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Defect in the generation of cytotoxic T cells in lepromatous leprosy

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SUMMARY

Cytotoxic T cells are consistently produced in normal individuals after *in vitro* stimulation by a pool of mitomycin-treated normal lymphocytes. Patients suffering from lepromatous leprosy (LL), presenting with large amounts of *Mycobacterium leprae* and without a history of erythema nodosum leprosum (ENL) are unable to generate such cytotoxic T cells, while lepromatous patients with ENL which, in the present study were all deprived of *M. leprae*, react normally.

INTRODUCTION

The immunological status of patients suffering from leprosy has been the subject of considerable investigation. Converging arguments indicate the existence of various humoral and cellular abnormalities. The question remains, however, of the primary or secondary nature of these immunological changes. A primary immunodeficiency could predispose the patient to develop leprosy and could modulate its clinical appearances (tuberculoid or lepromatous). Conversely, the prolonged presence of *Mycobacterium leprae* could induce significant changes in the immune system.

Previous immunological studies of cell-mediated immunity in leprosy have essentially been addressed to delayed hypersensitivity reactions, mitogen- and lepromin-induced proliferative responses and B and T lymphocyte markers (E rosettes, surface immunoglobulin). Variable results have been reported in lepromatous leprosy. Some authors (Convit, Pinardi & Rojas, 1971; Rea *et al.*, 1976; Ulrich, De Salas & Convit, 1972) reported no impairment of immunological responses to antigens other than depression of delayed skin reactions to lepromin. Others observed a more general defect in T cell-mediated immunity including decrease of T cell number, assessed by the E rosette test (Dwyer, Bullock & Fields, 1973; Nath *et al.*, 1974), depression of delayed hypersensitivity reactions to various antigens not related to lepromin (Bullock, 1968; Turk & Bryceson 1971), of mitogen- and antigen-induced proliferation (Bullock & Fasal, 1971; Sheagren, Block & Trautman, 1969) and lymphokine production (Katz, De Betz & Zaras, 1971; Talwar, Krishman & Mehra, 1972). Finally, other authors have found dissociated results with abnormalities of only some of the parameters listed above (Nelson *et al.*, 1971; Salazar-Mallen, Zamora & Montes, 1971; Sheagren *et al.*, 1969; Waldorf, Sheagren & Trautman, 1966). While difficulties remain, the presence of a general impairment in T cell-mediated immunity in lepromatous leprosy is the most widely accepted view (Bullock, 1979; Godal *et al.*, 1974; Kantor, 1975; Shepard, 1968).

We have investigated T cell function in lepromatous leprosy (LL) by use of the cell-mediated

Abbreviations: CML = Cell-mediated lympholysis; ENL = Erythema nodosum leprosum; LL = Lepromatous leprosy; LU = Lytic units.

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lympholysis (CML) assay which evaluates the capacity of T cells to generate cytotoxic T cells against allogeneic cells.

Lepromatous patients have been divided into two groups. Patients of the first group had no antecedent history of erythema nodosum leprosum (ENL) and presented with numerous intact bacilli. Those of the second group had presented with ENL in the recent past. Such patients had all received chemotherapy before immunological evaluation. They presented with fewer bacilli which appeared as granular. This clinical distinction was retrospectively justified by the differences observed in CML assays: patients of the first but not of the second group showed clear CML deficiency.

MATERIALS AND METHODS

Patients and control subjects. Twenty-six African patients with LL were investigated. These patients were followed-up at the 'Institut d'Hygiène Sociale' in Dakar, Sénégal. All patients presented with a lepromatous nodular form of the disease. They were all classified after examination of the skin biopsy in the LL form, as defined in Ridley–Jopling classification (Ridley & Jopling, 1966; Ridley & Waters, 1969). Clinical features of those patients are presented in Table 1.

Normal controls were African soldiers of the Senegalese Army, without known disease at the time of the test. The age spectrum and ethnic origins were not statistically different in patients and control groups.

Patients with LL were selected randomly from the clinics. Results obtained in these patients will be presented under two headings:

(1) Patients having presented with ENL (12 cases). Such patients had already been treated for a period of time longer than 10 months. Mycobacteria were not found in their nasal fluid but were sometimes present in limited numbers in skin biopsies (with a strong predominance of the granular form). Such biopsies showed hisiocyte infiltrations and the presence of lymphocytes. All of these patients had already presented one or several ENL episodes. Three of them presented with ENL at the time of the test. Eight were tested in the polyallogeneic system; four in the xenogeneic system.

