Does immunotherapy with heat-killed *Mycobacterium vaccae* offer hope for the treatment of multi-drug-resistant pulmonary tuberculosis?

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The ability of immunotherapy with heat-killed *Mycobacterium vaccae* (NCTC 11659), as an addition to the available chemotherapy, to improve the outcome in patients with multi-drug-resistant tubercle bacilli (MDRTB) who had not been cured by chemotherapy alone was evaluated in tuberculosis centres in Estonia, Iran, Kuwait, New Zealand, Romania, Vietnam and the U.K.

A total of 337 patients in the above countries received intradermal injections of *M. vaccae* in addition to chemotherapy. Patients were grouped according to the length of their histories of disease: less than or greater than 2 years duration. Initially, single doses of *M. vaccae* were given but subsequently up to 12 doses at 2-month intervals were given. Chemotherapy varied from isoniazid alone to drugs selected according to susceptibility tests. Most patients had failed to respond to repeated courses of chemotherapy and the majority were expected to die from their disease. Results were assessed by sputum smear and culture and by clinical observations. Cured patients were followed for 18–24 months to exclude relapse.

Eighteen of 22 (82%) patients with disease for less than 2 years were bacteriologically cured by one or two doses of *M. vaccae*. Among 315 chronic patients, 24 (7.6%) were cured after one dose, 37.9% after seven doses and 41.6% after 12 doses. Sixty-six chronic patients were lost to follow-up, or died, during the multi-dose regimens. Nine of 33 patients (27%) with advanced disease unaffected by several courses of chemotherapy and discharged on isoniazid alone in Vietnam were cured by 3–12 injections of *M. vaccae*.

The data provide preliminary evidence that the addition of immunotherapy with *M. vaccae* to chemotherapy improves the rate of cure of MDRTB, most effectively in patients with short histories of disease, but multiple dosing can have beneficial effects in chronic patients in whom chemotherapy has failed. A randomized clinical trial of this immunotherapy in MDRTB patients is therefore required.

Key words: tuberculosis; multi-drug resistance; *Mycobacterium vaccae*; immunotherapy.

Introduction

The treatment of tuberculosis caused by multi-drug-resistant tubercle bacilli (MDRTB), defined as resistant to isoniazid and rifampicin, with or without resistance to other drugs, remains a major problem, especially in the former USSR and the developing world (1). Effective management of MDRTB requires high quality laboratory support and individualized, fully supervised, therapy (2,3), but resources for such interventions are rarely encountered in the developing nations where the mortality rate of MDRTB is extremely high. Novel approaches to the therapy of MDRTB are urgently required, such as a combination of chemotherapy and immunotherapy (4). We have added from one to 12 doses of an immunotherapeutic agent based on heat-killed *Mycobacterium vaccae* (NCTC 11659) to the treatment of patients with different degrees of chronicity, receiving a variety of drug regimens.

Most previous studies of immunotherapy with *M. vaccae* in tuberculosis have been of single doses given early in the course of chemotherapy for newly diagnosed cases of predominantly drug susceptible disease (5–7). Two recently
published randomized (good clinical practice) trials have yielded disparate results. The study from South Africa showed no effect (8), and results from Uganda showed faster clearance of tubercle bacilli from the sputum 1 month after starting chemotherapy, and significantly improved clearance of lesions on chest X-ray at the end of chemotherapy and 6 months later, in the immunotherapy group (9).

It was a conclusion of the Uganda trial that a study of multiple injections of *M. vaccae* in the treatment of MDRTB should be carried out and the current paper reports the data already available on this subject.

**Materials and methods**

**PATIENTS**

Four patients in Estonia, 109 in India, 41 in Iran, two in Kuwait, two in New Zealand, 19 in Romania, 129 in Vietnam and 31 in the U.K. have been studied. All had MDRTB confirmed by drug susceptibility testing, according to the methods used by the reference laboratories in the respective countries. Patients were divided according to the length of their histories of tuberculosis. Twenty-two patients had developed tuberculosis during the preceding 2 years, many with initial multi-drug resistance. The remaining 315 patients had chronic disease, with histories of tuberculosis varying from 2 years to 11 years. Most of these had received repeated courses of treatment but had not been cured.

