Original Article

A SEQUENTIAL STUDY OF CIRCULATING IMMUNE COMPLEXES, COMPLEMENT AND IMMUNOGLOBULINS IN BORDERLINE TUBERCULOID LEPROSY PATIENTS WITH AND WITHOUT REACTIONS

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Sequential estimates of the levels of circulating immune complexes (CIC), complement catabolic fragment C3d, complement-mediated immune complex solubilization (CMS) and immunoglobulins were made in 24 newly diagnosed patients with borderline tuberculoid leprosy over a 20 month period after initiation of chemotherapy.

Fourteen of these patients had not suffered from reversal reactions either at the time of presentation or during the follow-up period. The levels of CIC were elevated in them from the third to the eleventh month after starting chemotherapy and immunoglobulin G (IgG) levels were elevated upto eight months. The concentrations of C3d and immunoglobulins A (IgA) and M (IgM) were normal in these patients.

The other ten patients had reversal reaction at the time of diagnosis which subsided by the third month after starting treatment. They did not have reversal reactions later. The levels of CIC and IgG were elevated and those of CMS were depressed throughout the study period. Serum C3d level was initially elevated but came down to normal by the third month while IgA and IgM levels were within normal limits.

The relevance of these findings to the genesis of reversal reaction is discussed in this communication.

INTRODUCTION

Antigen-antibody complexes or immune complexes (ICs) are thought to play a vital role in the occurrence of erythema nodosum leprosum (ENL) syndrome in leprosy. It is held that an increase in lymphocyte-mediated delayed hypersensitivity results in the production of the reversal (type 1) reaction. However, perturbations in complement and antibody levels during type 1 reaction suggest that ICs may be involved in the causation of some of the manifestations of this condition also (Ramanathan et al 1985, Chakrabarty et al 1988, Saha et al 1995).

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We had earlier found that circulating ICs (CICs) and complement catabolic fragment (C3d) were elevated during reactions in borderline tuberculoid leprosy (BT) patients (Ramanathan et al 1984). Further, it was noted that such patients had a lowered complement -mediated IC solubilization (CMS) (Ramanathan et al 1985, Chakrabarty et al 1988). Efficient solubilization by complement renders ICs innocuous by making them incapable of stimulating phlogistic responses, and this function of the complement system is thought to reflect the ability of individuals to metabolize ICs in vivo.

In order to better define the relationship between the humoral immune parameters and the occurrence of reversal reactions, we decided to sequentially estimate the levels of CICs, immunoglobulins, C3d and complement-mediated IC solubilization (CMS) in a group of untreated BT leprosy patients and follow them up for some time after initiating treatment.

MATERIAL AND METHOD

SUBJECTS

The subjects consisted of patients and controls. Twenty-four untreated adult BT patients (18 males and six females) diagnosed on the basis of Ridley-Jopling criteria (Ridley & Jopling 1966) were the subjects for this study. Their mean ages and durations of the disease at the time of presentation are given in Table I. They were put on multidrug regimen as per the schedule prescribed by the National Leprosy Eradication Programme (NLEP) of India (IAL 1984). Blood was collected from them at the time of inception of treatment, two months after starting chemotherapy and subsequently at three-monthly intervals up to 20 months.

Table I. Clinical features of borderline tuberculoid (BT) patients without and with (BTR) reactions at the time of presentation

Type of disease	M/F	Mean age (Years) ± SD	Disease duration at presentation (years)	Lepromin reaction (mm)	
				Erythema	Induration
BT	11/3	34.0 ± 12.2	2.29 ± 1.35	7.9 ± 4.0	2.57 ± 1.27
BTR	7/3	42.2 ± 16.7	3.31 ± 3.43	13.0 ± 6.0	3.11 ± 1.45

Twenty-four healthy laboratory volunteers and hospital staff of the Central JALMA Institute for Leprosy (Agra), matched for age and sex were used as control subjects. Sera from at least three of them were tested along with those of the patients in all the investigations. Serum pooled from a minimum of five volunteers (NHS) was used as the standard.

SERUM

Blood was collected from the patients and the controls and the separated serum was stored at -70 °C in aliquots. Each aliquot was used only once after thawing.

LEPROMIN TESTING

Patients were given intradermally 0.1 ml of standardized Dharmendra lepromin (Sengupta et al 1979) in the volar aspect of the forearm. Both erythema and induration were measured 24 hr after the test.

MATERIALS

Bovine conglutinin was prepared as described by Lachmann and Hobart (1978) from normal bovine serum which was kindly supplied by Dr VD Padmanabhan, Madras Veterinary College, Chennai. Conglutinin and bovine serum albumin (BSA) were labelled with ^{125}I (Bhabha Atomic Research Centre, Mumbai), using iodogen (Hudson & Hay 1980). Antiserum against BSA was raised in rabbits. Antisera against C3d (Dakopatts, Denmark) and immunoglobulins G, A and M (Hoechst India Ltd) were purchased as indicated.