(2) Patients without ENL (14 cases). Such patients had usually been treated for a shorter period, and often in a less regular fashion. In one case, chemotherapy had been totally ineffective. Three recently diagnosed patients had never received any anti-leprosy treatment. The majority of patients in this group had *M. leprae* in their nasal fluid and skin biopsies, often in large amounts and always with solid appearance. The cellular infiltrate included a majority of histiocytes and Virchow cells. Lymphocytes were rare or absent. Eleven were tested in the polyallogeneic system, and three in the xenogeneic system.

Allogeneic CML assay. Stimulating cells were obtained from a pool of five or six normal subjects, mixed in equal numbers. Cells were isolated as already described (Charpentier, Carnaud & Bach, 1979) and immediately treated with 50 μg/ml mitomycin C (Sigma, St Louis, Missouri) for 45 min at 37°C (10^7 cells/ml). The cells were then washed three times in the culture medium and the number of viable cells (60–90%) evaluated.

Responding cells were prepared as described above. Each experiment included a CML assay performed on a normal subject whose lymphoid cells were treated under identical conditions to those of the leprosy patients.

The culture medium was RPMI 1640 (Gibco, New York) containing 100 international units/ml penicillin, 100 μg/ml streptomycin, 2mM glutamine, 1% tryptose soy broth and 20% normal human plasma. Normal plasma was prepared from a pool of 10 normal plasma samples, that had been thawed, mixed, decomplemented by heating for 30 min at 56°C, and lastly centrifuged for 30 min at 30,000 ***g***.

The mixing of responding cells (20×10^6) and stimulated cells (20×10^6) was perfomed in 20 ml culture medium. The cell suspension thus obtained was placed in Falcon 3013 flasks which were incubated in a 5% CO_2 incubator at 37°C for 6 days. At the end of the incubation period, cells were pipetted, washed in Hanks medium and resuspended in RPMI 1640 (Gibco) containing penicillin, streptomycin and glutamine, as described above, but with 10% fetal calf serum (FCS) instead of human plasma. Cell recovery varied between 50% and 150% of initial values.

Table 1. Lepromatous patients under study

Patient	Age	Duration of treatment	Treatment	*M. Leprae* Skin	*M. Leprae* Nasal Fluid	ENL History	ENL Present	Total LU ($\times 10^{-3}$)
ENL Group								
1	26	unknown	DDS Rifamycin	0	0	+	0	486
2	49	5 years	DDS Rifamycin	0	0	++++	0	1250
3	34	12 years	DDS Rifamycin	0	0	++++	0	2560
4	16	2 years	DDS Rifamycin	0	0	+	0	333
5	69	10 years	DDS Rifamycin Clofazimine	0	0	+++	0	848
6	17	6 years	DDS Clofazime	Numerous granulous	0	+	+	110
7	30	2 years	DDS	0	0	+	+	324
8	28	5 years	DDS	0	0	+	+	168
Group without ENL								
9	38	10 years	DDS	0	0	0	0	1
10	29	2 years irregular	DDS	++	0	0	0	23
11	69	10 years	DDS but resistant	+++	+	0	0	1
12	14	6 months irregular	Rifamycine Suphamethoxy pyridazine	+++	+	0	0	34
13	29	0	0	+++	+	0	0	29
14	43	2 months	DDS		+	0	0	18
15	66	15 days	Rifamycin	+++	+++	0	0	64 (1st test) 1 (2nd test)
16	20	0	0	+++	+++	0	0	38
17	46	0	0		++	0	0	1
18	65	5 years	DDS	+	+	0	0	1
19	53	12 years irregular	DDS			0	0	1

The cytotoxicity assay was perfomed by adding the cultured cells to concanavalin A-stimulated lymphoblasts (Con A-blasts) derived from the donors of stimulating cells.

Two million mononucleated cells from each donor were cultured for 6 days ($2{\cdot}5 \times 10^5$ cells/ml) in the culture medium described above (with 10% FCS) to which was added 2·5 μg/ml Con A (Sigma). Cells were washed three times in Hanks medium, enumerated and resuspended in the same medium as effector cells. Con A-blasts from each donor were then mixed in equal proportion and adjusted to 5×10^6 total cells (in 0·1 ml medium).

Such cells were then radiolabelled by incubating them for 1 hr at 37°C in 5% Co_2 with 100 μCi of ^{51}Cr (sodium chromate, C.E.A., sp. act. 162 mCi/ml). The cells were washed three times in RPMI 1640 containing 10% FCS and resuspended in the culture medium. Lastly, they were distributed in

Table 2. Xenogeneic cytotoxicity

	Exp.1	Exp.2	Exp.3	Exp.4	Exp.5
Normal controls	28%	13%	12%	13%	13%
Lepromatous patients with ENL	17%		14%	29%	49%
Lepromatous patients without ENL	0%	0%	—	—	10%

% of cytotoxicity $\left(\frac{\text{effector}}{\text{target cell}} = 40/1\right)$.

micro tissue culture plates (Cooke Engineering) (10^4 cells per well in 0·1 ml). Graded amounts of effector cells were added (in 0·1 ml) in effector cell:target cell ratios ranging from 100:1 to 1:1.

