The use of immunomodulators as an adjunct to antituberculous chemotherapy in non-responsive patients with osteo-articular tuberculosis

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We studied 51 patients with osteo-articular tuberculosis who were divided into two groups. Group I comprised 31 newly-diagnosed patients who were given first-line antituberculous treatment consisting of isoniazid, rifampicin, ethambutol and pyrazinamide. Group II (non-responders) consisted of 20 patients with a history of clinical non-responsiveness to supervised uninterrupted antituberculous treatment for a minimum of three months or a recurrence of a previous lesion which on clinical observation had healed. No patient in either group was HIV-positive. Group II were treated with an immunomodulation regime of intradermal BCG, oral levamisole and intramuscular diphtheria and tetanus vaccines as an adjunct for eight weeks in addition to antituberculous treatment. We gave antituberculous treatment for a total of 12 to 18 months in both groups and they were followed up for a mean of 30.2 months (24 to 49). A series of 20 healthy blood donors served as a control group.

Twenty-nine (93.6%) of the 31 patients in group I and 14 of the 20 (70%) in group II had a clinicoradiological healing response to treatment by five months.

The CD4 cell count in both groups was depressed at the time of enrolment, with a greater degree of depression in the group-II patients (686 cells/mm\(^3\) (SD 261) and 545 cells/mm\(^3\) (SD 137), respectively; p < 0.05). After treatment for three months both groups showed significant elevation of the CD4 cell count, reaching a level comparable with the control group. However, the mean CD4 cell count of group II (945 cells/mm\(^3\) (SD 343)) still remained lower than that of group I (1071 cells/mm\(^3\) (SD 290)), but the difference was not significant. Our study has shown encouraging results after immunomodulation and antituberculous treatment in non-responsive patients. The pattern of change in the CD4 cell count in response to treatment may be a reliable clinical indicator.

Osteo-articular tuberculosis affects approximately 300 000 people worldwide.\(^1\) It is well recognised that a few patients are not responsive to first-line standard antituberculous drugs such as isoniazid, rifampicin, ethambutol and pyrazinamide.\(^2\)

Tuberculosis is characterised by immunological deficiency, mainly depletion of the CD4 cell count.\(^3,4\) Patients with pulmonary tuberculosis who show clinicobacteriological improvement have restoration of homeostasis of the peripheral lymphocyte subpopulation. The immune competence of the patients, together with chemotherapy are important determinants of outcome.\(^5,7\)

The requirement for a long course of antituberculous therapy (ATT) can lead to non-compliance of the patient which may contribute to the emergence of drug resistance of the organism.\(^8,9\) The combination of an immunopotentiating agent to augment the efficacy of ATT has been considered previously.\(^10,11\) The efficacy of intradermal BCG and of oral levamisole in the induction of a mycobacteria specific immune response has been reported.\(^12-16\)

Our study was undertaken to evaluate the role of intradermal BCG, oral levamisole and intramuscular diphtheria tetanus (DT) vaccines as adjuncts to ATT in patients with osteo-articular tuberculosis who were clinically non-responsive.\(^17\)

Patients and Methods
Ethical approval had been granted for the study and informed consent was obtained from all patients. Between March 2000 and May 2004 two groups of patients with osteo-articular tuberculosis were studied simultaneously. The diagnosis had been made on clinicoradiological grounds supplemented by bacteriological and histopathological findings wherever there was any doubt.\(^1\)
We also did not include patients with a doubtful diagnosis of diabetes, malignancy or a condition requiring steroid therapy. Were those with any detectable serious illness including pulmonary tuberculosis or HIV infection were excluded as abscess. Pott's paraplegia or immediate aspiration of a large cold abscess. The patients in group I had supervised ATT, namely isoniazid, rifampicin, ethambutol and pyrazinamide. The last was stopped after two months and ethambutol after six months. The remainder was continued for a total duration of 12 to 18 months.20

The patients in group II, who had been on ATT for three months or more were subjected to immunomodulation therapy for an initial period of eight weeks. In the patients with recurrence of disease ATT and an immunomodulation regime were given. The latter had those elements, as follows (Fig. 1).

1) BCG vaccination (BCG Laboratory, Guindy, Chennai, India; 0.1 ml (1.5 x 10^5 Mycobacterium bovis) intradermally).21,22 Two injections were given with an interval of one month between them.

