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Immunotherapy with *Mycobacterium vaccae* in the treatment of tuberculosis in Romania. 2. Chronic or relapsed disease

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In this study of 102 patients with culture-positive chronic treatment failure or repeatedly relapsed pulmonary tuberculosis receiving chemotherapy, 56 received an injection of killed *Mycobacterium vaccae* as immunotherapy after 1 month of treatment. At the start of treatment, there was little difference between those receiving immunotherapy and the 46 patients in the control group receiving chemotherapy alone. Thereafter, the two groups diverged so that 1 yr later, 43 of 56 (77%) patients receiving *M. vaccae* had a successful outcome, in comparison with 24 of 46 (52%) patients receiving chemotherapy alone ($P<0{\cdot}02$).

Successful results were obtained from patients infected with drug-resistant bacilli, 20 of 32 (63%) patients compared with 11 of 25 (44%) patients, respectively, as well as from fully drug-sensitive cases (23 of 24 compared with 12 of 21 patients; $P=0{\cdot}004$). At the final follow-up after 22 months, 13 of 56 patients receiving immunotherapy had an unfavourable outcome compared with 26 of 46 members of the control group ($P=0{\cdot}0006$). During the study, 16 patients died of tuberculosis (six after immunotherapy), and 12 were lost to follow-up.

Not only was bacteriological success improved by immunotherapy, chest X-rays showed markedly better resolution of cavities and other radiological lesions, recovery of body weight was improved, and the mean erythrocyte sedimentation rate returned almost to normal ($P<0{\cdot}001$) in comparison with those receiving chemotherapy alone. These changes were seen even in those failing bacteriological cure, suggesting that the immunotherapy had been effective, but that bacilli were replicating in an extracellular situation, protecting them from its effects.

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Introduction

Major problems resulting in patients developing chronic relapsed tuberculosis include poor treatment and the development of drug resistance by the bacilli. Some patients are first infected with drug-resistant bacilli, known as primary drug resistance, and they start with a considerable disadvantage and are more likely to fail treatment, or relapse to become chronic cases. Others, first infected with drug-susceptible bacilli, receive poor or inadequate treatment for a variety of reasons, and their infecting bacilli acquire secondary resistance, making them more difficult to treat. Still others remain infected with fully drug-susceptible bacilli, but either receive such poor treatment, or comply so poorly with properly prescribed treatment, that their disease is not cured and they too develop chronic relapsing disease.

Approximately 20 000 new cases of pulmonary tuberculosis are diagnosed in Romania each year, and there is a continuous backlog of about

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TABLE 1. Initial data on patients with sputum cultures positive for *Mycobacterium tuberculosis* entering the two arms of the study

	Mycobacterium vaccae recipients		Placebo recipients
Numbers	56		46
Sex	9F 47M		14F 32M
Mean age (years)	44·1 ± 12·9		40·3 ± 11·3
Bacteriology			
AFB visible in sputum	53/56 (95%)		45/46 (98%)
Cultures showing resistance to one or more drugs	32/51*(63%)		25/44*(57%)
Multi-drug resistance†	18/51*(35%)		14/44*(32%)
Clinical			
Mean weight (kg)	58·4 ± 10·1	$P=0·04$‡	53·9 ± 9·7
Mean ESR	56·8 ± 33·8		65·2 ± 27·5
Radiology			
No. with cavities	50/56 (89%)		41/45*(89%)
Mean cavity surface area	83·7 ± 99·7		63·7 ± 56·1
Mean lesional score	2·4 ± 0·6		2·5 ± 0·5

ESR, erythrocyte sedimentation rate; AFB, acid-fast bacilli. *Data not available for the missing numbers; †Cultures resistant to rifampcin and isoniazid, with or without other drugs; ‡Student's *t*-test.

1000 chronic patients, many with drug resistance, requiring further treatment at any time. Such patients are notoriously difficult to cure, even if they can be made to comply with their chemotherapy. Against this background, the present study was designed to investigate the contribution towards successful treatment that might be made by an immunotherapeutic injection of killed *Mycobacterium vaccae* (1).

