Granuloma formation in patients after injection of methanol extraction residue (MER-BCG)

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SUMMARY Nine patients with advanced cancer who were receiving the methanol extraction residue of BCG (MER-BCG) intradermally or intratumorally underwent biopsies from the injected sites or from locally enlarged lymph nodes. Most preparations showed a chronic granulomatous reaction consisting of lymphocytes, histiocytes, and epithelioid cells as well as either Langhans's or foreign-body type giant cells, or both. The degree of granuloma formation and giant cell infiltration varied. In only one case did the reactions consist merely of chronic lymphocytic and histiocytic inflammation with no granuloma formation. Examination of melanoma nodules injected with MER showed, in addition to granulomas, large numbers of giant cells penetrating the tumour.

Immunotherapy is a new form of cancer treatment under intensive investigation in many centres (Bast et al., 1973). Various bacteria and preparations derived from bacteria, such as living BCG (Muggleton et al., 1975), killed Corynebacterium (Fisher et al., 1976), and agents derived from BCG (Granger et al., 1976), have been shown to cause tumour regression in animals and in human cancer patients. These agents have also been reported to potentiate different parameters of the cellular immune response and thereby increase host resistance (Mitchell, 1976).

BCG has been found to cause multiple granulomata at the site of injection as well as in distant organs (Khalil et al., 1976; Whittaker et al., 1976) often associated with various related complications (Hunt et al., 1973). MER is a methanol extraction residue of BCG organisms killed by phenol and washed with acetone which has immunostimulatory properties (Weiss, 1972). It has been used recently for non-specific immunotherapy in cancer patients with beneficial effect on the cutaneous reactivity and on the in-vitro lymphocyte transformation responses (Robinson et al., 1975; 1977).

This study describes the histological changes found at sites of MER injection as well as in regional lymph nodes that react to it. To the best of our knowledge these histological changes have not yet been reported.

Patients

Nine patients with locally advanced or disseminated cancer received MER immunotherapy in addition to conventional radiotherapy or chemotherapy, or both. The disease had been confirmed histologically in all patients. Informed consent to immunotherapy had been obtained from the patients.

Age, sex, diagnosis, previous therapy, and cutaneous reactivity to recall antigens are shown in Table 1. Seven of the patients received intradermal injections of MER. Four of them had biopsies taken from the MER injection sites in the skin. In the other three local nodes enlarged. In two of them the nodes appeared in the skin near the injection sites, and in the third an axillary lymph node became swollen (Table 2). These nodes were excised for histological examination. The two remaining patients out of the nine studied, both suffering from metastatic malignant melanoma, were given intratumoral MER therapy. All biopsies were performed two to six weeks after MER injection, except in one patient who had an enlarged axillary lymph node excised nine weeks after the treatment.

Methods

A course of 10 intradermal injections of MER fraction of BCG 0.1 mg (from a batch prepared by
Merck, Sharpe and Dohme Research Laboratories, New Jersey, USA), making a total of 1 mg per course, was given in the back or in the arm. The number of courses each patient received before biopsy, the site of biopsy, and timing are shown in Table 2. The intralesional MER therapy in the two melanoma patients with skin metastases was given as follows: One patient (No. 8) was treated by daily injections in increasing doses, starting with 0.1 mg and gradually increasing to 0.8 mg per nodule. A total of six nodules were injected and each received 7-9 mg. After two weeks of treatment the nodules began to regress and they were excised.

The other patient (No. 9) received intralvesional MER injections once a week for four weeks, starting with 0.5 mg and finally reaching 1 mg per nodule. The total amount per nodule was 3 mg. Transient flattening of the nodules was noted but the patient succumbed later to disseminated disease. A necropsy was performed six weeks after termination of treatment. The intensity of the skin reaction to MER was determined according to a scale of five grades based on the degree of local induration, ulceration, secretion, and squama formation: -, no response; +1, weak response; +2 mild reaction; +3, moderate reaction; +4 strong reaction; and +5, hyperreactive response (Robinson et al., 1975). Most patients in this study had a moderate to strong reaction to MER with the exception of one patient who showed a weak reaction (Table 2). In patient 8, treated intralesionally only, the skin over the injected nodule showed initially a marked erythema progressing to induration and abscess formation.

**RECALL ANTIGENS**

The patients were skin tested during the MER therapy by intradermal injections of 0.1 ml of five recall antigens—purified protein derivative, streptokinase-streptodornase, candidine, mixed bacteria, and trichophyton. The diameter of induration at 48 hours was recorded as previously described (Robinson et al., 1975).

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**Table 1** Clinical characteristics of patients treated with MER

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/Sex</th>
<th>Site of tumour</th>
<th>Extent of disease</th>
<th>Treatment before MER injection</th>
<th>Cutaneous reactivity before biopsy</th>
<th>Site of MER injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>49 F</td>
<td>Epiglottis</td>
<td>Lymph nodes</td>
<td>S, R</td>
<td>+ + +</td>
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<td>2</td>
<td>40 F</td>
<td>Lung</td>
<td>Mediastinum</td>
<td>R, C</td>
<td>- + +</td>
<td>ND ND ID</td>
</tr>
<tr>
<td>3</td>
<td>78 M</td>
<td>Lung</td>
<td>Mediastinum</td>
<td>R</td>
<td>- ± ±</td>
<td>ND ND ID</td>
</tr>
</tbody>
</table>
| 4       | 60 F    | Melanoma       | Lymph nodes       | R, C                          | ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ± ····

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S = surgery; R = radiation; C = chemotherapy; ID = intradermal; IT = intratumoral; ND = Not done; PPD = purified protein derivative; CAN = Candida; SK/SD = streptokinase/streptodornase; M = mixed bacteria; T = trichophyton; Duke's C = tumour infiltrating serosa and lymph nodes.