2) Levamisole (2 mg/kg body-weight/day) was administered for three days as a single dose, followed by an interval of seven days. Six cycles were given.

3) DT (0.5 ml, intramuscularly) was given one month after the second injection of BCG.

After the immunomodulation therapy ATT alone was continued for a total of 12 to 18 months. Follow-up. Three months after starting treatment both groups had clinicoradiological assessment and the laboratory investigations were repeated. Because of the paucibacillary nature of skeletal tuberculosis the assessment of the response to treatment was primarily based on clinicoradiological grounds including resolution of constitutional symptoms, pain, spasm, swelling, healing of sinuses, improvement in the range of movement, radiological evidence of the re-appearance of bony margins and resolution of regional osteoporosis. Clinical follow-up was continued in both groups for a mean of 30.2 months (24 to 49) to confirm the long-term stability of the clinical response which was observed during the treatment.

Control group. A series of 20 age- and gender-matched healthy blood donors was included in the study for a comparison of laboratory parameters including measurement of the peripheral lymphocyte subpopulation. This group

**Group I (newly-diagnosed group).** This comprised 31 patients with newly diagnosed osteo-articular tuberculosis. They had no history of previous ATT. There were 14 men and 17 women with a mean age of 25.6 years (3 to 70).

**Group II (non-responder group).** This consisted of 20 patients with osteo-articular tuberculosis who fulfilled any of the following criteria.

1) Patients who were already on an uninterrupted supervised regime of ATT and who had already completed a two-month course of pyrazinamide, with any of the following clinical features: a) clinicoradiological non-responsiveness to ATT after at least three months; b) deterioration or spread of the disease while on ATT; c) non-healing or breakdown of a surgical wound; and d) repeated formation of a cold abscess despite repeated aspirations.

2) Recurrence of a clinically healed osteo-articular tubercular lesion.

There were nine men and 11 women with a mean age of 30.1 years (15 to 55).

**Exclusion criteria for both groups.** Patients having active pulmonary tuberculosis or HIV infection were excluded as were those with any detectable serious illness including diabetes, malignancy or a condition requiring steroid therapy. We also did not include patients with a doubtful diagnosis of tuberculosis or those requiring urgent decompression for Pott's paraplegia or immediate aspiration of a large cold abscess.

**Laboratory studies.** All patients had the following investigations at enrolment.

**Routine.** These included measurement of haemoglobin, serum levels of albumin and globulin, total and differential leucocyte counts, the ESR (erythrocyte sedimentation rate) and blood sugar (fasting and post-prandial).

**Peripheral lymphocyte profile.** This was carried out on peripheral blood smears using the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique.18,19 A total of 200 lymphocytes either stained (bright red) or unstained (clear blue) were counted in each of the slides. Counts which stained positive for CD4 or CD8 cells were expressed as a percentage of the total lymphocyte count. These percentage counts were converted into absolute counts using values derived from routine total and differential leucocyte counts. For an individual trained operator the coefficient of variation of counts, in sets of ten slides for each of the two markers, was 6.9% (SD 2.7; 4.2% to 9.6%) when observed in 20 healthy blood donors and in 40 patients with high or low counts. Similarly, counts obtained by eight different personnel, each counting the same slide, showed a coefficient of variation of 6.9% (SD 2.3; 4.6% to 9.2%).

**Regime of therapy.** The patients in group I had supervised ATT, namely isoniazid, rifampicin, ethambutol and pyrazinamide. The last was stopped after two months and ethambutol after six months. The remainder was continued for a total duration of 12 to 18 months.

The patients in group II, who had been on ATT for three months or more were subjected to immunomodulation therapy for an initial period of eight weeks. In the patients with recurrence of disease ATT and an immunomodulation regime were given. The latter had those elements, as follows (Fig. 1).

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Control group. A series of 20 age- and gender-matched healthy blood donors was included in the study for a comparison of laboratory parameters including measurement of the peripheral lymphocyte subpopulation. This group
Results

shows an improvement in all of the parameters (Table II). Repeat assessment of these parameters after three months serum globulin level compared with the treatment group. The globin level, a raised mean ESR and an increased mean haemoglobin level, a decreased mean haemoglobin level, and the point of enrolment they had a decreased mean haematological response to the standard ATT at three months. At the involved site (Table I).

