2RHEZ/4RH. Except for improved weight gain over the first 8 weeks of treatment, little benefit resulted from the immunotherapy in this region where results of treatment for tuberculosis were found to be poor. In both study arms sputum culture conversion at 8 weeks was around 40%, there was a 25% treatment failure rate and deaths during treatment ran at 7%. At a 4-year follow-up 25% of the patients had died, three fifths of them from recurrent tuberculosis. An explanation for the very poor performance of ostensibly good chemotherapy in this study has never been found, though it seems to be a uniquely South African experience. Whether is was due to the drugs being of poorer quality than was purported (such as reported for the drugs used in Nigeria (62) and South Africa (63), to some racial characteristic of a predominantly Zulu population, to their particular environmental situation or to the worm infestations and parasitaemias prevalent there, are interesting speculations. Nonetheless an explanation still needs to be found. The poor response to therapy, despite supervision, was not unique to this investigation, having been reported in other studies in this region (64).

7.4. The study in Nigeria

Although a small minority of the patients enrolled in the trial in The Gambia were infected with the human immunodeficiency virus (HIV), the study in Nigeria, carried out in 1993-4, was the first in which attention was specifically directed towards such patients. This single-blind, randomised, placebo-controlled trial suffered from being carried out on a partly nomadic population, which restricted the numbers that could be followed up (65). Nonetheless the results were better than anticipated and very different from those described from Kwa Zulu above. Notable recovery was found in the HIV-infected patients (66), and there was a dramatic reduction in deaths in all patients followed immunotherapy. The chemotherapy prescribed was 2SHRZ/6HR and only those patients who had obtained their first month of chemotherapy were recruited into the trial, even though shortages and costs of drugs allowed few to complete the regimen. The single injection of M.v. hk was given to those randomised to receive it, one, two or three weeks after starting chemotherapy. Parameters of assessment included sputum smear stained for acid fast bacilli by the Ziehl-Neelsen method; culture was not available. Blood was collected on entry into the trial and after 10-14 months, both for HIV serology, for measurement of agalactosyl immunoglobulin (60) and routine haematology including ESR. Body weight was measured at trial entry and on any

Table 5. Studies on M. vaccae immunotherapy in Nigeria

A. Results of the trial in Nigeria				
	Smear +ve for AFB	Fall in ESR	Increase in body-weight	Change in Gal 0 (60)
Placebo group	53/65 (82%)	4±2.9mm	0.55±0.17kg	+0.11±1.35
	P<0.00001	p<0.001	p<0.001	p<0.05
M.v. hk group	20/75 (27%)*	25.4±2.5mm*	2.9±0.24kg*	-3.61±0.91*
Follow-up 1-2 weeks after intervention and 20+8 days of chemotherapy This was a randomised comparison of a single dose of heat-killed M.vaccae or saline placebo given after 1 to 2 weeks after starting a very poor course of chemotherapy (all that was available), carried out under difficult conditions at the Infectious Diseases Hospital, Kano, Nigeria. (65)Note: All patients were sputum smear positive at the start of this study and all had a raised ESR and a raised level of agalactosyl IgG (Gal 0). The results shown are the changes that occurred in relation to the initial values. Comment. Under these conditions of very restricted availability of antituberculosis drugs, in which most patients only took a few weeks of chemotherapy, the addition of a single dose of M.v.hk had a remarkable effect on these markers of clinical improvement. * p<0.001 from initial values.				
B. Follow-up 10-14 months after diagnosis				
	Smear +ve for AFB	Fall in ESR	Increase in body-weight	Deaths
Placebo group	22/26 (85%)	14.9mm	2.04kg	19/47 (40%)
	p<0.00002	p<0.001	p<0.003	p<0.00001
M.v. hk group	11/33 (33%)	42mm	7.91kg	0/34
Note: The mean fall in ESR and increase in body-weight is in relation to the initial values. Comment. Although a very limited follow-up (81/140, 58%) could be achieved in the difficult conditions of Kano, the evidence for an improved cure-rate in immunotherapy recipients is striking.				
C. 10-14 month follow-up for HIV+ve patients				
	Nos. HIV seropositive	Smear +ve for AFB	Deaths	
Placebo group	9/47 (19%)	3/3	6/9	
	n.s.	p<0.02	p<0.03	
M.v. hk group	5/34 (15%)	0/5	0/5	
Comment. This study provides further evidence for the efficacy of immunotherapy in patients taking an abbreviated course of chemotherapy. Despite the small numbers of HIV seropositive patients in the group followed up, the effect of the single dose in reducing sputum-smear positivity for AFB in survivors and in reducing mortality is significant. The efficacy of immunotherapy in HIV seropositive tuberculosis was shown for the first time.				