METHOD

Circulating ICs, complement-mediated IC solubilization (CMS), serum C3d and immunoglobulins were measured as indicated. Circulating ICs were estimated using ^{125}I-labelled conglutinin as described previously (Ramanathan et al 1984). Conglutinin was prepared, as suggested by Lachmann and Hobart (1978) from normal bovine serum (obtained from Madras Veterinary College, Chennai 7). The result was expressed as a number obtained by dividing conglutinin binding in the patient's serum by the binding in the pooled normal serum run the same day as control (Ramanathan et al 1991).

Complement-mediated IC solubilization was performed using ^{125}I-labelled bovine serum albumin (BSA)-anti BSA immune complexes. The amount of ICs solubilized was expressed as a proportion of the ICs solubilized in the pooled serum control included in the same batch (Ramanathan et al 1985, 1991).

Complement C3d levels were quantitated with a two-stage procedure involving precipitation with 22% polyethyelene glycol and rocket immuno electrophoresis using anti-C3d antiserum (Dakopatts, Denmark). The results were measured as "arbitrary Units" (aU) which were derived from the standard C3d generated in pooled NHS treated with 20mg/ml yeast at 37 °C for two hours (Ramanathan et al 1984).

Serum immunoglobulins G, A and M were measured using immunoglobulin class -specific antisera (Hoechst India Ltd, India) by the single radial immunodiffusion procedure.

ANALYSIS OF RESULTS

The results were analysed at the end of the study period, after completing all the laboratory tests, to avoid bias. Student's two-tailed unpaired t-test was used for inter-group comparisons. The paired t-test was used for comparisons within a group at different periods relative to a fixed time point.

RESULTS

CLINICAL FEATURES OF REACTIONS

Of the 24 patients, ten had features of reactions (consisting mainly of erythema and oedema of the lesions) which were the presenting symptoms in these patients. There was a tendency for an increased occurrence of neuritis in these patients (4/10) compared to patients without reaction (2/14) but, this difference was not statistically significant. Chloroquine and aspirin were given to control reactions and when there was neuritis, that was treated with corticosteroids. These drugs were discontinued after the subsidence of the reaction. The reaction usually subsided by three months and these patients did not suffer from any more episodes during the follow-up period. When patients were tested with Dharmendra lepromin, at 24 hours, slightly greater erythema ($p>0.05$) was seen in BTR patients (Table I).

CIRCULATING IMMUNE COMPLEXES

The levels of CIC in the patients are expressed as a fraction of the levels observed in normal human serum (NHS). We found that there was an increase in their levels in BT patients without reactions from the third month onwards upto the eleventh month which then came down to levels comparable to those observed in NHS by the fourteenth month after initiation of treatment. The CIC levels of BTR patients were elevated throughout the period of study, even after the subsidence of reactions (Fig. 1).

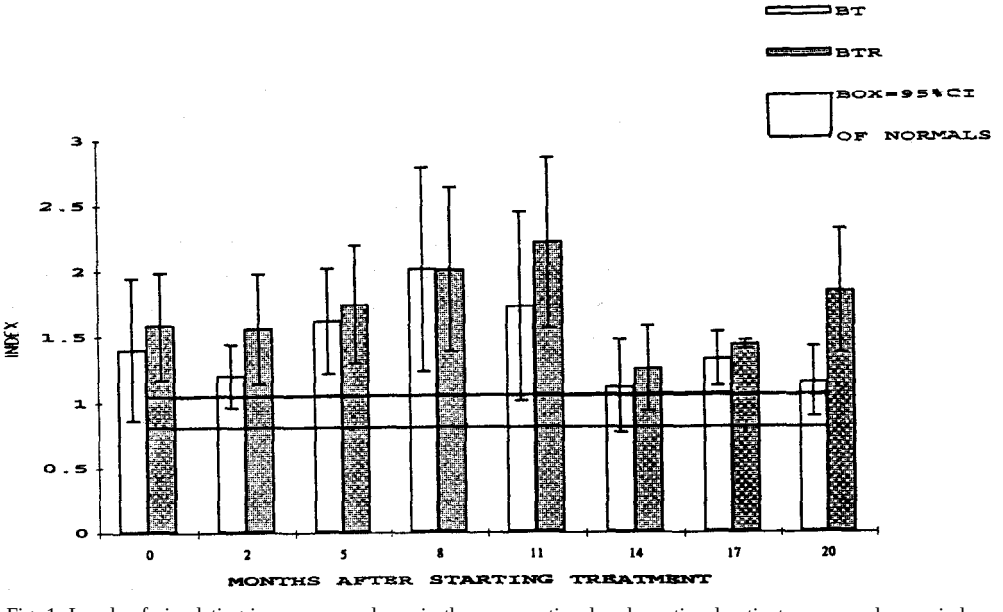

Fig. 1. Levels of circulating immune complexes in the non-reactional and reactional patients expressed as an index (Mean ± 95% CI). Horizontal box indicates the 95% CI of values seen in normal human serum.