Anatomical sites of involvement in group-I and group-II patients, by number and percentage

<table>
<thead>
<tr>
<th>Site</th>
<th>Group I (n = 31)</th>
<th>Group II (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spine</td>
<td>12 (38.7)</td>
<td>12 (60)*</td>
</tr>
<tr>
<td>Calcaneum</td>
<td>3 (9.7)</td>
<td>3 (15)</td>
</tr>
<tr>
<td>Elbow</td>
<td>3 (9.7)</td>
<td>-</td>
</tr>
<tr>
<td>Wrist</td>
<td>3 (9.7)</td>
<td>-</td>
</tr>
<tr>
<td>Shoulder</td>
<td>3 (9.7)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Hip</td>
<td>2 (6.5)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Tibia</td>
<td>1 (3.2)</td>
<td>-</td>
</tr>
<tr>
<td>Clavicle</td>
<td>1 (3.2)</td>
<td>-</td>
</tr>
<tr>
<td>Humerus</td>
<td>1 (3.2)</td>
<td>-</td>
</tr>
<tr>
<td>Metacarpal</td>
<td>1 (3.2)</td>
<td>-</td>
</tr>
<tr>
<td>Scapula</td>
<td>1 (3.2)</td>
<td>-</td>
</tr>
<tr>
<td>Ilium</td>
<td>-</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Knee</td>
<td>-</td>
<td>2 (10)</td>
</tr>
</tbody>
</table>

* not significant

provided the control values for the CD4 and CD8 cell counts from our local population, where tuberculosis is an endemic disease.

Statistical analysis. A paired Student t-test was used to assess changes in the laboratory parameters between enrolment and follow-up. The chi-squared test was used to compare the frequency of involvement of anatomical sites between the two groups.

Results

Both groups had a predominance of patients between 15 and 40 years of age with means of 25.6 and 30.1 years, respectively. Group II had a statistically insignificant preponderance of women. The spine was the most commonly involved site (Table I).

Of the 31 patients in Group I, 29 showed a clinicoradiological response to the standard ATT at three months. At the point of enrolment they had a decreased mean haemoglobin level, a raised mean ESR and an increased mean serum globulin level compared with the treatment group. Repeat assessment of these parameters after three months showed an improvement in all of the parameters (Table II).

The CD4 cell count at enrolment in group I was depressed (mean 686 cells/mm$^3$ blood (SD 261)) as compared with that of the matched control group (1102 cells/mm$^3$ blood (SD 156)) (Table II). After treatment for three months, the mean CD4 cell count of 29 patients in this group was restored to the normal level, with a variable degree of increase from 2.8% to 359% compared with baseline values. Two patients (tuberculosis of the shoulder and of the spine (L4-L5) showed a decrease in the CD4 cell count of 30.6% and 21.7%, respectively), despite their baseline values being similar to those responding to treatment (within mean ± 1 SD). These patients did not respond to ATT (Fig. 2).

In group II, 14 patients showed an initial clinicoradiological improvement at three months under the influence of immunomodulation. However, on further clinical follow-up, one of these patients with involvement of the calcaneum had recurrence of discharge from a sinus within two months which failed to respond to extended ATT. One of the six patients (tuberculosis of the spine (D12-L1)) not showing a clinicoradiological response at three months had healing after a further two months which was stable at 48 months. Therefore, 14 of 20 patients (70%) had a successful long-term response to the combination of immunomodulation and ATT (Fig. 2).

The patients in group II were characterised by a decreased mean haemoglobin level, an increased mean total leucocyte count, a raised mean ESR and an increased mean serum globulin level (more marked when compared with group I) at the point of enrolment. However, they did not show a statistically significant change in the mean haemoglobin value at follow-up although the remaining parameters improved significantly (Table II).

Group II also had greater impairment of the CD4 cell count at enrolment compared with group I (p < 0.01). After three months (i.e. about one month after completion of immunomodulation), the mean CD4 cell count of group II was lower than that of group I. All 14 patients in group II who showed a stable response to treatment were character-

![Table II. Laboratory investigation data (mean ± SD) of group I. Group II at enrolment and in response to therapy at three months](image-url)
ised by a varying degree of augmentation of the CD4 cell count which was greater than that in group I (143% to 306%). However, analysis of the CD4 cell count of six patients who failed to show a stable response to combined immunomodulation plus ATT on follow-up showed either a decrease (ranging from 13.5% to 17%) in five patients or a minimal increase (8.2%) although their baseline values at enrolment were comparable with those of the responders in the same group.