occasion when the patient attended an out-patients clinic. Chest radiographs were taken of most patients on diagnosis and after one month, and wherever possible at around one year. Although not written into the study, a radiologist reviewed the x-ray plates and noted a much better clearance of opacities and closure of cavities in M.vaccae recipients. A summary of the results of follow-ups is shown in table 5 a, b and c.

7.5. The studies in Romania

Two randomised, placebo-controlled, partly blinded studies were carried out on pulmonary tuberculosis in Romania in 1994-5, one in newly diagnosed patients (67) and the other in chronic, relapsed and drug-resistant patients (68). Except for 2 sentinel patients with multi-drug-resistant disease who received 2 doses of M.vaccae (M.v. hk) with a 2-month interval between them, all patients received a single dose of M.vaccae or placebo one month into a first, or repeat, course of chemotherapy. The regimen used for newly diagnosed patients was $2H_2R_2Z_2S_2/4H_2R_2$ (drugs given twice-weekly throughout) and that for treatment failures and relapsed cases was the same, since second-line drugs were not available in Romania at the time. Although immunotherapy improved the completeness and rate of recovery in new patients, the greatest effects were observed in the chronic and, relapsed and drug-resistant patients, many of whom had advanced disease. The results of both studies are summarised in table 6, a, b and c.

7.6. The Argentina Studies

These were two small studies of newly diagnosed HIV seronegative pulmonary tuberculosis patients (40 in all) carried out in 1996-7 to assess the effects of a single dose of M.v. hk, given either on the first day of chemotherapy or a month later (69). Besides showing reduced sputum smear positivity for AFB a month after immunotherapy and a greater reduction in ESR at two months, improvements in weight gain ($p<0.05$) and time to become apyrexial ($p<0.05$) were observed. Radiological improvement ($p<0.05$) after M.vaccae was observed in the first of the two studies.

The real significance of the Argentinian studies was that cytokine levels and IgG antibody titres to stress proteins (65kDa, 70kDa) were measured. These findings, shown in table 7, support the original hypothesis that immunotherapy with M. vaccae induces an enhancement of Th1, and a reduction of Th2, mechanisms.

7.7. The studies in China

Following papers presented on the Vietnamese and other studies at meetings in 1994-5 (70), Chinese scientists took up the investigation of M.vaccae in the treatment of tuberculosis (71,72). Their reagent was produced from the type strain of M.vaccae obtained from the American Type Culture Collection (ATCC). It was grown on liquid Sauton's medium and, after harvesting, washed cells were broken open with a press before sterilisation by autoclaving. Instead of intradermal injections, the Chinese used deep intramuscular injections, giving 0.1mg 2 weeks after starting chemotherapy with 2RHZE/4RH, and injections of 0.5mg every 3-4 weeks