For the present study, 102 patients with chronic treatment failure or multiply-relapsed pulmonary tuberculosis were recruited to receive a course of the chemotherapy standard for Romania, with or without the addition of an injection of killed *M. vaccae* 1 month after starting treatment. Although it was intended that the study should be randomized, compassion broke through design and a higher proportion with more severe disease received the immunotherapy.

Materials and Methods

THE PATIENTS

The study population consisted of 102 adult patients (≥ 18 years) with pulmonary tuberculosis attending hospitals in Bucharest and Brasov in Romania for treatment of their chronic treatment failure or multiply-relapsed disease. They were selected for being sputum culture positive for *Mycobacterium tuberculosis*, and for having a history of previous unsuccessful treatment, the majority having started many such courses. Although the authors cannot be sure that none of the patients had completed previous courses, most were regular defaulters, failing to comply with treatment after the first few weeks of each course had produced some clinical improvement. In the present study, these patients were started on a new course of treatment, encouraged to comply, and followed-up in their homes when necessary. The initial data obtained from the patients at the start of their course of chemotherapy is shown in Table 1. With the degree and severity of tuberculous disease present in such a group of patients, no exclusion criteria were applied, except that those who did not agree to participate were omitted.

TREATMENT

Treatment consisted of the standard course used in Romania of 2 months of rifampicin, isoniazid,

streptomycin and pyrazinamide taken twice weekly, followed by 4 months of rifampicin and isoniazid twice weekly ($2HRZS_2/4HR_2$). The dosage was: isoniazid and rifampicin, 15 mg kg^{-1} body weight to a maximum of 900 mg; pyrazinamide, 60 mg kg^{-1} to a maximum of 4·5 g; streptomycin, 1 g. As far as possible, patients were hospitalized for the first 2 months of treatment. Chemotherapy was not strictly supervised but compliance was encouraged. Ideally, patients found to have resistance to a drug would have that drug substituted for another in their course of treatment, but at the time of the present study, second-line drugs were not generally available in Romania.

The immunotherapy was the same as that described previously (1–4), consisting of an intradermal injection of 0·1 ml *M. vaccae* strain NCTC 11659, Batch A5, given over a deltoid muscle. The reagent contained 10 mg ml^{-1} wet weight of whole, killed (autoclaved) bacilli in borate-buffered saline. Patients allocated to the control group received a similar injection, but of saline. Injections were given after 1 month of chemotherapy had been completed. Patients were not told which injection they received, but this was known to the physicians who gave the injections. Rather fewer women (nine of 56) received immunotherapy than received placebo (14 of 46), probably because the most severely ill patients tended to be directed towards immunotherapy. This sex imbalance was responsible for the lower average body weight in the placebo group. The same randomization schedule was used for this study and that of newly-diagnosed patients (2), thus numbers are not quite evenly balanced in the two studies.

CLINICAL STUDIES

Patients were weighed on the first day of their current course of treatment, and were clinically examined. This was repeated at occasional intervals, after 2 months of treatment, at the end of chemotherapy and 6 months after its completion.

RADIOLOGICAL INVESTIGATION

Chest X-rays were performed at the start of the study, and were repeated at the end of treatment and 6 months later. The results were reported as approximate areas of inner cavity wall, calculated by measuring cavity diameters on radiographs and applying the formula: Pi times the square of each diameter, and as a score of the extent of lesions from 0 to 3. This was based on methods developed by Simon (5) modified by the American Thoracic Society, and further modified locally. Radiographs were read by experienced hospital staff who did not know that the patients were in a special study, or which intervention they had received.

LABORATORY INVESTIGATIONS

Sputum samples were taken for culture and microscopy for mycobacteria at the start of the study, and were repeated at intervals. Results of those obtained after 2 months of treatment, at the end of chemotherapy and 6 months later are reported. Drug-susceptibility studies were performed on initial cultures, and results are available for 95 of 102 patients. Venous blood samples were taken at the start, after 2 months of treatment, at the end of treatment and 6 months later for erythrocyte sedimentation rate (ESR, by the Westergren method). These investigations were also carried out by staff who did not know which intervention patients had received.

STATISTICS

The two-tailed Fisher's exact test, Student's *t*-test, and the two-tailed, unpaired Mann–Whitney test were used to evaluate the results.

