Clin. exp. Immunol. (1971) **8**, 901–909.

A STUDY OF CELL MEDIATED IMMUNITY IN LEPROSY: CHANGING TRENDS IN THE IMMUNOLOGICAL SPECTRUM OF THE DISEASE

KUNAL SAHA AND M. M. MITTAL

Department of Microbiology, G. B. Pant Hospital and Maulana Azad Medical College, Department of Medicine, Sir Ganga Ram Hospital, New Rajender Nagar, New Delhi

(*Received* 25 *November* 1970)

SUMMARY

Skin tests of delayed hypersensitivity were performed on thirty-eight patients of leprosy with a bacterial antigen (tuberculin), a hapten (dinitrochlorobenzene) and allogenic lymphocytic transplantation. The results have indicated that leprosy is associated with a generalized depression of delayed allergic response. The depression is of greater severity in patients with lepromatous leprosy and less among tuberculoid patients. The depression of cell mediated immunity in these cases is not absolute but is relative and depends upon the dose and potency of an antigen and the severity of the disease.

INTRODUCTION

The clinical characteristics of patients with lepromatous leprosy at one end of a spectrum and tuberculoid leprosy at the other, are well known. Studies of immunologic reactivity in these patients have also demonstrated diverse alterations in the two polar forms of the disease. The lepromatous type of disease goes virtually unchecked by the host with his macrophages laden with lepra bacilli, a high titre of serum antibody bathing the tissues and no reaction to bacilli or their products in his skin or elsewhere in contrast to the benign self-limited course of tuberculoid type, with few lepra bacilli detectable, little or no serum antibody and markedly positive delayed hypersensitivity to lepromin. The anergy to lepromin observed in cases of lepromatous leprosy is not due to the presence of 'anticutins', as has been postulated in the past, but is a manifestation of central immunological deficit consequent to an unspecified aberration of immunocompetent cells that may either cause or result from the disease itself. The latter explanation is supported by the observations that these patients had shown poor response to intradermal injections of bacterial and fungal antigens (Bullock, 1968; Guinto & Mabalay, 1962; Buck & Hasenclever, 1963; Guinto, 1963), had difficulty in achieving primary contact sensitization with haptens (Bullock, 1966; Waldorf *et al.*, 1966; Turk & Waters, 1969) and their lymphocytes had decreased ability to be transformed into blast cells in culture by phytohaemagglutinin, streptolysin O or specific

Correspondence: Dr Kunal Saha, Department of Microbiology, G. B. Pant Hospital, New Delhi, India.

bacterial antigens (Sheagren *et al.*, 1967, 1969; Paradisi, DeBonaparte & Moragenfeld, 1968; Dierks & Shepard, 1968).

The study of delayed hypersensitivity in leprosy has given variable but interesting results. Against the common belief, more and more evidence is accumulating to suggest that not only lepromatous cases but also a variable number of tuberculoid patients are associated with depression of delayed inflammatory allergic responses (Bullock, 1968). Furthermore Turk & Waters (1969) have recently shown that those cases of lepromatous leprosy which could not achieve primary sensitization with dinitrochlorobenzene, could be induced to show delayed hypersensitivity to keyhole-limpet haemocyanin, a more powerful antigen, thereby suggesting that depression of cell mediated immunity even in these cases is not absolute.

The purpose of this communication is to present the results of a study designed to examine the phenomenon of delayed immune mechanism by various skin testing techniques in lepromatous and tuberculoid leprosy. Apart from the tuberculin test and primary sensitization with dinitrochlorobenzene, normal lymphocyte transfer tests have also been studied, as the NLT test is a manifestation of natural state of allograft immunity. It has also been aimed to find out whether the depression of cell mediated immunity in a case is absolute against a specific antigen or is dose dependent. Further, on simultaneous analysis of all the three tests, a new trend in the immunological pattern of the disease appears to have evolved.