Table 6. Studies on M. vaccae immunotherapy in Romania

A. Summarised results of the two studies carried out in Romania in which immunotherapy with heat-killed M.vaccae or placebo was administered 1 month into a short-course of chemotherapy with $2H_2R_2Z_2S_2/4H_2R_2$ (67,68)			
	Culture +ve	Body-weight	ESR
New cases (67)			
Placebo group	26/107 (24%)	58.7±9.6kg	31.3±23.7mm
	(p<0.06)	n.s.	n.s.
M.v. hk group	14/97 (14%)	59.6±9.2kg	27.4±21.4mm
Chronic and relapsed cases (68)			
Placebo group	32/45 (72%)	55.8±10.5kg	44.4±24.6mm
	p<0.01	n.s.	p<0.02
M.v. hk group	25/56 (45%)	59.7±9.1kg	34.9±30.3mm
Data obtained one month after placebo or immunotherapeutic injection and two months after starting chemotherapy			
B. Data obtained at the end of chemotherapy, five months after placebo or immunotherapy			
	Culture +ve	Body-weight	ESR
New cases			
Placebo group	9/105 (9%)	59.4±9.3kg	24.9±19.5mm
	p<0.05	n.s.	p=0.002
M.v. hk group	2/91 (2%)	60.9±9.5kg	16.7±12.1mm
Chronic and relapsed cases			
Placebo group	12/43 (28%)	57.6±8.7kg	38.0±24.3mm
	n.s.	n.s.	p<0.02
M.v. hk group	10/50 (20%)	62.3±9.5kg	26.0±20.5mm
C. Radiological assessment at the end of chemotherapy, five months after placebo or immunotherapy			
	Still with cavities	Cavity surface area	Mean lesional score
New cases			
Placebo group	50/77 (65%)	31.9±33.4cm^2	1.6±0.6
	n.s.	n.s.	n.s.
M.v. hk group	47/73 (64%)	26.2±34.0cm^2	1.4±0.6
Chronic and relapsed cases			
Placebo group	36/43 (84%)	50.6±46.8cm^2	2.1±0.7
	(p<0.07)	n.s.	p<0.01
M.v. hk group	32/47 (68%)	47.2±56.8cm^2	1.7±0.7
Comment. Two months after starting chemotherapy and one month after M.v.hk or placebo numbers with sputum cultures positive for tubercle bacilli are significantly reduced among the immunotherapy recipients, weight-gain shows an upward trend and ESR is reduced compared with the placebo group. At the end of treatment fewer patients remain sputum culture positive, weight-gain is better and ESR is lowest in the patients receiving immunotherapy. All three parameters of chest x-ray have been improved by the immunotherapy, especially in the chronic and relapsed cases.			

until the end of chemotherapy. The results obtained were very similar to those produced elsewhere as shown in table 8.

8. THE THREE MAJOR TRIALS OF SINGLE-DOSE IMMUNOTHERAPY

The first of these was carried out in Durban, South Africa, jointly by the South African MRC, King George V Hospital and the University of Natal Medical School and funded by Stanford Rook Holdings Ltd. (UK). The second was a trial carried out in Uganda by the Ugandan National Tuberculosis authorities and Case Western Reserve University (US) and was sponsored by the National Institutes of Health (NIH, US). The third was carried out in Zambia and Malawi by the LUSKAR Trial group and University College London Medical School, supported by the Department for International Development, Health and Population Division (UK).

Batches of heat-killed M.vaccae (referred to as SRL 172) and placebo used in all 3 trials were prepared to the standards of Good Manufacturing Practice (GMP) and approved by both the Medicines Control Agency (MCA), UK and the Food and Drug Administration (FDA), USA. In all 3 trials the M.vaccae SRL 172 was administered as a single intradermal dose within the first 2 weeks of chemotherapy.

8.1. The Durban Trial

This was a phase III, randomised, placebo-controlled and near-blinded trial carried out to the standards of Good Clinical Practice (GCP) between 1994 and 1996. The patients, all HIV seronegative, had newly diagnosed, smear and culture positive pulmonary tuberculosis and attended hospitals in Durban, South Africa (73). Patients received a single dose of SRL 172 or placebo on day 8 of a 6-month course of 2RHZ/4RH, given under direct supervision. For the first eight weeks sputum samples were examined weekly by smear and culture. Thereafter monthly samples were obtained on two consecutive days whenever sputum was still being produced. Clinical observations, regular body-weight and ESR measurements and

