

✓ CELL MEDIATED AND HUMORAL IMMUNITY IN "REVERSAL REACTIONS"

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There has been comparatively little research into the mechanisms involved in "reversal reactions" occurring in borderline leprosy, in the past. It seems likely that they result from a delayed hypersensitivity reaction, as there is usually a dense lymphocytic infiltration on histological examination of skin and nerve, and there is a marked increase in lymphocyte transformation responses (LTTs) with whole Mycobacterium leprae (M. leprae) used as antigen.(1)

The clinical presentation of patients with reversal reactions may vary considerably and may be divided into three groups.

1. Those with predominantly skin hypersensitivity where there is erythema and oedema of the skin lesions without obvious involvement of the nerves.
2. Those with predominantly nerve hypersensitivity with swelling and tenderness of the nerves, but no change in the hypopigmented skin lesions.
3. Those with both skin and nerve hypersensitivity.

This paper describes a study of 24 patients who developed reversal reactions. Fifteen were included in a prospective study of the immunology of borderline leprosy and pre-treatment assessments were available. Nine further patients were first seen at the start of their reactions. Sixteen were borderline tuberculoid, 2 borderline and 6 borderline lepromatous as classified on the Ridley-Jopling scale.(2)

All patients received anti-leprosy treatment with dapsone, which was continued in unaltered dosage throughout the study. When patients developed reactions they were treated with prednisolone. The initial dosage was 30-40 mg daily, which was gradually reduced over a period 1-9 months according to the duration and severity of the reaction.

Patients were examined repeatedly during the period of reaction and the post-reaction period. Skin involvement was assessed on the degree of erythema and edema of the skin lesions. Nerve involvement was assessed by three criteria:

- (a) by clinical estimation of nerve tenderness and swelling,
- (b) by motor nerve conduction studies of the ulnar and median nerves.
- (c) by voluntary muscle tests (3)

LTTs were performed at least twice during the reactions using as antigens whole and sonicated preparations of M. leprae, at 3 concentrations standardised to the bacillary content of the original homogenate, i.e. 10^3 , 10^7 and 10^8 bacilli per ml. The LTTs were carried out by a micro-method as described by Closs. (4)

Serum immunoglobulin (Ig) levels (IgG, IgA and IgM) were also determined in 12 of the patients included in the prospective study of the immunology of borderline leprosy, who developed reversal reactions. Quantification of the Igs was performed by means of agar diffusion plates containing specific antisera as described by Mancini et al (5) and was carried out at baseline, during reaction and post reaction. Two of the baseline serum samples were unfortunately lost before analysis.

The maximal LTT responses (responses to whole M. leprae and sonicated M. leprae added, with controls subtracted) of the 15 patients who were studied from the inception of anti-leprosy treatment, at baseline, during reaction and post reaction are shown in Fig. 1. In 13 of the patients there was a marked rise in LTT responses with these antigen preparations from baseline to reaction, with a comparable fall after reaction. When the results were analysed using the Students' 't' test, both rise and fall were highly significant ($p < 0.005$). (See Fig. 1)

The rise in LTT response was due, in different patients, to increased responses to whole or sonicated or both preparations of M. leprae. When the total LTT responses of these patients when in reaction, and of the nine further patients who also were in reaction though they had not been followed from inception of treatment, were studied, it was found that the clinical presentation correlated with the rise in LTT with the two different antigen preparations. In the 10 patients who were judged to have a "skin reaction" clinically, there was a marked increase in LTT responses using whole M. leprae, but a smaller or negligible increase using sonicated M. leprae. In those judged to have a "nerve reaction" clinically, the reverse was true. Those who had a "mixed reaction" showed a high LTT response using both antigen preparations. The ratio of LTT results - whole/sonicated M. leprae (total responses) are shown in fig.2. Statistical significance was estimated using the non-parametric test of Kruskal