Of the eight patients who failed to respond to conventional ATT (two patients in group I) and combined immunomodulation and ATT (six patients in group II), seven did not have an increase in the CD4 cell count and the remaining patient had only a minimal increase. The baseline CD4 cell count in all of these patients did not differ from that of patients who showed a clinicoradiological response to therapy in their respective group.

At the point of enrolment the CD8 cell count was within normal limits in group I but was marginally depressed in group II. Unlike the levels of CD4 cells, the level of CD8 cells did not significantly change in either group after treatment for three months (Table II). However, there was a significant increase in the CD4:CD8 ratio in both groups after treatment for three months because of the significant increase in the CD4 cell count.

**Discussion**

In our series the preponderance of patients between 15 and 40 years of age and the frequent involvement of spine, is in accordance with the observations of other authors. CD4 cells produce a variety of cytokines considered to be the central mediators in the genesis of specific T-cell immunity, macrophage activation and granuloma formation, which are the three major recognised components of the protective response against infection with tubercle bacilli. While numerous reports on peripheral CD4 cell counts in patients with pulmonary tuberculosis suggest low or normal values, relatively fewer reports on the CD4 cell count in extrapulmonary tuberculosis have consistently documented lowering of the CD4 cell count which substantiated the immunopathogenic postulation that dissemination of tuberculosis beyond the extrapulmonary site is an indication of failure of the host’s immune system to contain the infection at a local site. In our study patients from both groups had a low mean CD4 cell count, as compared with the control group. Most of the patients in group I (93.6%) and in group II (70%) had a significant increase in the CD4 cell count after treatment for three months.

It has been postulated that trapping of CD4 cells at a local site of involvement is more selective than that of CD8 cells. Thus, the changes in the profile of peripheral lymphocyte subsets may be brought about by the re-trafficking of cells from the local site to the circulatory pool after healing.

Failure of two patients in group I (tuberculosis of the shoulder and of the spine (L4-5)) to show clinical improvement and recurrence of symptoms in one in group II (tuberculosis of the calcaneum) were associated with lack of improvement in the CD4 cell count in response to treatment. Conversely, the late clinicoradiological response in one patient in the non-responder group with tuberculosis of
the spine (D12-L1), despite lack of improvement at three months showed improvement of the CD4 cell count at follow-up at three months. These observations gave valuable support to the hypothesis that the CD4 cell count is a reliable predictor of clinical outcome. The observed relationship between the increase in the peripheral CD4 cell count and a favourable clinical outcome was further strengthened by the concomitant increase in the haemoglobin level as well as by a decrease in the ESR, total leucocyte count and the serum globulin levels. These changes are all considered to be indicators of a successful response to treatment in tuberculosis.

Chandra observed that cellular and humoral immunocompetence was depressed in patients who were undernourished. The serum albumin level in both of our groups of patients was found to be within normal limits indicating that their nutritional status was not the reason for the suboptimal immune status of these patients (Table II).

A study by Bose et al from New Delhi on patients with pulmonary tuberculosis refractory to ATT showed that those who showed failure of normalisation of blood CD4/CD8 homoeostasis ran a risk of relapse, even if they became sputum-negative after ATT. These observations are further validated in our study since the normalisation of the CD4 cell count as well as the CD4/CD8 ratio showed a correlation with clinical improvement on a long-term basis.

Clinical non-responsiveness to conventional first-line antituberculous drugs is an increasing worldwide problem. In developing countries, modern diagnostic techniques such as BACTEC (a radiometric system of mycobacterial culture which allows early detection of tuberculous infection) and the polymerase chain reaction as methods of identification of the organism and determination of the sensitivity of antituberculous drugs are available at very few centres. Moreover, the positive culture for drug sensitivity by routine methods (e.g. on Lowenstein Jansen media) is low since skeletal tuberculosis is a paucibacillary disease. The problem is further compounded by the ill-advised use of broad-spectrum antibiotics such as quinolones before a definitive diagnosis has been made leading to further reduction in positive culture yields. This makes identification of the type of Mycobacterium and testing for its sensitivity to antituberculous drugs difficult. For these reasons, we used clinical parameters such as non-responsiveness rather than identification of the organism or the results of drug sensitivity as the basis for inclusion in group II.