Table 7. Immunological results from the study in Argentina for IgG antibodies to heat shock proteins 65kDa and 70kDa and for the cytokines IL-4, IL-10, IFN-gamma and TNF-alpha (69)

	Immunotherapy Group (n=13)		Placebo group (n=11)	Healthy control group (n-12)
IgG to hsp 65kDa (ELISA absorption)				
On admission	0.30±0.03		0.23±0.04	0.20±0.04
	p<0.001		p<0.05	
After 1 month	0.19±0.02	n.s.	0.20±0.02	
% decrease	32.0±5.5	p<0.05	15.6±5.4	
IgG to hsp 70kDa (ELISA absorption)				
On admission	0.59±0.05		0.62±0.06	0.25±0.06
	p<0.001		n.s	.
After 1 month	0.31±0.03	p<0.001	0.53±0.06	
% decrease	48.0±3.6	p<0.0001	17.0±2.6	
Cytokines in pg/ml				
Interleukin-4				
On admission	685±77		586±63	69±9
After 1 month	342±36	p<0.02	495±58	
% decrease	47.0±4.7	p<0.001	15.0±4.9	
Interleukin-10				
On admission	3800±302		3863±270	35±6
After 1 month	2292±187	p<0.002	3663±286	
% decrease	38.0±5.3	p<0.007	16.5±5.8	
Interferon-gamma				
On admission	524±76		553±57	157±7
After 1 month	1172±173	p<0.05		700±99
% increase	124.0±21.0	p<0.005		41.0±20.0
TNF-alpha				
On admission	86.0±6.0		85.5±3.3	None detected
After 1 month	52.0±5.0	p<0.001	74.0±3.7	
% decrease	38.0±3.6	p<0.01	14.0±4.1	

Note: In each case the values for healthy controls are significantly lower than for patients. Comment. Decreases in titres of the IgG antibodies especially to hsp 70kDa are greater following immunotherapy with M.vaccae and after 1 month the value for the immunotherapy group is not significantly different from that of the healthy controls, Values for each of the cytokines measured were grossly raised on admission and those of IL-4, IL-10 and TNF-alpha showed significantly greater falls over a month than did placebo recipients. The value for IFN-gamma more than doubled in immunotherapy recipients compared with a much smaller rise in the placebo group. Taken together these results show a faster return to normal in those receiving M.vaccae.

Table 8. Data from studies of patients with culture p ositive newly diagnosed pulmonary tuberculosis treated in China (72)

	Negative culture			Radiological absorbance		
After	Placebo		M.vaccae	Placebo		M.vaccae
1 month of treatment	33/171 (19%)	p<0.0001	81/171 (47%)	-	-	-
2 months of treatment	116/171 (68%)	p<0.0001	147/171 (86%)	31/171 (18%)	p<0.0001	63/171 (37%)
4 months of treatment	163/171 (95%)	n.s.*	159/171 (93%)	69/171 (40%)	p<0.0001	113/171 (66%)
6 months of treatment	169/171 (99%)	n.s.	170/171 (99%)	130/171 (76%)	n.s.	135/171 (79%)

Probabilities assessed by Fisher's exact test. * n.s.= not significant. Comment. These results confirm the benefits of immunotherapy with a preparation of M.vaccae completely different from that used in the other studies. The early improvement in sputum conversion and the improved radiological resolution are well shown in this study.

radiological assessment at day 56 were secondary endpoints. The well-publicised results failed to show any benefit associated with the immunotherapy, and the results remain contentious, though the radiological findings have still to be fully assessed.

8.2. The Uganda Trial

This trial, carried out in 1996-7, was also limited to HIV negative, newly diagnosed, smear and culture positive, pulmonary tuberculosis patients (74). Patients received an injection of SRL 172 or placebo after one week of a chemotherapy regimen of 2RHZ/4RH. Patients were followed-up by sputum smear and culture, by body weight, by some *in vitro* immunological parameters, and by chest radiology.

The results, which showed significant benefits in bacteriological culture and in radiology, are summarised in table 9. Regain of body weight showed no improvement with immunotherapy and the immunological measurements were, surprisingly, uninfluenced.