MATERIALS AND METHODS

The study consisted of forty-five individuals, of which thirty-eight suffered from leprosy and the remaining ones were normal persons. Two population samples of patients with leprosy were chosen for study; a lepromatous sample (twenty-four patients); and a tuberculoid sample (fourteen patients). Classification of leprosy type was based on clinical history, physical examination and skin biopsy. Patients with intermediate lesions, as defined by Ridley-Jopling classification (1962), were not included. All the control patients and patients with leprosy underwent X-ray examination of the chest and were screened carefully to exclude those in whom tuberculosis, usually poor dietary history, cachexia, prior BCG vaccination or steroid therapy, might have affected skin test procedures.

Normal lymphocyte transfer (NLT) tests were done in all cases. Separation of lymphocytes was done after the method described by Goldsmith (1965). The final suspension of the cells was made in dextran-ringer solution so as to give 2·5 million lymphocytes in 0·1 ml. Viability of lymphocytes was checked by trypan blue test. Each recipient was given 0·1 ml of dextran-ringer solution on the flexor surface of the left forearm (as a control) and 0·1 ml of suspension on the volar surface of the right forearm. The measurement of erythema and induration at each test site were made by a Glogau's vernier caliper and recorded immediately after the injection and daily thereafter for a period of at least 10 days. In each case measurement of diameters was done by taking two readings at right angles to each other and finding out the mean of the two.

Out of thirty-eight cases, twenty subjects were tested for tuberculin sensitivity. Initially the patients were injected with 10 u of old tuberculin in 0·1 ml amounts on the flexor surface of the forearm using 1 ml tuberculin syringe with a 26 gauge needle. If this preparation gave negative results (induration less than 6 mm) the subjects were tested with 100 u and finally with 1000 u when necessary. Each time reading was taken 48 hr after the injection.

Induction of contact type delayed hypersensitivity was performed with dinitrochlorobenzene (DNCB) in sixteen out of thirty-eight leprosy patients and in five normal individuals. An area on the volar surface of each forearm was cleared with acetone. 2000 μg of DNCB in 0·1 ml acetone was applied on the flexor surface of the right forearm with an area enclosed by a 2 cm diameter copper coin ring. At the same time, in order to determine preexisting sensitivity to the agent, a challenge dose of DNCB (100 and 50 μg DNCB in 0·1 ml acetone) was applied similarly to the areas on the left forearm. All the areas were then properly covered. The 2000 μg site was covered for 5 days and then left open to the air; The 100 μg and 50 μg sites were opened at 48 hr, then at 72 hr for signs of delayed hypersensitivity. 21 days after application of the 2000 μg sensitizing dose the patients were rechallenged by the application of 100 μg in 0·1 ml acetone to a site 2 cm in diameter on the forearm adjacent to the initial challenge site. The area was then covered for 48 hr and read at 48 and 72 hr. Patients were considered positive if erythema and/or induration and/or vesiculation with or without bullae and ulceration appeared. In case challenge with 100 μg did not induce hypersensitivity, rechallenge doses of 400 μg and 1000 μg in 0·1 ml acetone were applied in the same way as described before, at an interval of 3 months and the findings were noted as forementioned. In another five normal persons, after ruling out the pre-existing sensitivity to the agent, 100 μg, 200 μg, 400 μg, 500 μg, 750 μg, 1000 μg, 1500 μg and 2000 μg of DNCB in 0·1 ml acetone were applied simultaneously at a distance of 4 cm apart on the forearms in order to determine the minimum dose of the antigen producing the nonspecific reaction occurring within 48 hr of its application. No reaction to the antigen was observed up to the dose of 400 μg. The reaction appeared only in three cases with 500 μg and thereafter the intensity of the reaction increased invariably with the increasing dose of the antigen.

RESULTS

After intradermal injection of 2·5 million lymphocytes in normal individuals, two peaks of induration were observed: the initial peak appeared by 24 hr, reached its maximum height between 48 and 72 hr and regressed between 3 and 5 days; the second peak began to appear by the 3rd to 5th day, reached its maximum height between 6 and 8 days and disappeared by the 10th to 13th day. In all the cases the initial peak was associated with local pain, itching and/or erythema while no such phenomenon was observed with the second peak. Erythema appeared between 24 hr and disappeared by 72–96 hr but it was not universally present in all the cases. The diameters of induration of initial and secondary peaks varied respectively between 6–9 mm and 8–20 mm. No reaction to dextran-ringer solution was observed in any case.