Results

The majority of results are shown in a series of tables based on information obtained 2 months after starting chemotherapy and 1 month after intervention (Table 2), at the end of 6 months chemotherapy (Table 3), and after 1 yr (Table 4). More detailed analyses of progression of cavities seen on chest X-rays are shown in Table 5.

At the time of admission to the study, every patient had tubercle bacilli demonstrable in sputum by culture, and in most cases by microscopy (98 of 102 patients). Drug-sensitivity tests to streptomycin (S), isoniazid (H), rifampicin (R), ethambutol (E) and pyrazinamide (Z) were performed on cultures from 95 of the patients. These showed 21 patients to be infected with bacilli resistant to at least one drug (11 *M. vaccae*

TABLE 2. Results obtained 2 months after starting chemotherapy, and 1 month after intervention

	Mycobacterium vaccae recipients		Placebo recipients
Bacteriology			
Microscopy positive	16/53*(30%)	$P<0{\cdot}02$‡	25/45*(56%)
Culture positive	25/56 (45%)	$P=0{\cdot}008$‡	32/45*(72%)
Culture+ve DR	16/32 (50%)	$P=0{\cdot}05$‡	19/25 (76%)
Culture+ve MDR	10/18 (56%)		11/14 (79%)
Clinical			
Body weight	$59{\cdot}7 \pm 9{\cdot}1$ (1·3 kg)†		$55{\cdot}8 \pm 10{\cdot}5$ (1·9 kg)†
ESR	$34{\cdot}9 \pm 30{\cdot}3$	$P<0{\cdot}02$§	$44{\cdot}4 \pm 24{\cdot}6$

DR, drug resistance; MDR, multi-drug resistance; ESR, erythrocyte sedimentation rate. *Data not available for the missing numbers; †Increases in body weight; ‡Two-tailed Fisher's exact test; §Student's *t*-test.

TABLE 3. Results obtained at the end of chemotherapy and 5 months after intervention

	Mycobacterium vaccae recipients		Placebo recipients
Total patients	56		46
Lost to follow-up	1		1
Assessed	55		45
Deaths	4		2
Treatment success	40/55 (73%)		31/45 (69%)
Require re-treatment	10		12
Bacteriology			
Microscopy positive	7/47*(11%)	$P=0{\cdot}02$§	14/43 (30%)
Culture positive	10/50*(20%)		12/43 (28%)
Culture+ve DR	8/26*(31%)		8/23 (35%)
Culture+ve MDR	5/14*(36%)		4/12 (33%)
Clinical			
Body weight	$62{\cdot}3 \pm 9{\cdot}5$ (3·9 kg)†		$57{\cdot}6 \pm 8{\cdot}7$ (3·7 kg)†
ESR	$26{\cdot}0 \pm 20{\cdot}5$	$P<0{\cdot}02$‖	$38{\cdot}0 \pm 24{\cdot}3$
Radiology			
Still with cavities	32/47*(68%)		36/43 (84%)‡
Cavity surface area	$47{\cdot}2 \pm 56{\cdot}8$ cm^2		$50{\cdot}6 \pm 46{\cdot}8$ cm^2
With reduced cavities	45/47 (96%)	$P<0{\cdot}02$§	33/43 (77%)
With larger cavities	2/47 (4%)	$P<0{\cdot}05$§	8/43 (19%)‡
Mean lesional score	$1{\cdot}7 \pm 0{\cdot}7$	$P<0{\cdot}01$‖	$2{\cdot}1 \pm 0{\cdot}7$

DR, drug resistance; MDR, multi-drug resistance; ESR, erythrocyte sedimentation rate. ‡Including two additional patients developing cavities; *Data not available for the missing numbers; †Increases in body weight; §Two-tailed Fisher's exact test; ‖Student's *t*-test.

recipients), 26 were resistant to two drugs (14 *M. vaccae* recipients), and 10 were resistant to three or four drugs (seven *M. vaccae* recipients). Thirty-two patients were infected with multi-drug resistant bacilli, defined as resistant to both R and H at least (18 *M. vaccae* recipients).