Table 9. Bacteriological and radiological results from the Uganda trial (74)

Sputum culture positive			
	Placebo group		Immunotherapy group
Day 36*	7/52 (14%)	p=0.01	19/53 (35%)
Month 2	37/52 (73%)	n.s.	46/53 (85%)
Month 4	47/49 (96%)	n.s.	51/52 (98%)
Month 6	46/47 (98%)	n.s.	50/50 (100%)
Radiographic improvement			
	Placebo group		Immunotherapy group
Month 1*	16/50 (32%)	n.s.	23/58 (40%)
Month 3	31/53 (59%)	n.s.	40/59 (68%)
Month 6	40/52 (77%)	p=0.04	52/57 (91%)
Month 12	39/49 (80%)	p=0.04	46/49 (94%)

In this trial, which was conducted by Case Western Reserve and Makerere Universities, patients were randomised to receive immunotherapy with M.v. hk (SRL 172) or placebo, after one week of a course of 2RHZ/4RH. * Time after starting chemotherapy. Comment. The rapid conversion of sputum culture to negative in the recipients of SRL 172 is accompanied by significantly better radiographic improvement. The similarity should be noted between the results of this trial and the Chinese study shown in Table 8.

8.3. The Zambia/Malawi Trial

This trial, primarily aimed at reducing the death rate amongst tuberculosis patients who were HIV seropositive, recruited over 1200 newly diagnosed smear-positive patients (75) between 1998 and 2000. Two thirds of those recruited were HIV seropositive, and amongst them no benefit of the immunotherapy was detected, either in mortality or bacteriological parameters. However, among the HIV seronegative minority there was a near-significant ($p<0.06$) benefit of immunotherapy in sputum bacteriology after 2 months of treatment.

Analysis of the x-rays from all 3 trials has just been completed and is being prepared for publication.

9. IMMUNOTHERAPY FOR MULTI-DRUG-RESISTANT TUBERCULOSIS (MDRTB)

One of the most important potential widespread applications of immunotherapy is in the treatment of infections with multi-drug-resistant bacilli. Such organisms appear to rapidly acquire resistance to other drugs but it is hard to imagine a way in which bacilli could acquire resistance to immunotherapy, which works on the host's immune system. There may, however, be patients with immune systems so depressed that they cannot respond to immunotherapy, or those who cannot respond for genetic reasons. Fortunately these latter appear to be a small proportion of people.

There has recently been a review of the results obtained with heat-killed M.vaccae SRL 172 in 337 patients with MDRTB collected from many countries (76), summarised in table 10a. Since that time an account has appeared in the Chinese literature (see below, Section 9.3) of a placebo-controlled trial in MDRTB, the results of which are summarised in table 10b.

9.1. The study in Iran

Other than a few isolated cases investigated elsewhere, this was the first test of immunotherapy with M.vaccae in patients infected with MDRTB (70,77,78). At the time the study was carried out (1991-2) the only drugs available at the hospital concerned were streptomycin, isoniazid, rifampicin, ethambutol and kanamycin. These were drugs to which the bacilli in many cases were fully resistant. In this setting only 1 patient over the preceding 2 years had been bacteriologically cured out of more than 100 cases seen.

Forty-one patients volunteered to receive doses of M.v. hk and all received from 1 to 4 doses (77,78), as a result of which 9 (22%) became consistently sputum negative by smear and culture ($p<0.0001$ from the immediate historical control).

9.2. The studies in Vietnam

These included two small studies that exemplify the value of multiple injections of M.vaccae in the treatment of this disease carried out between 1994 and 1999. In one of these studies 28 patients with chronic pulmonary tuberculosis who had failed several courses of chemotherapy and had been sent home on isoniazid monotherapy, were treated with a course of up to 12 doses of M.vaccae given at 2-month intervals. Nine of these 28 patients (36%) became consistently sputum negative by smear and culture for tubercle bacilli after 3 to 7 doses.