**CHEMOTHERAPY**

Patients received a variety of regimens depending on the drugs available and national treatment policies. Three patients with short histories were receiving standard first-line anti-tuberculosis chemotherapy, 142 (including five with short histories) were on re-treatment regimens, 79 (including 14 with short histories) were receiving regimens tailored to the resistance patterns of their infecting organisms and 113 chronic patients were receiving 5 mg kg⁻¹ daily isoniazid alone. Despite these various treatments, the great majority of chronic patients entering our study were those who had failed to be cured by these chemotherapeutic regimens.

**IMMUNOTHERAPY**

Heat-killed *M. vaccae* NCTC 11659 in borate buffered saline, pH 8, was used. Treatment consisted of intradermal injections over a deltoid muscle of 0.1 ml containing 1 mg wet weight of bacilli. Three recent and 96 chronic cases, of whom 80 were receiving isoniazid alone, entered studies in which only one immunotherapeutic injection was given. Up to four doses were given to 41 patients in Iran, including five recent cases. Up to five doses were given to 85 patients with chronic disease in Vietnam and up to seven doses were given to 29 similar patients in India. In the most recent studies, up to 12 injections have been given to 83 patients, including 14 recent cases, in several countries. The reagent has an excellent safety record and reactions to the twelfth dose were no different from those to the first.

**FOLLOW-UP AND OBSERVATIONS**

Follow-up after immunotherapy was by clinical examination and sputum smear microscopy for acid-fast bacilli. Apparent bacteriological cures were confirmed by culture and, in almost all cases, by subsequent follow-up for 18–24 months.

**Results**

The proportion and cumulative numbers of patients cured bacteriologically after each injection of *M. vaccae* are shown in Table 1.

**PATIENTS WITH SHORT HISTORIES OF DISEASE (2 YEARS OR LESS)**

Fourteen of the 22 patients with short histories of disease (2 years or less) became sputum culture-negative after a single dose of SRL 172; four more became negative after a second dose given 2 months later and one patient became negative after seven doses given at 2-month intervals. One patient was lost to follow-up after a single dose and two died, despite receiving two and five doses of immunotherapy, respectively. Eight patients received standard or failure/relapse chemotherapy regimens and seven of these were cured; 14 received chemotherapy tailored to their drug susceptibility pattern and 12 were cured.

**Table 1. Numbers of doses of *M. vaccae* after which patients with chronic MDRTB became bacteriologically cured**

<table>
<thead>
<tr>
<th>Number of doses of immunotherapy</th>
<th>Proportion cured after each dose</th>
<th>Cumulative numbers and percentage cured</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24/315 (7.6%)</td>
<td>24 (7.6%)</td>
</tr>
<tr>
<td>2</td>
<td>18/193 (9.3%)</td>
<td>42 (16.9%)</td>
</tr>
<tr>
<td>3</td>
<td>14/164 (8.5%)</td>
<td>56 (17.8%)</td>
</tr>
<tr>
<td>4</td>
<td>15/129 (11.6%)</td>
<td>71 (19.2%)</td>
</tr>
<tr>
<td>5</td>
<td>9/92 (9.8%)</td>
<td>80 (29%)</td>
</tr>
<tr>
<td>6</td>
<td>1/36 (2.8%)</td>
<td>81 (31.8%)</td>
</tr>
<tr>
<td>7</td>
<td>2/33 (6.1%)</td>
<td>83 (37.9%)</td>
</tr>
<tr>
<td>8–12</td>
<td>1/27 (3.7%)</td>
<td>84 (41.6%)</td>
</tr>
</tbody>
</table>
PATIENTS WITH CHRONIC DISEASE
(HISTORIES OF 2 YEARS OR MORE)

After a single dose of *M. vaccae* many of the 315 patients with chronic disease became temporarily sputum smear-negative but 24 became sustainedly culture-negative. After a second dose given 2–6 months later 18 additional patients became culture-negative. Increasing the number of doses given at 2-month intervals led to 38 more patients becoming culture-negative after five doses and an additional four patients receiving up to 10 doses were also cured. Twenty-six sputum-positive patients went on to receive 12 doses, but without achieving bacteriological cure. During the study, 66 patients with chronic disease either died or were lost to follow-up whilst still sputum-positive for tubercle bacilli.