The behaviour of NLT was not uniform in any type of leprosy (Table 1). Out of twenty-four cases of lepromatous variety, in eighteen cases (75%) no erythema or induration was observed at the site of injection within 24 hr and thereafter within a period of ten days, meaning thereby that NLT reaction was flat. In three cases (12·5%) only the first peak was observed. In these cases the reaction was depressed in intensity as well as in duration. Out of the remaining three cases in one case each (4·2%) the following reactions were observed: (1) normal NLT pattern with depressed reaction; (2) only a second peak; (3) the first and second peak merged with each other. Of the fourteen cases of tuberculoid leprosy the first peak only occurred in ten cases (71·5%). Out of these, in six cases (42·9%) the pattern of the

TABLE 1. NLT behaviour in leprosy cases*

Type of leprosy	Normal NLT behaviour (reaction depressed)	Flat NLT	First and second peak merged		First peak only			Second peak only
			Normal	Very much exaggerated	Normal	Depressed	Exaggerated	
Lepromatous (24 cases)	1 (4·2%)	18 (75%)	1 (4·1%)	—	—	3 (12·5%)	—	1 (4·2%)
Tuberculoid (14 cases)	1 (7·1%)	—	—	1 (7·1%)	6 (42·9%)	2 (14·3%)	2 (14·3%)	2 (14·3%)

* Lymphocyte transfer tests were done by injecting intradermally 2·5 million lymphocytes in 0·1 ml dextran-ringer solution. Observations were made daily for at least 10 days. In normal persons an initial reaction (first peak) of moderate induration and erythema reached its maximum diameter between 24 and 48 hr and a secondary reaction (second peak) occurred on the 3rd–5th day, reached its peak on the 6th–7th day and disappeared between the 10th and 13th day. The average induration of the first and secondary peaks in normals varied between 6–9 mm and 8–20 mm respectively. In flat NLT there was not observed any initial or secondary reaction.

peak was normal, in two cases (14·3%) it was depressed while in another two it was exaggerated. Of the remaining four cases, only the second peak was noticed in two cases (14·3%); a normal NLT pattern with depressed intensity occurred in one case (7·1%); and in the other case both the peaks merged into each other with exaggerated reaction.

Tuberculin sensitivity tests were done in fourteen cases of lepromatous and six cases of tuberculoid leprosy (Table 2). Only five cases of lepromatous variety reacted positively to 10 u of tuberculin. Of the remaining nine cases, two exhibited positive sensitivity when

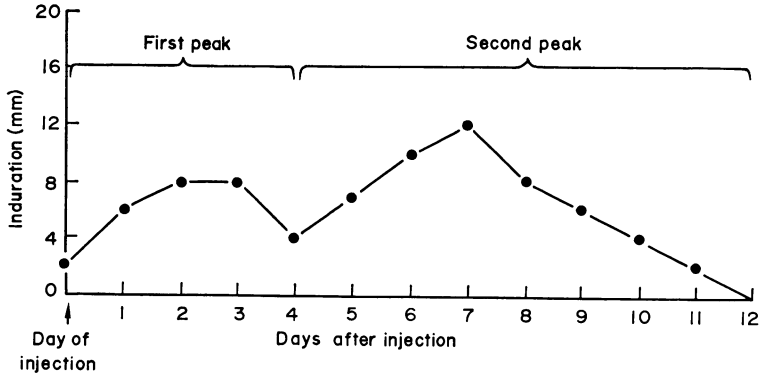

FIG. 1. The pattern observed after intradermal injection of 2·5 million allogenic lymphocytes in a normal individual.