In the second study, eleven patients with proven MDRTB were treated with a similar course of up to 12 doses of M.vaccae at 2-month intervals. These were patients who had been given visas for the United States and had failed to respond to chemotherapy tailored to the susceptibility pattern of their bacilli and supplied from the US. (Otherwise these patients would have been sent home on isoniazid monotherapy). All eleven became consistently sputum culture negative after 2 to 12 doses of immunotherapy and were allowed to take up their visas and migrate to the United States.

These 2 studies, included in the recent review (76) but awaiting full publication, illustrate both the ability of immunotherapy to be effective in the absence of effective chemotherapy, and the benefits of combining

Table 10. The use of immunotherapy with M.vaccae in the treatment of multi-drug resistant tuberculosis

A. Results of studies in Romania, Iran and Vietnam on the treatment of MDRTB with M.v. hk SRL 172			
Romania (68). Single dose of SRL 172 or placebo plus $2H_2R_2Z_2S_2/4H_2R_2$.			
Numbers (%) becoming Culture Negative			
	Placebo group		Immunotherapy group
2 months*	4/16 (25%)		5/15 (33%)
6 months	10/15 (67%)		10/12 (83%)
12 months	8/14 (57%)	($p<0.08$)	10/11 (91%)
* After starting chemotherapy			
Iran (77). Up to 4 doses of M.v. hk plus a long-course of HRSE. An immediate historical control group achieved success in only 1 out of 100 similar patients.			
	Became culture –ve	Remained culture +ve or were lost or died	
Numbers	11/41	30/41	
Body-weight increase*	5.3kg (10%)	1.1kg (2.3%)	
ESR change*	32.1mm decrease	5.4mm increase	
* After 1 year			
Vietnam (76).			
Up to 12 doses of M.v. hk plus Isoniazid monotherapy	9/28 patients became consistently culture –ve		
Up to 12 doses plus chemotherapy tailored to susceptibility pattern	11/11 patients became consistently culture –ve		
B. Results obtained 6 months after starting chemotherapy in the Chinese study of patients with multi-drug-resistant tuberculosis. Six intramuscular doses of heat-killed M.vaccae were given plus 6 months of chemotherapy (79)			
	Placebo group		Immunotherapy group
Sputum culture negative	5/28 (18%)	$p<0.03$	13/28 (46%)
Radiography: good absorption	14/28 (50%)	$p<0.03$	22/28 (79%)
Radiography: deterioration	10/28 (36%)	$p<0.03$	3/28 (11%)
Comment. All these studies show that repeated doses of M.vaccae significantly improve the recovery of patients with multidrug-resistant tuberculosis.			

immunotherapy with chemotherapy tailored to the susceptibility pattern of the bacilli.

9.3. The study in China

This was a trial in which the Chinese preparation of disrupted, heat killed bacilli as described above was tested on patients with MDRTB (79). Injections were given by deep muscular injection at approximately monthly intervals during antituberculosis chemotherapy. The results reported were very similar to those obtained with SRL 172 and are shown in table 10b.

10. IMMUNOTHERAPY FOR TUBERCULOSIS IN HIV-SEROPOSITIVE PATIENTS

Although the only trial planned to include a large number of tuberculosis patients who were HIV seropositive was that carried out in Zambia and Malawi (74), two earlier studies, those in the Gambia and in Nigeria (65,66), included a number of such patients. The Nigerian data was particularly impressive with all those doubly infected becoming cured from their tuberculosis and the only 2 patients from whom serum samples were available from both before and after treatment, reverting to HIV seronegativity. The samples were taken from the same patients and it has not been possible to disprove the data, but neither has it been possible to confirm it from elsewhere.

In addition a number of studies (80,81) have been performed in which the effect of immunotherapy with M.vaccae has been assessed in HIV patients without tuberculosis. These showed that the immunotherapy improved immune reconstitution of the patients and as a result a trial is underway in Tanzania in which HIV patients without evidence of tuberculosis infection are being given 5 doses of SRL 172 to prevent their acquiring the disease.