Resulting from a preponderance of females in the placebo group (14 of 46, compared with nine

TABLE 4. Results obtained 6 months after the end of chemotherapy

	Mycobacterium vaccae recipients		Placebo recipients
Total patients	56		46
Lost to follow-up	2		2
Died during treatment	4		4
Given re-treatment	10		12
Re-treatment success	5/10		3/12
Assessed	40		30
Further deaths	0		2
Remain cured	38/39*(97%)	$P<0{\cdot}003$§	21/30 (70%)
Bacteriology			
Microscopy positive	1/39*(3%)	$P<0{\cdot}02$§	7/28 (25%)
Culture positive	1/39*(3%)	$P<0{\cdot}02$§	7/28 (25%)
Culture+ve DR	0/17	$P=0{\cdot}04$§	4/13 (31%)
Culture+ve MDR	0/8		2/6 (33%)
Clinical‡			
Body weight	64·5 ± 9·6 (6·1 kg)†		58·5 ± 9·2 (4·6 kg)†
ESR	19·9 ± 15·3	$P<0{\cdot}001$‖	41·2 ± 22·7
Radiology†			
Still with cavities	17/45 (38%)	$P<0{\cdot}01$§	22/32 (69%)
Cavity surface area	32·7 ± 31·2		45·4 ± 39·4
Cavities reduced	40/45 (89%)		23/32 (72%)
Cavities increased	1/45 (2%)	$P<0{\cdot}01$§	7/32 (23%)
Mean lesional score	1·4 ± 0·6	$P<0{\cdot}001$‖	2·0 ± 0·7

DR, drug resistance; MDR, multi-drug resistance; ESR, erythrocyte sedimentation rate. *Data not available for the missing numbers; †Increases in body weight; ‡Based on all patients followed up at 1 yr; §Two-tailed Fisher's exact test; ‖Student's *t*-test.

of 56), the mean body weight of this group was somewhat lower than that of immunotherapy recipients. The same proportion of patients in each treatment group had radiologically detectable cavities, although those of immunotherapy recipients tended to be larger.

RESULTS AT 2 MONTHS

A month after its injection, significantly fewer patients receiving *M. vaccae* had positive cultures for tubercle bacilli ($P=0{\cdot}008$) or acid-fast bacilli visible in their sputum. Only 16 of 32 patients infected with bacilli showing resistance to R and H were still culture positive in the immunotherapy group, compared with 19 of 25 in the placebo group ($P<0{\cdot}05$). Body weight increased by over 1 kg in both groups, but ESR was significantly lower amongst immunotherapy recipients ($P<0{\cdot}02$).

RESULTS AFTER 6 MONTHS

By the end of chemotherapy, the differences in bacteriological results between the two groups showed a trend in favour of immunotherapy recipients, but only sputum microscopy remained statistically significant ($P<0{\cdot}02$, see Table 3). Regain of body weight was more than 3 kg in both treatment groups. Erythrocyte sedimentation rates of the *M. vaccae* recipients were significantly lower than those of the control group ($P<0{\cdot}02$). Radiological differences between the two groups were apparent with 45 of 47 immunotherapy recipients showing a significant reduction ($P<0{\cdot}05$) in cavity surface area, and only two of 47 showing an increase. In comparison, a smaller proportion, 33 of 43 ($P<0{\cdot}02$), of placebo recipients showed a non-significant reduction in cavity surface area, and eight of 43 showed an increase in cavitation

TABLE 5. Changes in cavitation seen in patients with a full set of three X-rays, one taken at the start of treatment, another at the end of treatment and the third 6 months after this

	Numbers	Area of cavity walls		
		At start	After 6 months	After 1 year
Patients showing progressive resolution of cavities				
M. vaccae	36/42 (86%) $P<0{\cdot}02$‡	72·3 ± 77·7	34·6 ± 55·9	8·3 ± 19·1*
Placebo	18/31 (58%)	71·6 ± 68·0	39·4 ± 42·3	16·0 ± 24·6†
Patients showing moderate resolution of cavities				
M. vaccae	4/42 (9·5%)	91·0 ± 69·9	60·0 ± 33·6	42·5 ± 43·5
Placebo	5/31 (16%)	40·0 ± 14·1	26·0 ± 16·7	27·2 ± 12·0
Patients with worsening cavities				
M. vaccae	1/42 (2·4%) $P<0{\cdot}02$‡	48	68	68
Placebo	7/31 (23%)	28·9 ± 27·7	64·3 ± 32·0	83·9 ± 41·1

The figures shown are for calculated internal surface areas of cavities. *M. vaccae*, *Mycobacterium vaccae*. Cavities completely resolved: *23/36 (64%) and † 8/18 (44%). ‡Two-tailed Fisher's exact test. Note that results are not shown in this table for the six patients in the immunotherapy group and two patients in the placebo group who did not show cavitation.