**Table 2** Clinical data in relation to MER injection and microscopic findings

<table>
<thead>
<tr>
<th>Patient</th>
<th>Cutaneous reaction to MER</th>
<th>No. of MER courses before biopsy</th>
<th>Site of biopsy</th>
<th>Time of biopsy after injection (weeks)</th>
<th>Extent of granuloma formation</th>
<th>Type of giant cells</th>
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<tbody>
<tr>
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<td>+1</td>
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<td>6</td>
<td>I</td>
<td>F</td>
</tr>
</tbody>
</table>

*Necropsy study.*

0 = chronic inflammation without granuloma; I = granuloma present; II = well-formed granuloma; III = numerous well-formed granulomas; F = foreign-body type giant cells; L = Langhan's type giant cells; IS = injected skin; ASN = adjacent skin nodule; RLN = reactive lymph node; ITN = injected tumour nodule.
Granuloma formation in patients after injection of methanol extraction residue (MER-BCG)

Results

The general histological appearance of most of the lesions induced by MER was of a chronic granulomatous inflammation (Figs. 1 and 2). The inflammatory infiltrate consisted of lymphocytes, histiocytes, and epithelioid cells; occasionally there were also a few eosinophils and granulocytes. Eight of the nine preparations contained giant cells.

The giant cells were divided, according to their appearance, into two cell types—giant cells of the Langhans's type with nuclei disposed about the
periphery of the cell in a complete circle or horse-shoe-shaped pattern (Figs. 1 and 2), and foreign-body giant cells with nuclei scattered erratically throughout the cytoplasm (Fig. 2). In three cases the cells had the appearance of typical Langhans’s giant cells, in four cases they were classified as foreign-body giant cells, and in one case both types were identified (Table 2).

The extent of the granuloma formation was of a different degree in each case (Table 2). Thus in two cases, one a skin preparation (patient 4) and the other (patient 7) from a regional lymph node, there was a marked granulomatous reaction with a large number of giant cells as well as areas of central necrosis and foci of fibrosis. In two other cases (patients 2 and 8) there was considerable granulation inflammation with a sufficient number of giant cells.

In four patients (1, 3, 6, and 9) moderate chronic inflammation of the skin was observed with one or two granulomas with a low to moderate number of giant cells in the infiltrate. In only one patient out of the nine (patient 6, who had the specimen excised nine weeks after the injections) did the biopsy show a chronic lymphocytic and histiocytic inflammation without granuloma formation. In addition, evidence of acute inflammation was demonstrated in three out of the nine preparations (patients 3, 5, and 9).

In all our cases the Ziehl-Neelsen stain for acid-fast bacilli was negative.

Discussion

We found chronic granulomatous inflammation associated with either Langhans’s or foreign-body type giant cells after intradermal injection of MER. The findings were similar after injection of MER into melanoma nodules. In one patient (patient 8) the infiltrate penetrated into the tumour and the giant cells were said to intermingle with the tumour cells. In most cases there was a correlation between the cutaneous response to MER and the extent of granulomatous inflammation.

Skin reactivity to recall antigens seemed to be of importance since anergic reactions were associated with only slight granulomatous inflammation. The time of biopsy should also be considered because in specimens excised more than six weeks after the injection the reaction was less intense (Table 2). There is no sharp division between the Langhans’s and foreign-body type giant cells. The Langhans’s type cell is always thought to be associated with granulomatous inflammation, particularly when caused by the tubercle bacillus.

The granuloma formation properties of MER/BCG are similar to those reported after the injection of living BCG organisms (Khalil et al., 1976) or whole BCG cell walls (Granger et al., 1976) as well as massive lymphocyte invasion, giant cells, and local necrosis (Muggleton et al., 1975). This indicates that MER/BCG contains those structural entities of the mycobacterium responsible for inducing granuloma.

Animal experiments have shown that the delipidated cell wall skeleton of BCG (CWS-I), which is a peptidoglycolipid, induced little granulomatous inflammation in the lungs of mice (Meyer et al., 1974, 1975). Since P₃ is a trehalose mycolic acid ester derived from the cell wall of BCG it is also a poor inducer of such lung granuloma. Nevertheless, CWS-I and P₃ combined caused extensive lung granuloma, showing that several subfractions of mycobacterial cell walls are needed to cause this reaction.

The appearance of granulomas was correlated with the antitumoral activity of the agents (Bast et al., 1974).

MER has a considerable advantage over living BCG and it does not cause the many undesirable side effects that it does when used for treating cancer patients (Hunt et al., 1973). The similarity in the histological findings as well as its ability to stimulate immunological reaction suggest that clinical trials of MER should continue.

References


A. Bartal, H. Kerner, Y. Cohen, and E. Robinson

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