11. SIDE EFFECTS OF IMMUNOTHERAPY WITH M.VACCAE, DISADVANTAGEOUS AND ADVANTAGEOUS 11

There is always a local response to the intradermal injection of heat-killed M.vaccae, variable in severity in different individuals. This reaction may be partly associated with the intensity of the immune response of the individual, partly with the number of injections that have been given and the time interval between them and partly with the particular disease that is being treated. In the great majority of people the local reaction is less severe than the response of the average British teenager to BCG vaccination, and when a single or small series of injections are to be given, this has not proved a problem. Just as with BCG, a small permanent scar is left at the injection site. Hence it is unlikely to prove a problem in the treatment of tuberculosis. The Chinese workers preferred to use intramuscular injection of their preparation of M.vaccae, though this too was sometimes associated with adverse side effects.

The real area of need to reduce local responses arises in the treatment of chronic disease in which injections at 1-2 month intervals may be needed over long periods of time, for example in the treatment of asthma and

cancer. Early investigation suggests that there are no problems associated with oral doses given as capsules and these appear to be very effective in asthma, though therapeutic trials have yet to be carried out. Other ways of potentially decreasing local response, such as isolating the active principle or principles of M.vaccae required in different diseases are being investigated in the laboratory and in preclinical models (26,28,29). A comparative study of intradermal, intramuscular and oral administration for a range of diseases would be worthwhile

Other disadvantageous side effects following intradermal injection have been remarkably few and the induction of Th1 autoimmunity expected by some authorities has not occurred, probably because M.vaccae is an immune regulator, not a simple Th1 adjuvant. Flu-like symptoms, so frequently associated with immunising vaccinations, occur in between 1 and 2% of individuals, and then usually only following the first injection. Tiredness, lasting a few days after each injection, probably because of changing levels of interleukin-1, is experienced by some patients, usually those with advanced cancers.

Advantageous side effects are many, and it has been as a result of them that a number of new potential uses of immunotherapy with M.vaccae have been found. The only ones of these that have been reported in the literature are asthma and psoriasis, both of which were discovered in the course of treatment for other conditions (82-85). Another beneficial side effect found in a phase III trial on the use of M.vaccae in the treatment of non-small cell lung cancer has been a significantly improved quality of life (see **www.srpharma.com** section on cancer therapy). Other potential benefits beginning to be investigated include improved arterial and arteriolar blood flow (86,87) and improved wound healing.

12. PERSPECTIVE AND PROSPECTIVE

The data from studies of immunotherapy with a single dose of M.vaccae given early in a course of modern chemotherapy for newly diagnosed pulmonary tuberculosis give a clear message. There is faster clearance of bacilli from the sputum, whether this is measured by smear or culture, which is most clearly seen during the first 2 months of treatment (tables 8 & 9). This is then obscured by the continuing bactericidal activity of the chemotherapy. Some of the studies also show that there is a better recovery from disease in immunotherapy recipients shown by a faster fall in ESR and better regain of body weight (tables 5 & 6b), as the toxic effects of TNF become less marked in the presence of less interleukin-4 (table 7). Radiology shows a more rapid closure of cavities and clearance of lung opacities, as fibrosis resolves under the influence of changing cytokine levels (tables 8 & 9).

Thus immunotherapy with heat-killed Mycobacterium vaccae offers a unique advance in the treatment of tuberculosis. Using this very safe treatment there are opportunities for overcoming problems of the emergence of drug resistance and of treating MDRTB without the possibility of bacilli developing resistance to the intervention. Problems of non-adherence to chemotherapy could be further overcome by reducing the length of modern short-course chemotherapy if immunotherapy was included.