COMPLEMENT

The mean C3d in NHS was 3.17 aU/ml (95% confidence interval: 0.99 to 5.35). The concentrations of C3d in both groups of patients (BT and BTR) were estimated only up to the fourteenth month. The levels of serum C3d (Table II) and CMS (Fig. 2) in the non -reactional BT patients were within normal limits. On the other hand, there were alterations in both C3d and CMS levels in BTR patients. Serum C3d which was elevated at the onset of reaction, was not significantly different from normal levels by the second month after the initiation of antileprosy treatment. However, the ability to solubilize ICs through the complement system consistently remained low compared to NHS.

Table II. Levels of C3d in patients expressed as aU/ml

Month	BT		BTR	
	Mean	95% CI	Mean	95% CI
0	5.63	3.15 - 8.11	16.4	11.13 - 21.67
2	4.81	1.94 - 7.72	10.8	0.79 - 20.87
5	6.41	2.49 - 10.33	15.0	0.14 - 30.14
8	2.49	0.87 - 4.11	4.3	0.29 - 8.87
11	3.64	2.32 - 4.96	14.3	0.71 - 37.21
14	10.50	0.24 - 21.24	6.0	0.11 - 11.89

95% CI = 95% confidence interval. Value in normal serum was 3.17 aU/ml. (95% CI = 0.99-5.35)

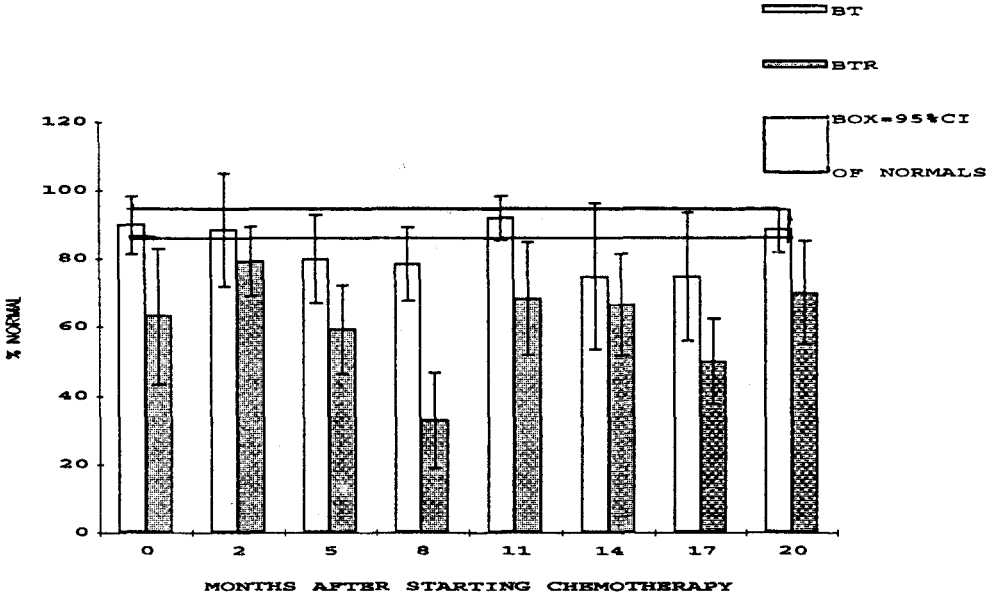

Fig. 2. Levels of complement-mediated immune complex solubilization in non-reactional and reactional patients. Results are expressed as percentage of solubilization seen in pooled normal human serum (mean ± 95% CI). Horizontal box indicates the 95% CI of values in normal human serum.

IMMUNOGLOBULINS

The levels of IgG which were significantly elevated to begin with, returned to normal levels from the eighth month onwards, in the non-reactional BT patients. In the BTR patients, they were elevated throughout the period of observation (Fig. 3).