TABLE 2. Results of tuberculin sensitivity tests

Type of leprosy	Result	Sensitivity tested with 10 u (14*+6† cases studied)	Sensitivity tested with 100 u (9*+1† cases studied)	Sensitivity tested with 1000 u (7* cases studied)
Lepromatous	+ve	5 (35·6%)	2 (22·2%)	5 (71·4%)
	−ve	9 (64·4%)	7 (77·8%)	2 (28·6%)
Tuberculoid	+ve	5 (83·3%)	1 (100%)	—
	−ve	1 (16·7%)	—	—

* Lepromatous. † Tuberculoid.

tested with 100 u and five more when challenged with 1000 u. Two of the lepromatous patients were negative to all the three doses. Of the six cases of tuberculoid leprosy five became tuberculin positive when tested with 10 u and the other one when challenged with 100 u.

Contact sensitivity to dinitrochlorobenzene was induced in ten cases of lepromatous and six cases of tuberculoid leprosy (Table 3). None of the cases exhibited evidence of pre-existing sensitivity. All the normal individuals and one case each of the tuberculoid and lepromatous variety were sensitive to challenge with 100 μg of DNCB. All the tuberculoid

TABLE 3. Results of sensitization with DNCB

Type of leprosy	Result	Challenging dose 100 μg (10* + 6† cases studied)	Challenging dose 400 μg (9* + 5† cases studied)	Challenging dose 1000 μg (9* + 5† cases studied)
Lepromatous (10 cases)	+ve	1 (10%)	6 (66%)	7 (77·7%)
	−ve	9 (90%)	3 (34%)	2 (22·3%)
Tuberculoid (6 cases)	+ve	1 (16·6%)	5 (100%)	5 (100%)
	−ve	5 (83·4%)	—	—

* Lepromatous. † Tuberculoid.

cases and six of the nine lepromatous patients reacted to 400 μg. When all these patients were challenged with 1000 μg, only one more lepromatous patient reacted positively in addition to all those who had already exhibited sensitivity with 400 μg. The intensity of reaction observed with 1000 μg was invariably higher than that observed with 400 μg.

DISCUSSION

A preliminary study of normal lymphocyte transfer tests has already been reported from this laboratory (Kunal Saha & Mittal, 1970). The present communication is the continuation of the same study. The behaviour of allogenic lymphocytic graft in both the types of leprosy has been variable without any definite pattern. In most of the cases of lepromatous leprosy the NLT reaction has been flat. Out of the remaining cases the NLT pattern either has been normal or there have been observed first or second peak only or both the peaks combined. In tuberculoid leprosy in most of the subjects only the first peak was noticed. In the remaining patients, the patterns observed were a normal NLT reaction, the second peak only or both the peaks combined. In none of the tuberculoid patients was the NLT pattern flat. These findings suggest that a variable number of cases of both lepromatous and tuberculoid leprosy are associated with depression of cell mediated immunity. The frequency and severity of depression is much more common in lepromatous than that in tuberculoid variety. Furthermore the intensity of depression of delayed hypersensitivity in lepromatous cases may approach a state of anergy as is observed in cases of thymic aplasia (Lischner, Dacou & DiGeorge, 1967) or in animals pretreated with antilymphocytic sera (Levey & Medawar, 1966). Similar impressions have been expressed by Bullock (1968) who studied allergic inflammatory responses in leprosy by skin testing techniques. The findings are further supported by the observations of Dierks & Shepard (1968) who found markedly depressed lymphocytic response to PHA and mycobacterial agents in lepromatous patients while the response was moderately depressed in tuberculoid patients. The variable patterns of NLT observed in these cases also throw some light on the immunogenesis of NLT. Brent & Medawar (1963) speculated that in guinea-pigs the first reaction observed after NLT test is a graft versus host reaction. The appearance of only the first peak in most of the cases of tuberculoid leprosy and disappearance of even this peak in most of the cases of lepromatous leprosy, suggest the possibility of host component in eliciting the first peak response of NLT. The appearance of various components of NLT in different stages of the disease further suggests that leprosy patients might also act as models for the study of genesis of NLT.