Spontaneous ^{51}Cr release by target cells was determined in wells containing medium alone (0–1 ml). Maximum release was evaluated by addition of 0·1 ml 10% detergent (RBS 25, Traitement Chimique des Surfaces, Lille, France).

The microplates were incubated at 37°C in 5% CO_2 for 4–6 hr, at the end of which 0·1 ml supernatant was collected by means of an automatic pipette (Gilson).

Radioactivity was measured by liquid scintillation by use of the technique described by Herscowitch & McKillipe (1974) which enables one to detect ^{51}Cr radioactivity in a sensitive way by use of a beta counter; 0·1 ml of supernatant was added to 1·9 ml Lumagel (Kontron), agitated and placed into 3 ml plastic flasks (Kontron). Such flasks were themselves placed in glass scintillation vials. Radioactivity was evaluated in a Kontron scintillation counter. Cytotoxic index was defined as follows:

$$\frac{\text{educated lymphocytes c.p.m.} - \text{spontaneous c.p.m.}}{\text{maximum c.p.m.} - \text{spontaneous c.p.m.}} \times 100.$$

Each well was studied in triplicate. The c.p.m. values are the average of the three experimental determinations.

The calculation of lytic units (LU) was performed as described by Cerottini & Brunner (1972).

Xenogeneic cytotoxicity. The xenogeneic cytotoxicity assay was performed as described by Carnaud *et al.* (1977). Stimulating cells were spleen cells from DBA/2 mice (C.E.A.L., Orléans la Source, France). Such mouse cells were treated with 50 μg/ml mitomycin for 45 min at 37°C. Human–mouse mixed lymphocyte cultures were performed by adding 20×10^6 responding cells to 20×10^6 stimulating cells. The cell mixture was placed for 6 days in a 5% CO_2 incubator at 37°C in 3013 Falcon Flasks in the vertical position. Target cells were P-815 mastocytoma cells with the same $H\text{-}2^d$ haplotype as DBA/2 stimulating cells. ^{51}Cr labelling and the cytotoxicity assay were performed as described above for the allogeneic assay.

RESULTS

Allogeneic cytotoxicity

The use of a pool of four to five different donors (polyallogeneic cytotoxicity) was tested in preliminary studies in normal subjects. At variance with 'monoallogeneic' CML assays, where fortuitous HLA identity (or other factors) may lead to negative responses in spite of normal level of immunity, consistently good responses were obtained in the polyallogeneic system (cytotoxicity index over 40% at the highest lymphocyte:target cell ratios, LU over 85×10^3). In each of

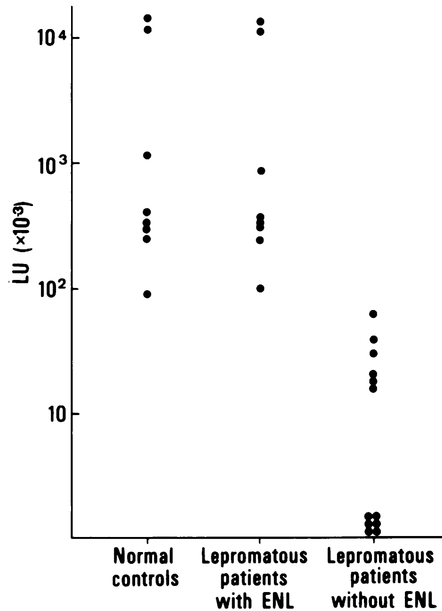

Fig. 1. Polyallogeneic cell-mediated lympholysis in lepromatous patients (lytic units per 10^3 cells).

subsequent experiments with lepromatous patients, a normal control was included and only the result of such controls studied simultaneously with lepromatous patients will be presented. The results presented in Figs 1 & 2 show that patients with ENL generate cytotoxic T cells with an efficiency comparable to normal subjects (no significant difference). Conversely, patients without ENL and with the highest number of *M. leprae* show a highly statistically significant deficiency in the generation of cytotoxic T cells. No cytotoxic T cells are detected in five of these patients at any effector: target cell ratio. This abnormality is detectable whether results are expressed in cytotoxic indices or lytic units but is even more clearcut using lytic units. One may hypothesize that the few patients with depressed LU but normal cytotoxicity indices have a reduced number of cytotoxic cells, but that such cells have normal individual cytotoxic capacity.