In the treatment of tuberculosis most existing clinical information concerns the tentative introduction of single doses of M.vaccae early in the course of chemotherapy. Single doses were used initially, after the demonstration in mice that excessive dosing might be prejudicial (88), and because of the theory for its mode of action. It was postulated that the immunotherapy acted as a switch from potentially tissue-necrotising immunity to more protective antibacterial immunity (16). The description and general acceptance of two types of helper T-cell, Th1 and Th2 provided a better framework for understanding its activity. M.vaccae acts as a Th1 adjuvant and enhances production of regulatory T-cells (Tr) effective in suppressing Th2 mechanisms reducing the necrotising and cachectic actions of TNF (26-29). Although first thought of as a switch, requiring only one dose, it is now recognised that the amount of regulation required depends on the force of the drive to wards Th2.

Meta-analysis (89) of the efficacy of single doses of M.vaccae have failed to take account of the evidence relating to multiple dosing in all the major problem areas of tuberculosis therapy. It is in the treatment of these that the present problems lie and the future conquest of the disease depends.

Single doses of M.vaccae were effective in Argentina, The Gambia, Kuwait, Nigeria, Romania and Uganda, but notably not in South Africa. Differences in the efficacy of single doses may reflect the different prevalence of conditions with a Th2-driving propensity underlying the tuberculous process that would influence the regulation of cellular immunity. These include parasitic diseases, chronic viral infections, constant contact with allergens and auto-antigens, and the presence of cancer cells. All of these enhance Th2 responses and repeated doses of M.vaccae are required to overcome their effects.

Multiple doses have been used to offset these problems, notably in the treatment of cancers (90), allergies such as asthma, certain autoimmune conditions and in the treatment of multidrug-resistant tuberculosis (76-79). Nonetheless, single doses have contributed to the recovery of many patients with drug-sensitive tuberculosis and have proved curative in some patients with short histories (less than 2 years) of infection with MDRTB, and in a few chronic cases. Studies with multiple doses in newly diagnosed, presumed drug-sensitive, tuberculosis are now being discussed. The first trial of three doses of M.vaccae given during the 2-month intensive phase of chemotherapy is nearing completion in Vietnam, building on their preliminary evidence that the continuation phase of chemotherapy can be safely reduced when immunotherapy is included in the regimen. This trial includes the option of giving further injections at monthly intervals if the patient has not become sputum culture negative by a month after the third injection.

Some remarkable examples of the successful use of multiple doses of M.vaccae can be taken from the results achieved in MDRTB and shown in table 10 (76). The two small studies carried out at the Pham Ngoc Thach Tuberculosis and Lung Disease Centre in Ho Chi Minh City, Vietnam, on a compassionate basis exemplify the value of multiple injections. In these situations it is almost impossible to use anything but historical controls and these suggest that without immunotherapy all the patients in both studies would have died. Currently, multiple doses of M.vaccae are being investigated in the treatment of DOTS-Plus failures in the Philippines.

The use of repeated doses could also play a role in the treatment of tuberculosis in HIV seropositive individuals. It has not been possible to confirm or repeat elsewhere the very optimistic data from Kano (64,65) on HIV+ve tuberculosis. However, published data from the group at the Dartmouth-Hitchcock Medical Centre in New Hampshire (80,81), and unpublished data from Lille and Tourcoing provide evidence of partially restored immune responsiveness in HIV+ve persons without tuberculosis. A study is just starting in Rosario, Argentina, in which patients with pulmonary tuberculosis, some of whom will be HIV seropositive, will receive doses of M.vaccae at the beginning of chemotherapy and after 1 and 2 months of their course.

After interesting preliminary data, the results of an on-going trial of the use of 5 doses of M.vaccae in HIV patients in Tanzania to prevent the emergence of tuberculosis are keenly awaited.

Embracing multiple-dose immunotherapy as part of the routine treatment of tuberculosis (91) would reverse the current tendency to consider the worldwide tuberculosis situation as hopeless. The intervention has an exemplary safety record and has been accepted both by the MCA in the UK and the FDA in the US for use in clinical trials, though a production licence has yet to be obtained. The problems of non-adherence to chemotherapeutic regimens, as well as those of relapse and the emergence and spread of untreatable forms of the disease could be halted. Indeed the treatment of tuberculosis would be revolutionised by the proper use of immunotherapy with killed M.vaccae in combination with modern chemotherapy.

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