Skin sensitivity tests done with tuberculin and induction of primary contact sensitization

with DNCB have shown that most of the lepromatous patients and all tuberculoid patients have shown positive sensitivity when higher doses of the same antigen is used. Similar observations have been made by Bullock (1968). The findings not only confirm the impressions of Turk & Waters (1969), who reported that more potent antigenic stimulation can induce delayed hypersensitivity in cases of leprosy who could not be sensitized by a comparatively weaker antigen, but also suggest that the higher dose of the same antigen can induce the delayed immune mechanism on most occasions in the same individual who was anergic to the small dose of the respective antigen. The forementioned findings indicate that the depression of cell mediated immunity in cases of leprosy is not absolute but is relative, and depends upon dose of the antigenic stimulation and severity of the disease. It further confirms the impressions of Leiker (1968) who reported that the majority of lepromatous patients also show a very weak response to lepromin of slightly more than usual strengths. In many patients, a stronger reaction is seen after the use of more concentrated lepromin. A minority of patients with severe diffuse type of lepromatous leprosy are truly anergic to lepromin.

In sixteen patients comprising ten cases of lepromatous and six cases of tuberculoid variety all three tests were undertaken. Analysis of various findings has resulted in the following general pattern: (i) flat NLT has been observed in most of the lepromatous leprosy patients (90%) and in none of the tuberculoid leprosy patients, (ii) 40% of lepromatous leprosy cases do not respond to 100 u of old tuberculin in contrast to all the cases of tuberculoid leprosy, (iii) 30% of lepromatous leprosy individuals do not develop hypersensitivity on being challenged with 400 μg of DNCB while all the six tuberculoid patients have responded to the similar challenge. These findings indicate that transplantation antigen on the lymphocytes is a weaker antigen in comparison to both tuberculin and DNCB. Thus 90% of patients with lepromatous leprosy do not exhibit signs of delayed hypersensitivity when tested with 2·5 million lymphocytes in contrast to 40% and 30% cases when challenged with 100 u of old tuberculin and 400 μg of DNCB respectively. In only one case did both tuberculin and DNCB tests give negative skin reactions when tested with 100 u of old tuberculin and 400 μg of DNCB where the NLT test has been positive; thus pointing out that an absolute immunological pattern may not be observed even in histologically well classified cases. Again, when the results of challenge with 100 u of tuberculin and 400 μg of DNCB are compared, it appears that the latter agent is stronger than the former as only three patients have not responded to this assault in contrast to four patients with 100 u of tuberculin. These observations indicate that not only the dose of antigen and immunological status of patients but also the affinity of antigen–antibody system in the elicitation of delayed inflammatory allergic response (Karush & Eisen, 1962) might play an equally important role. Dierks & Shepard (1968) studied the effect of phytohaemagglutinin and various mycobacterial antigens on the lymphocyte culture from leprosy patients and normal individuals and found that patients with active lepromatous disease had a very low lymphoblastic response while those with inactive lepromatous or tuberculoid form had intermediate responses. If the impressions of these authors are analysed in the light of the present study, the investigators are tempted to postulate that the 'stimulus' caused by an antigen, which depends on its dose and affinity for ligand; and the number of 'healthy' immunological competent cells, which depends upon the immunological state of the disease (active, inactive or progressive form of the disease with or without bacteriology positivity), are responsible for the expression of cell mediated immunity. Thus if a weak stimulus has

not been sufficient to attract the necessary 'number' of 'healthy' immunocompetent cells out of the total lymphocyte population crowded at the test site, then in the same case a stronger 'stimulus' may attract the required number of immunologically competent cells necessary for the expression of delayed allergic response, explaining thereby why a bigger dose of an antigen or a higher potency antigen could produce a positive skin test in a case in which a lesser dose of the same antigen or a lower potency antigen has failed to induce it.

In the last, the authors like to mention the discrepancy observed in the results of skin tests with old tuberculin and DNCB when the findings are analysed on the basis of arithmetical logic. Out of sixteen patients with leprosy, 63% have exhibited hypersensitivity on being tested with 10 u of old tuberculin while only 12% have developed the delayed reactions when challenged with 100 μg DNCB. On using ten times more dose of tuberculin and only four times more dose of DNCB the maximum number of cases should have developed positive reactions with 100 u of old tuberculin instead of 400 μg of DNCB. In fact this has not been so and the reverse has been true.