($P<0{\cdot}05$), including two patients who developed cavities during treatment. Fifteen patients in the immunotherapy group starting with cavities no longer had them, compared with seven patients in the control group. The radiological lesional score index was also significantly lower in the group receiving *M. vaccae* ($P<0{\cdot}01$) in comparison with the group receiving placebo.

Ten patients from the immunotherapy group and 12 from the placebo group required courses of re-treatment, coming out from further analysis.

RESULTS AFTER 1 YR

Six months after the end of chemotherapy (see Table 4), 39 patients from the immunotherapy group and 30 from the placebo group, apparently cured at the end of chemotherapy, were available for re-assessment. Amongst immunotherapy recipients, one patient had relapsed to smear and culture positivity, compared with seven relapses in the placebo group ($P=0{\cdot}02$), and two deaths. The relapsed patients were prescribed courses of re-treatment.

For all patients followed-up at 1 year, ESRs were significantly lower among *M. vaccae* recipients ($P<0{\cdot}001$), and body weights had increased by 6·1 kg in the *M. vaccae* group compared with 4·6 kg in the placebo group. Radiological parameters showed much greater improvement in the immunotherapy group, with significantly fewer patients still with detectable cavities ($P<0{\cdot}01$), and only one patient showed increased cavitation in comparison with seven patients in the placebo group ($P<0{\cdot}01$). The mean X-ray lesional score of the immunotherapy group was significantly further reduced since the end of chemotherapy ($P<0{\cdot}03$), whereas the small improvement in this parameter was far from significant ($P>0{\cdot}5$) amongst placebo recipients.

RESULTS FOR THOSE INFECTED WITH DRUG-RESISTANT BACILLI

In the immunotherapy group, 20 of 32 (63%) patients infected with bacilli resistant to one or more drugs were bacteriologically cured, three of them after re-treatment, and all four who died were infected with bacilli resistant to R and at least one other drug, in two cases H. In the placebo group, 11 of 25 (44%) patients infected with bacilli resistant to one or more drugs were

cured, two of them after re-treatment, and three of the four deaths were of patients infected with bacilli resistant to both R and H.

REGAIN OF BODY WEIGHT

Besides the mean body weights shown in the tables, in those patients for whom a complete set of four data points is available, whatever their bacteriological outcome (47 in the *M. vaccae* group and 33 in the control group), there are differences in progression of weight regain. In the immunotherapy group, 42 of 47 (89%) patients improved in weight, compared with 22 of 33 (67%) members of the placebo group ($P<0{\cdot}02$). Of these, 13 of 47 (28%) showed a steady increase at each time point in the immunotherapy group, compared with three of 33 (9%) from the placebo group ($P<0{\cdot}02$). Only two of 47 (4%) *M. vaccae* recipients showed an overall fall in weight between the first and last time points, in comparison with 10 of 33 (30%) placebo recipients ($P<0{\cdot}005$).

RADIOLOGICAL PROGRESSION

Data illustrating this, and based on patients for whom a complete set of three radiographs is available (48 in the *M. vaccae* group, 33 in the placebo group), are shown in Table 5 for the progression of cavities in the two intervention groups. The six patients in the immunotherapy group and two in the placebo group who never showed cavitation are left out of the table and calculations.

At the start of the study, there was no difference in the extent of cavitation between the two groups (Mann–Whitney $U=563$, $P=0{\cdot}33$), and neither was there at the end of chemotherapy ($U=545$, $P=0{\cdot}24$). At 12 months, however, the extent of cavitation in the *M. vaccae* recipient group was significantly less than in the placebo group ($U=398$, $P=0{\cdot}005$).