It is well known that natural immunity against tuberculosis is very effective. This is attested by the fact that only 5% of infected individuals develop the clinical disease and about another 5% develop post-primary tuberculosis later in life. In the pre-chemotherapy era 30% to 50% of patients with clinical tuberculosis showed spontaneous recovery with general measures aimed at improving the general health and immunity of the patient. In the light of these facts, the combating of immunosuppression and the enhancing of the host’s immune response have always been considered to be a desirable approach to augment conventional chemotherapy.

Levamisole initially introduced as a laevorotatory isomer of tetramisole with antihelminthic activity has been used as an immunomodulator since 1976 as an adjunct to dapsone. Lepromatous leprosy patients whose skin smears remained persistently positive after long-term treatment with dapsone responded well after the addition of levamisole, as indicated by the bacteriological clearance and lepromin conversion. The most convincing evidence in this regard was the in vitro finding of enhancement of the interaction between macrophages and lymphocytes as well as the increase in absolute lymphocyte count and E-rosette-forming cells.

Two recent studies using Mycobacterium vaccae as a means of immunotherapy in patients with pulmonary tuberculosis have shown enhanced radiological improvement and reversal of the Th2-dominated serum cytokine profile, i.e. lowering of serum levels of interleukin (IL)-4 and IL-10 along with elevation of γ-interferon levels. We could not use M. vaccae, a more popular agent for the treatment of patients who have failed to respond to chemotherapy. Nevertheless, in our study the use of BCG which also contains the immunopotentiating chemical substance muramyl dipeptide which is identical to M. vaccae, has apparently been equally effective as shown by the normalisation of the peripheral CD4 lymphocyte count indicating diversion of the pool of these cells away from an active site, a property considered to be an important parameter for evaluating the success of an immunotherapeutic agent. This possibility is further strengthened by more recent studies which relied on a more functional in vitro assay i.e. a study of secreted cytokines in supernatants and quantitation of cytokine-secreting cells in lymphocyte cultures stimulated by mycobacterium-specific antigens in healthy volunteers vaccinated with intradermal BCG. A previous study has shown the successful role of BCG with killed M. leprae as adjuncts to standard multidrug therapy for the treatment of lepromin-negative multidrug-resistant leprosy patients which agreed with in vitro evidence suggesting augmentation of cell-mediated immunity. An earlier study has shown the immunopotentiating role of BCG alone in offering protection in lepromin-negative household contacts of leprosy patients on long-term follow-up accompanied by lepromin conversion. It is possible that the combination of levamisole and BCG may have a synergistic action. Recently reported 35 patients with osteoarticular tuberculosis who were considered to be resistant to multidrug therapy. He supplemented standard chemotherapy in these patients with oral levamisole, BCG and DPT vaccination. Of the 35 patients, 31 showed a favourable clinicoradiological response within six to eight weeks after beginning treatment. However, he did not present supportive laboratory data indicating immunomodulation.

Forms of M. tuberculosis which do not carry drug resistance are easy to treat by virtue of the ready diffusion of...
antituberculous drugs into body fluids. However, intracellular persisters (latent bacilli which can reactivate themselves when the host is immunocompromised), pose a significant problem necessitating prolonged intake of drugs because of their slow metabolism. This problem is further compounded by non-compliance, which may occur in up to 50% of patients in developing countries. Thus, another important advantage of immunomodulation may be the reduction in the total duration of chemotherapy required to combat the disease. In developing countries, where the disease is rampant and resources are minimal, this approach would hopefully reduce the number of drug defaulters.

We conclude that the CD4 cell count is a reliable indicator of host immunity in patients with osteo-articular tuberculosis. These patients have a low CD4 cell count in the peripheral blood. The number of CD4 cells increases in the peripheral blood in parallel with a favourable clinical response to ATT. The pattern of change in the peripheral CD4 cell count in response to treatment may be a reliable indicator of the clinical response. Immunomodulation using BCG, oral levamisole and DT as an adjunct to ATT holds promise for patients who are not responsive to conventional therapy. Pending the general availability of M. vaccae vaccine or more efficient treatment, the immunopotentiating action of this vaccine may be of importance for patients, especially in developing countries.9 Thus, another significant problem necessitating prolonged intake of drugs (e.g., isoniazid because of their slow metabolism. The problem is further complicated by multi-drug resistant cases of osteo-articular tuberculosis.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References