Xenogeneic cytotoxicity

Seven patients have been tested in the xenogeneic CML assay using mouse cells. The results,

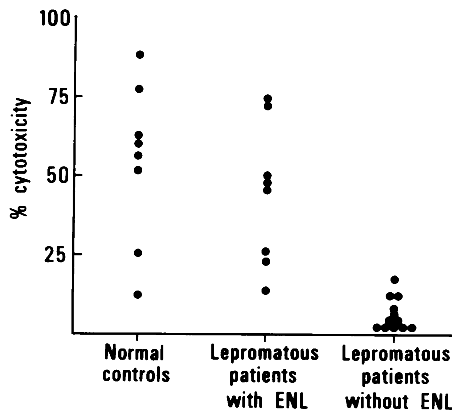

Fig. 2. Polyallogeneic cell-mediated lympholysis in lepromatous patients (cytotoxicity index).

presented in Table 2, are similar to those just presented for allogeneic cytotoxicity. The four patients with ENL generate cytotoxic T cells normally, whereas the three patients with large amounts of *M. leprae* (without ENL) are clearly deficient in the CML assay.

DISCUSSION

The data presented in this article show that some leprosy patients show a clear deficiency in the generation of cytotoxic T cells after exposure to allogeneic or xenogeneic lymphocytes. This deficiency had not yet been reported in human leprosy, but had already been noted in another disease due to intracellular parasites: trypanosomiasis (Pearson *et al.*, 1978; Roelants *et al.*, 1979). This deficiency is observed in patients presenting with large amounts of bacilli in nasal fluid and skin and no history of ENL. It is not observed in those lepromatous patients with history of ENL. Some tuberculoid patients tested (results not shown) behave as normal subjects.

The difference in immunological status between the two groups of leptomatous patients had not been noted previously by any author. One report by Lim *et al.* (1975) showed normal proliferative responses to PHA in ENL patients contrasting with depressed responses of patients without ENL. Rea, who previously reported no impairment in cellular immunity of lepromatous patients (Rea *et al.*, 1976) found recently a significant difference in delayed hypersensitivity to DNCB between 'non-reactional' and 'reactional' (including ENL) lepromatous patients (Rea & Levan, 1976).

In our work, the separation in 'ENL group' and 'group without ENL' may be questionable because of the many differences between the two groups. In particular, the patients of the group without ENL are in majority heavily loaded with *M. leprae*, while the patients of the ENL group are generally free of *M. laprae*. However, the bacterial load does not appear as a determining element since some patients in the group without ENL have only few bacilli in their lesions and still are deficient in the generation of CTL. In our view, bacterial load and non-reactional state act synergistically. A lepromatous patient is deficient in the generation of CTL. When treated, *M. leprae* disappear in lesions and progressively a recovery of immune functions takes place. The time of recovery is drastically shortened if ENL occurs. ENL is usually considered to represent an Arthus-like phenomenon, due to immune complex (IC) deposition in the vessels. Our results could suggest that ENL is associated with T cell-mediated immunity. Alternatively, the normal capacity of generating cytotoxic T cells as well as increased antibody and IC production in ENL patients could both be linked to a decreased level of suppressor T cells. Such interpretation is corroborated by the recent observation of decreased numbers of suppressor T cells evaluated by use of monoclonal anti-T cell antibodies in lepromatous patients with ENL (Bach *et al.*, 1980). In any case, it will be interesting in the future to follow-up CML capacity in lepromatous patients in order to determine whether normalization of CML precedes or follows the onset of ENL. The effect of anti-leprosy chemotherapy should also be evaluated, particularly since in this series ENL patients had been treated for a longer time than the other patients.

The mechanism of CML depression in lepromatous patients without ENL is still essentially unknown. Several hypotheses may be put forward.

(1) A decrease in the precursors of cytotoxic T cells could be involved. The normal percentage of the 'suppressor–cytotoxic' T cell subset evaluated by monoclonal antibodies (Bach *et al.*, 1980) in patients without ENL does not exclude this hypothesis since cytotoxic T cell precursors may only represent a minority of this subset and, in any case, T cell phenotypes were not determined in the patients studied in this work. Alternatively, cytotoxic T cell precursors could be present in normal number but functionally abnormal (unable to differentiate normally into effector cells).