Of those receiving immunotherapy, 36 of 42 (86%) showed progressively less cavitation with time, in comparison with 18 of 31 (58%) members of the placebo group ($P<0{\cdot}02$). Only one *M. vaccae* recipient showed worsening cavitation compared with seven of 31 placebo recipients ($P<0{\cdot}02$), two of whom developed cavities after starting treatment. Cavitation was not detectable on the 12-month radiographs in 24 of 42 (57%) members of the immunotherapy group, and eight of 31 (26%) placebo recipients ($P<0{\cdot}001$).

RESULTS AT LATEST FOLLOW-UP

Data was available after a mean of 22 months on 43 immunotherapy recipients and 34 placebo recipients who had been fully followed-up to 1 yr. These included 28 and 18, respectively, of those cured at 6 and 12 months. Three of 28 members of *M. vaccae* group and five of 18 members of the placebo group had relapsed to sputum culture positivity. Eleven *M. vaccae* recipients, four sputum positive for AFB, and eight placebo recipients, seven sputum positive for AFB, died between the 1 yr and final follow-ups ($P=0{\cdot}06$).

In general, out of all patients entering the study, 13 of 56 *M. vaccae* recipients and 26 of 46 placebo recipients had unfavourable outcomes due to tuberculosis ($P=0{\cdot}0006$) at the last analysis.

Discussion

The results obtained support the contention that immunotherapy with *M. vaccae* makes a considerable contribution towards the treatment of tuberculosis (3,4), particularly in patients in whom chemotherapy alone has been repeatedly unsuccessful, and in those infected with drug-resistant bacilli (6). Even so, it is likely that patients receiving chemotherapy alone have done better in the present study than they would have in standard practice. Since the patients enrolled knew they were in a special study, they may well have complied better, and their attendants may have been more encouraging, than under routine conditions. Despite this, it is not possible to know the degree to which defaulting occurred without directly observed therapy. The evidence from Nigeria (4) suggests that immunotherapy recipients tend to stop chemotherapy earlier because their disease resolves at a faster rate and they feel better sooner. The authors cannot be sure that this was the case in Romania, but suspect that those for whom data is missing when they failed to keep appointments might also have complied least well with their treatment.

In the authors' study of newly-diagnosed pulmonary tuberculosis in Romania (2), 182 of 193 (94%) patients remained culture negative

6 months after the end of treatment; 88 of 91 (97%) receiving immunotherapy plus chemotherapy, and 94 of 102 (92%) receiving chemotherapy alone. In comparison, in the current study, if those successfully re-treated are included, 67 of 102 (66%) patients were culture negative at 1 yr; 43 of 56 (77%) receiving immunotherapy, and 24 of 46 (52%) receiving placebo ($P<0{\cdot}02$).

Patients entering the two arms of the study were very similar in their characteristics (Table 1), but by 1 month after intervention, those receiving immunotherapy showed clear advantages bacteriologically and in reduction in ESR (Table 2). It is not surprising that the bacteriological advantage was no longer apparent by the end of chemotherapy, although regain in body weight was significantly improved, and there was continued reduction in ESR (Table 3). Striking from this table was the better radiological improvement seen after immunotherapy. Six months after completion of chemotherapy, the advantages of immunotherapy are again apparent, partly due to a 15% relapse rate in the placebo group, compared with only a 3% relapse after *M. vaccae* ($P<0{\cdot}05$).

As well as putting on more weight, a greater number of immunotherapy recipients than placebo recipients showed progressive regain of body weight ($P<0{\cdot}02$), and fewer showed weight loss ($P<0{\cdot}005$). Table 5, showing radiological changes taking place during and after chemotherapy, also shows distinct advantages for those receiving immunotherapy. Thirty-six of 42 (86%) and 18 of 31 (58%) patients showed progressive resolution of cavities in the immunotherapy and placebo groups, respectively ($P<0{\cdot}02$). Cavities had completely closed by 12 months in 24 of 42 (57%) *M. vaccae* recipients and eight of 31 (26%) placebo recipients ($P<0{\cdot}01$). This improved radiological appearance associated with immunotherapy was noted in an early study of *M. vaccae* in Kuwait (7), in a pilot study in Argentina (8), and in the study in Nigeria (4) where improved closure of cavities was particularly mentioned. Both the increased rate of regain of body weight and the improved resolution of chest X-rays are likely to be the result of changes in circulating cytokines. Notable amongst these may be a reduced influence of Th2 cytokines on the toxicity of circulating tumour necrosis factor, and a reduction in fibrosis, perhaps associated with increased levels of IL12 (9).