ACKNOWLEDGMENTS

We are grateful to Dr S. N. Chaudhuri, Professor of Biochemistry, Maulana Azad Medical College, for his valuable suggestions and criticism and also to Dr P. B. Majumdar, Medical Superintendent, G. B. Pant Hospital, for his kind help and encouragement during the period of study.

REFERENCES

BRENT, L. & MEDAWAR, P.B. (1963) Tissue transplantation: A new approach to the 'Typing' problem. *Brit. med. J.* **ii**, 269.

BUCK, A.A. & HASENCLEVER, H.F. (1963) Influence of leprosy on delayed-type skin reactions and serum agglutination titers to Candida albicans: Comparative study of patients with lepromatous and tuberculoid leprosy and controls in Ethiopia. *Amer. J. Hyg.* **77**, 305.

BULLOCK, W.E. (1966) Depression of the delayed-type allergic response by leprosy. *Clin. Res.* **14**, 337.

BULLOCK, W.E. (1968) Studies of immune mechanisms in leprosy. *New Engl. J. Med.* **278**, 298.

DIERKS, R.E. & SHEPARD, C.C. (1968) Effect of phytohemagglutinin and various mycobacterial antigens on lymphocyte culture from leprosy patients. *Proc. Soc. exp. Biol.* **127**, 391.

GOLDSMITH, K.L.G. (1965) Donor selection and compatibility typing. *Brit. med. Bull.* **21**, 162.

GUINTO, R.S. (1963) Skin reactions of patients with lepromatous and tuberculoid leprosy and of normal controls to non-mycobacterial antigen (*Candida albicans*). *Annual Report, Leonard Wood Memorial*, **3**.

GUINTO, R.S. & MABALAY, M.C. (1962) Note on tuberculin reaction in Leprosy. *Int. J. Leprosy*, **30**, 278.

KARUSH, F. & EISEN, H.N. (1962) A theory of delayed hypersensitivity. *Science*, **136**, 1032.

KUNAL SAHA & MITTAL, M.M. (1970) Lymphocyte transfer tests in leprosy. *Clin. exp. Immunol.* **6**, 971.

LEIKER, D.L. (1968) Intradermal tests with mycobacterial substances and normal tissue suspensions. *Int. J. Leprosy*, **36**, 52.

LEVEY, R.H. & MEDAWAR, P.B. (1966) Some experiments on action of antilymphocytic sera. *Ann. N.Y. Acad. Sci.* **129**, 1, 164.

LISCHNER, H.W., DACOU, C. & DIGEORGE, A.M. (1967) Normal lymphocyte transfer test: Negative response in a patient with congenital absence of Thymus. *Transplantation*, **5**, 555.

PARADISI, E.P., DEBONAPARTE, Y.P. & MORAGENFELD, M.C. (1968) Blasts in lepromatous leprosy. *Lancet*, **i**, 308.

RIDLEY, D.S. & JOPLING, W.H. (1962) Classification of leprosy for research purposes. *Leprosy Rev.* **33**, 119.

SHEAGREN, J.N., BLOCK, J.B., TRAUTMAN, J.R. & WOLFF, S.M. (1967) Immunologic reactivity in leprosy. *Clin. Res.* **15**, 300.

SHEAGREN, J.N., BLOCK, J.B., TRAUTMAN, J.R. & WOLFF, S.M. (1969) Immunologic reactivity in patients with leprosy. *Ann. intern. Med.* **70**, 295.

TURK, J.L. & WATERS, M.F.R. (1969) Cell mediated immunity in patients with leprosy. *Lancet*, **ii**, 243.

WALDORF, D.S., SHEAGREN, J.N., TRAUTMAN, J.R. & BLOCK, J.B. (1966) Impaired delayed hypersensitivity in patients with lepromatous leprosy. *Lancet*, **ii**, 773.