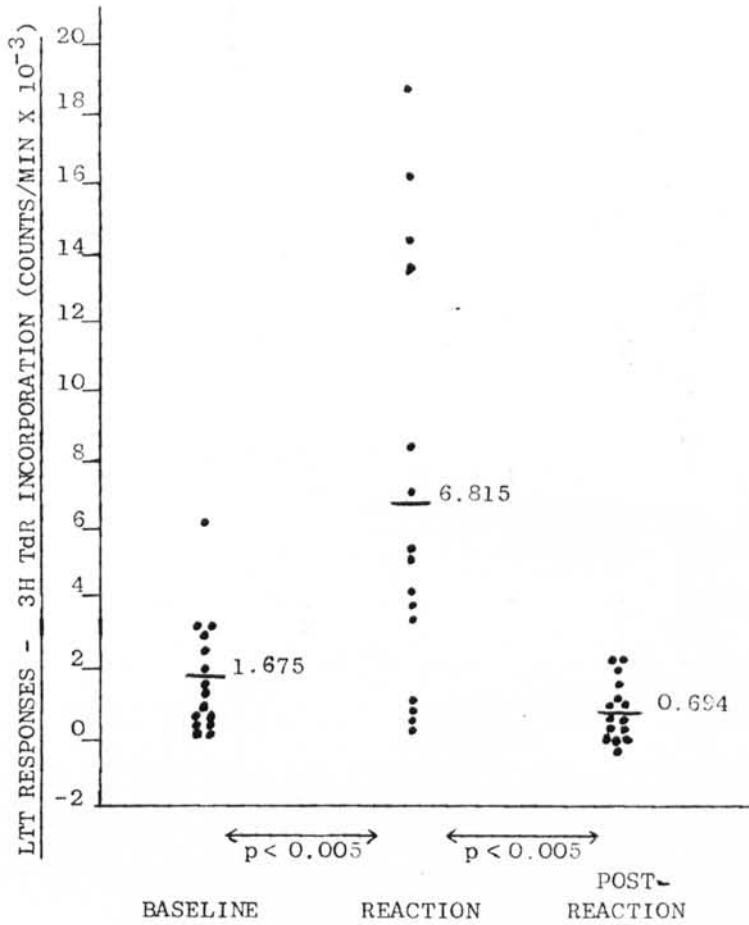


Fig.1 LTT Responses in 15 patients before reaction, during reaction, and after reaction. The responses to "whole" *M. leprae* and sonicated *M. leprae* are added, with the controls subtracted.

and Wallis (6) for independent samples and the differences between the three groups were found to be highly significant ($p < 0.001$).

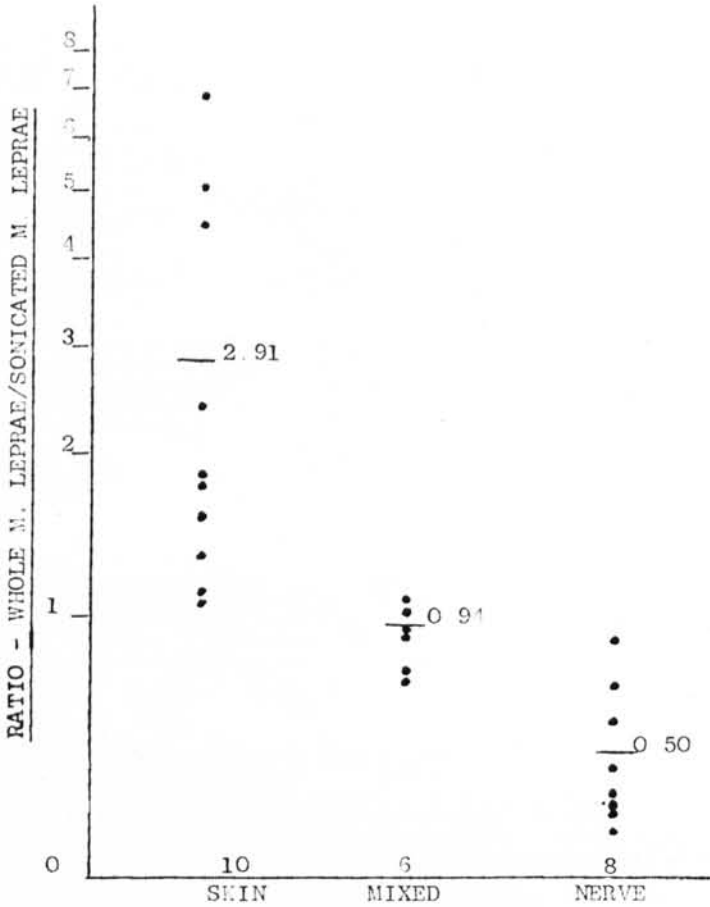


Fig.2. Ratio of total LTT responses - response to "whole" *M. leprae*/response to "sonicated" *M. leprae* in 24 patients during reaction. related to clinical presentation.

Serum Ig levels in 12 of the patients studied from inception of treatment, who developed reversal reactions. at baseline. during and post reaction are shown in Fig 3. When the values of each Ig at baseline were calculated and compared with those during reaction. in each case there was a significant rise (IgG, $p < 0.025$; IgA, $p < 0.01$; IgM, $p < 0.025$). When the values of these Igs during reaction were compared with the mean values post reaction. there was a significant fall in IgG and IgA only (IgG, $p < 0.001$; IgA, $p < 0.005$).