The present study may contribute to knowledge of who does not respond to immunotherapy, and how this immunotherapy may work, in that regain of body weight, reduction in ESR and reduction in the size of cavities may occur without a successful bacteriological outcome being achieved. Even in treatment failures, better progression is seen in the immunotherapy recipients in comparison with those receiving chemotherapy alone. A likely explanation for bacteriological failure in patients showing successful responses in other parameters is that their bacilli are multiplying outside of cells on the denuded walls of cavities and damaged airways. Immunotherapy with *M. vaccae* induces cell-mediated antibacterial mechanisms which could not be expected to eliminate extracellular bacilli, but they would be expected to limit the damage mediated by circulating tumour necrosis factor.

In the study of newly-diagnosed patients in Romania (2), the authors found 25 of 175 (14%) patients to be initially infected with bacilli showing some drug resistance, many of them probably true cases of primary drug resistance. Among them were seven of 175 patients infected with bacilli resistant to R and H, complying with the modern definition of multi-drug resistance. In the present study of patients with chronic tuberculosis, 57 of 95 (60%) patients showed resistance to at least one drug, and 32 (34%) of them were multi-drug resistant, the majority of them likely to be of secondary, or acquired, type. This illustrates the strong association of drug resistance, even to single drugs, with the development of chronic disease.

There is no reason to think that bacilli with resistance to anti-tuberculosis drugs should be able to resist the immune system. However, difficulty in lowering the tissue load of such bacilli would leave many more alive and multiplying than would be the case with fully drug-susceptible bacilli, of which the majority would be killed by early bactericidal action (10) before the *M. vaccae* is administered. Thus, at the end of a year amongst immunotherapy recipients and including those re-treated, 20 of 32 (63%) patients infected with bacilli resistant to at least one drug were cured, compared with 23 of 24 (96%) patients infected with fully drug-sensitive

bacilli. Of placebo recipients, 11 of 25 (44%) patients infected with bacilli showing some drug resistance were cured, including two after re-treatment, compared with 12 of 21 (57%) patients infected with drug-sensitive bacilli. Results previously published from Iran (6) showed patients with a history of disease of less than 2 yr, and with bacilli showing initial multi-drug resistance, to be curable with a combination of drugs to which they showed resistance plus a single injection of killed *M. vaccae*. Patients with longer histories in that study required repeated injections of *M. vaccae*, at intervals of between 2 and 6 months, before a proportion of them were cured. All of the present patients had histories of disease longer than 2 yr, yet the cure rate was significantly improved by a single injection. This was particularly obvious in those infected with drug-sensitive bacilli (23 of 24 compared with 12 of 21; $P<0{\cdot}004$), and showed a trend amongst those showing drug resistance (20 of 32 compared with 11 of 25; $P=0{\cdot}13$).

Of all the patients entering the present study, 14 receiving *M. vaccae* and 24 receiving chemotherapy alone required at least one course of re-treatment because of treatment failure or relapse ($P<0{\cdot}01$). This re-treatment was successful in five of 14 and two of 24 patients, respectively, suggesting that the beneficial effects of immunotherapy extend to subsequent courses of re-treatment, as well as to the course of treatment during which it was administered. Very similar results were obtained in the authors' study of newly-diagnosed tuberculosis patients (2).

There was no significant difference in numbers of patients dying from tuberculosis, whether they received *M. vaccae* or not, but it remains possible that administering *M. vaccae* earlier in treatment or giving second or further injections might have reduced mortality. The improvements seen in weight regain, ESR and reduced cavitation, even in those who were not cured after immunotherapy, does suggest that a further injection, given perhaps 2 or 3 months after the first, might have improved the outcome (6). Pilot studies are already in progress to investigate this and to find out whether immunotherapy would be more effective if given earlier in the course of chemotherapy. All of the parameters investigated were found to be useful measures of the efficacy of immunotherapy.

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