None of the 80 Indian patients receiving isoniazid alone and a single injection of *M. vaccae* achieved bacteriological cure but nine of the 33 Vietnamese receiving isoniazid monotherapy plus up to 12 doses of *M. vaccae* were cured: two after three doses; five after five doses; one after six doses and one after seven doses. Among the patients receiving failure/relapse regimens: 32 of 137 (23%) were cured: 12 after a single injection; two more after a second injection and a further 18 after three to five injections. The best results were obtained in the 65 patients who received chemotherapy tailored to their drug susceptibility patterns: 43 (66%) achieving bacteriological cure after one to 10 injections of *M. vaccae*.

For chronic patients the combination of chemotherapy tailored to the drug susceptibility pattern of the infecting bacilli with multiple-dosing with immunotherapy produces the best results (Table 2). Thus, none of the 80 patients receiving isoniazid alone and a single dose of *M. vaccae* were cured but nine of a further 33 patients receiving isoniazid alone were cured after multiple doses: 12 of 137 (8.8%) receiving treatment-failure/relapse chemotherapy were cured after one dose of *M. vaccae* and 12 of 65 (18.5%) receiving chemotherapy tailored to their pattern of drug susceptibility were cured after one dose of *M. vaccae*. Better bacteriological cure rates were observed in patients in these three treatment categories who received multiple doses of immunotherapy. Thus, nine of 33 (27.3%) receiving isoniazid alone, 21 of 121 (17.4%) receiving treatment-failure/relapse chemotherapy and 43 of 65 (66.2%) receiving tailored chemotherapy. These figures do not take into account the 36% of patients lost to follow-up, or dying before all the available doses of *M. vaccae* had been given.

### Table 2. Bacteriological cures achieved in patients with chronic tuberculosis according to chemotherapy regimen and number of doses (single or multiple) of immunotherapy

<table>
<thead>
<tr>
<th>Type of therapy</th>
<th>Single-dose</th>
<th>Multiple-dose</th>
<th><em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid alone</td>
<td>0/80</td>
<td>9/33 (27%)</td>
<td>0.005</td>
</tr>
<tr>
<td>Re-treatment regimen</td>
<td>12/137 (8.8%)</td>
<td>20/120 (17%)</td>
<td>0.13</td>
</tr>
<tr>
<td>Tailored therapy</td>
<td>12/65 (18.5%)</td>
<td>31/53 (58%)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

*Statistical analysis by Chi-squared test with Yates’ correction or Fisher’s exact test, as appropriate, using EPI-INFO 6.

### Discussion

The series of small pilot studies presented here have many weaknesses, not least the fact that they were not controlled with placebo recipients. Their purpose was to determine whether anything could be achieved by the addition of immunotherapy and whether a prospective, randomized, placebo-controlled, trial was justified.

Notwithstanding, they give an insight of what might be achievable. Eleven successful cures out of 41 patients in Iran with up to four doses of *M. vaccae* can be compared with the historical control of no more than one in 100 such patients cured with the same drug regimen over the previous 2 years (5,6). In Vietnam, cure was achieved in 14 of 85 chronic patients receiving up to five doses of *M. vaccae* during their second or third course of the retreatment regimen that had failed to cure them on previous occasions. Also, in Vietnam, nine of 33 patients who were sent home on isoniazid monotherapy and were given up to 12 doses of *M. vaccae* were successfully cured. These both indicate important treatment benefits (these groups are included but not distinguished in Table 2).

As expected, patients with short histories (less than 2 years) responded much better to the immunotherapy than did chronic cases. Those with short histories are likely to have less lung damage and fibrosis and the proportion of intracellular bacilli is likely to be relatively high. Thus, 19 of these 22 patients were cured, all but one after only one or two doses of *M. vaccae*, which is better than has been recorded for the use of chemotherapy alone, even where surgical resection has been available (7).

Analysis of the results for chronic patients was complicated by the different chemotherapy regimens they were receiving, even though these had been found unsuccessful in the majority of those entering our studies. In most places, the patients were those who had problems with adherence to treatment and who were generally considered untreatable. The studies were also complicated by the varying dosage schedules of immunotherapy offered to them. As shown in Table 2, the outcome is related to the expected effectiveness of the drug regimen but in all cases the outcome was significantly better in patients receiving multiple doses of immunotherapy. This is even the case in patients receiving isoniazid monotherapy that would be expected to have no significant antibacterial effect.

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References