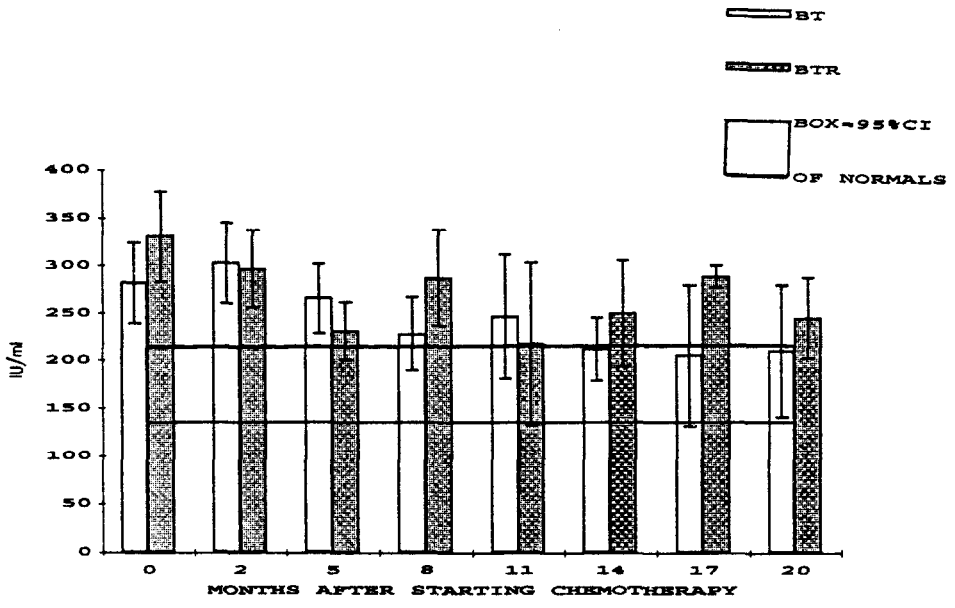

Fig. 3. Levels of immunoglobulin G in the non-reactional and reactional patients (Mean ± 95% CI). Horizontal box indicates the 95% CI of values seen in normal human serum.

The levels of immunoglobulins A and M were largely unaltered in both groups of patients and were comparable with those of NHS (data not shown).

DISCUSSION

This is a long-term follow-up study of serum immune complexes and complement activation in BT patients with and without reaction following treatment. The findings of the present study are in agreement with those made earlier that reactional states are seen only in a proportion of the leprosy patients and that elevated CICs and complement catabolic fragments are noted during such states (Saha et al 1982, Ramanathan et al 1984, 1985). Further, this is accompanied by a lowered capacity to solubilize preformed ICs. This reduced solubilization persists even after evidence for complement activation (indicated by increased C3d) was absent in the circulation (Fig. 2 and Table II). Although the mean levels of C3d in the BT patients at the fourteenth month and in the BTR patients at the eleventh month were high, they were not significantly different from that in the normal serum. As anti-reaction treatment with steroids was terminated three months after subsidence of reaction,

the persistently reduced CMS cannot be attributed to the effect of these drugs (Packard & Weiler 1983). Similarly, as these patients were treated with rifampicin and DDS only, the anticomplementary effect of clofazimine (Saha et al 1982) cannot be the reason for the persistant reduction of CMS.

Reversal (type 1) reactions in BT leprosy patients are conventionally attributed to an increase in delayed hypersensitivity response. However, there are reports of perturbation in the humoral immune system in patients undergoing reversal reactions. These mainly involve an increase in immunoglobulins and antibody levels (Lanjendijk et al 1983) increase in CIC and C3d and a depressed CMS (Ramanathan et al 1984, 1985, Chakrabarty et al 1988). Recently, it has been reported by Saha and his co-workers (1995) that anti-PGL antibodies of IgG class remain elevated even after the subsidence of reversal reaction. The findings presented in this study further confirm these reports.

When BT reactional lesions are examined histologically, lymphocytes, epithelioid cells, giant cells and plasma cells are seen. This would imply that cell-mediated hypersensitivity plays a major role in the genesis of reversal reactions. However, in view of the findings reported here and the presence of 20% B lymphocytes documented by Nilsen and his coworkers (1986) in nerve lesions in BT patients, it is clear that humoral mechanisms also play an important role in the causation of this type of reactions. Since a persistently reduced CMS appears to be a distinguishing feature of reactions in leprosy, the following explanation could account for the findings.

The immune complexes which are formed in situ are not cleared due to poor complement-mediated solubilization. It is known that unsolubilized complexes are capable of binding to various cellular receptors (Takahashi et al 1980, Law & Dodds 1997). It was noted in the present study that although the CIC levels were elevated in the non-reactional BT patients, since the CMS capacity remained intact it is likely that CICs had been fully solubilized and therefore were not pathogenic. The unsolubilized complexes in the reactional BT patients probably stimulate T cells which are responsible for delayed hypersensitivity. Although further proof is necessary for substantiating this hypothesis, it can be used as a working premise from which to proceed further to understand the pathogenesis of reversal reactions.

ACKNOWLEDGEMENT

We thank Dr VD Padmanabhan of Madras Veterinary College, Chennai 7 for providing normal bovine serum which was used for the preparation of conglutinin.

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