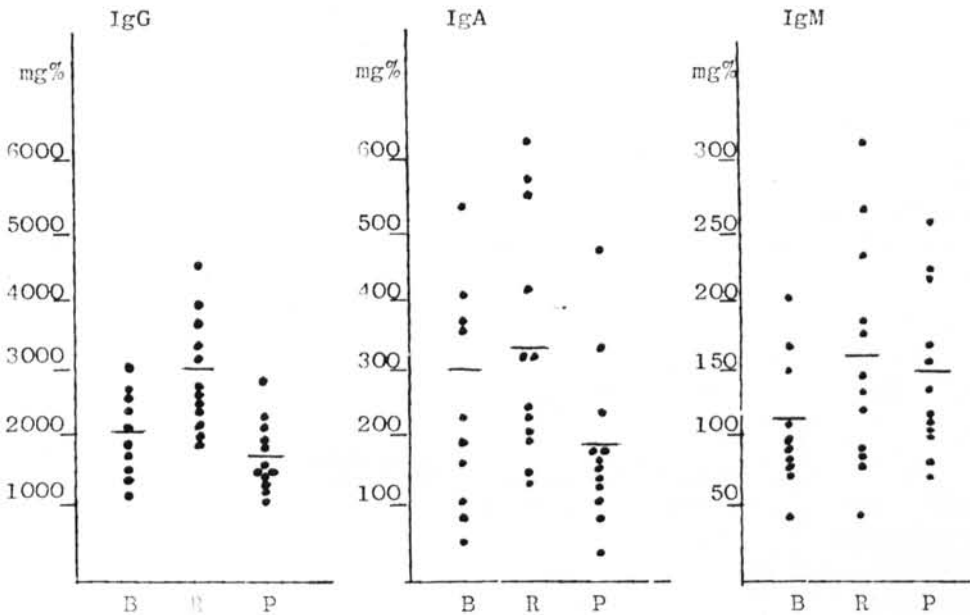


Fig 3 Serum Ig levels in 12 patients with borderline leprosy, at baseline, during reaction and post-reaction.

The prospective study of 15 borderline leprosy patients demonstrated that there was a marked rise in LTT responses to antigens of *M. leprae* during reversal reaction, and confirms that the mechanism of these reactions is an increase in cell mediated immunological reactivity.

The results of LTTs in the 24 patients in reaction show that nerve and skin involvement are associated with responses to different antigens. The failure of whole washed bacilli to elicit a high response in nerve reactions suggests that cytoplasmic antigens play an important role in this condition.

In skin reactions, the increased response is chiefly directed at whole washed bacilli, indicating that surface antigens are more important. The lesser rise to sonicated preparations (in which surface antigens are present) may be a dilution affect

The mechanism of nerve reactions may therefore be as follows. It has previously been noted (7) that in some borderline tuberculoid leprosy patients, where the host has reasonably good immunological competence to recognise and destroy the leprosy bacillus, many bacilli

may be present in the nerve but few if any in the skin. The same phenomenon has also been noted in borderline lepromatous patients particularly after one or two years of treatment. This is presumably because the Schwann cells of nerves, in which most of the bacilli are contained, are relatively long lived. In this situation it is likely that the surface antigens of the bacillus are rarely exposed; soluble cytoplasmic antigens are more likely to be exposed but they are probably released slowly, and so few lymphocytes are sensitised. In reactions there would appear to be a sudden increase in release of these cytoplasmic antigens, though the trigger mechanism is unknown. Subsequent destruction of the Schwann cells may then lead to increased exposure of bacillary surface antigens, and it was noted that in five of the eight patients who had nerve reaction, there was a rise in LTT responses using whole M. leprae as antigen in the weeks following the start of the reaction.

In skin reactions, the LTT results indicate that there may be an increased exposure of bacillary surface antigens. In borderline tuberculoid leprosy the bacilli are present in the skin in small numbers, and are predominantly found in the dermal nerves. It appears that the lymphocytes and macrophages in the skin suddenly recognise the bacilli in the dermal nerves with destruction of the Schwann cells and consequent exposure of surface antigens. This possibility is borne out by the observation that in eight out of ten of our patients with "skin" or "mixed" reactions, who had skin biopsies during reaction, there were present large nerves containing degenerate bacilli within granuloma.

In mixed reactions, hypersensitivity reaction in nerve often follows some weeks after the reaction in skin. This may be due to the fact that some cytoplasmic antigens are released during the "skin phase" with increased lymphocyte sensitisation to these antigens leading to increased recognition of such antigens elsewhere - particularly those in nerve.

Although the trigger mechanism for these reactions is still unknown, it has been suggested that coincidental exposure to other mycobacteria (1) could be a possible cause. However there is no good evidence for this, and in those of our patients where LTTs were performed using BCG and Mycobacterium duvalii as antigens during reactions, there was no consistent concomitant rise in responses.

In view of recent research demonstrating cooperation between T and B lymphocytes, it is interesting that there is a rise in IgG, IgA and IgM during reversal reactions. Investigations in 4 of the 12 patients using agarose gel and crossed immuno-electrophoresis suggest that this is a non specific rise. Several studies have demonstrated

that proliferating, non-secreting B cells can be converted into antibody producers by activated T cells. (8, 9, 10) In reversal reactions there is a marked increase in the number of circulating T lymphocytes sensitised to antigens of the leprosy bacillus. It would follow that these lymphocytes when activated might be liable to stimulate the production of serum Igs by non specific action in proliferating lymphocytes. Thus the rise in Igs is more likely to be an effect rather than a cause of the reaction.

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