(2) An abnormality in regulatory cells could also intervene (excess of suppressor T cells and macrophages, or decrease of helper T cells) as already suggested by Saha *et al.* (1979) in human LL using another experimental system. In particular, leprosy T cells might not produce the T cell growth factor (TCGF) normally known to mediate part or all the T cell help in CML. Phenotypic studies already mentioned (Bach *et al.*, 1980) do not argue in favour of this hypothesis but again they do not provide absolute information and cells may be only functionally abnormal. One should note, in favour of a suppressor cell excess, that suppressor cells are observed in murine leprosy

(Bullock, Carlson & Gershon, 1978; Rojas-Espinosa, Mendez-Casuluengo & Villanueva, 1976; Waison, Slijivic & Brown, 1975) and that certain bacteria such as *Corynebacterium parvum* (Kwok-Choy, Kay & Wong, 1979; Murahata & Zigelboim, 1979) and BCG (Klimpel & Henney, 1978) can stimulate suppressor cells as might *M. leprae*.

(3) An abnormality of T cell circulation may be conceived. It has been shown in murine leprosy that T lymphocytes may be trapped in the spleen and in lymph nodes of *M. Lepraemurium* infected mice (Bullock, 1976a; 1976b). The circulating pool of the various T cell subsets contributing to the CML reaction could be thus selectively depleted.

(4) A deficiency of leprosy macrophages could be incriminated since the involvement of macrophages (or monocytes) in the CML reaction is well established (Davidson, 1977). Conversely, macrophages might also intervene as suppressor cells, as described in murine leprosy (Waison *et al.*, 1975).

(5) The role of serum inhibiting factors is unlikely since incubation of normal lymphocytes with lepromatous sera inconsistently inhibits the generation cytotoxic T cells (data not shown).

(6) A direct inhibitory effect of chemotherapy is also unlikely. It has been reported (Beiguelman & Pisani, 1978; Sengupta *et al.*, 1979) that 4–4′ diamino-diphenyl-sulphone (DDS) may despress some parameters of cell-mediated immunity such as PHA-induced proliferative responses. It does not seem, however, that DDS or other drugs play a major role since several patients, not taking DDS (and in some cases any other drug) also show a CML defect. In addition, all patients with ENL showed normal CML under DDS–clofazimine treatment.

The relevance of the CML abnormality, described in this work, to the pathogenesis of LL is uncertain. The role of T cells in the elimination *M. leprae* is likely, probably through lymphokine production and macrophage activation. The contribution of cytotoxic T cells (as studied in the CML assay) is more dubious but could be envisaged. In other words, it is difficult to determine whether the deficiency of generation of cytotoxic T cells is part of the disease pathogenesis or a hallmark of the underlying T cell abnormality as are depressed PHA responses or delayed skin graft rejection (Bullock, Evans & Filameno, 1977; Han, Weiser & Kau, 1971), itself perhaps a direct consequence of the cytotoxic T cell abnormality. These concepts are reminiscent of those already discussed in a number of autoimmune diseases, for example in lupus, where selective CML depression has also been observed (Charpentier, *et al.*, 1979).

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REFERENCES

BACH, M.A., CHATENOUD, L., WALLACH, D., PHAN DINH TUY, F. & COTTENOT, F. (1980) Studies on T cell subsets and functions in leprosy. *Clin. exp. Immunol.* **44,** 491.

BEIGUELMAN, B. & PISANI, R.C.B. (1978) Effect of DDS on phytohemagglutinin-induced lymphocyte transformation. *Int. J. Lepr.* **42,** 412.

BULLOCK, W.E. (1968) Studies of immune mechanisms in leprosy. I. Depression of delayed allergic responses to skin test antigens. *N. Engl. J. Med.* **278,** 298.

BULLOCK, W.E. (1976a) Perturbation of lymphocyte circulation in experimental murine leprosy. I. Description of the defect. *J. Immunol.* **117,** 1164.

BULLOCK, W.E. (1976b) Perturbation of lymphocyte circulation in experimental murine leprosy. II. Nature of the defect. *J. Immunol.* **117,** 1171.

BULLOCK, W.E. (1979) Immunology and the therapeutics of leprosy. *Ann. Int. Med.* **91,** 482.

BULLOCK, W.E. & FASAL, P.F. (1971) Studies of immune mechanisms in leprosy. III. The role of cellular and humoral factors in impairment of the *in vitro* immune responses. *J. Immunol.* **106,** 888.

BULLOCK, W.E., CARLSON, E.M. & GERSHON, R.K. (1978) The evolution of immunosuppressive cell populations in experimental mycobacterial infection. *J. Immunol.* **120,** 1709.

BULLOCK, W.E., EVANS, P.E. & FILAMENO, A.R. (1977) Impairment of cell-mediated immune responses by infection with *Mycobacterium lepraemurium. Infect. Immunol.* **18,** 157.

CARNAUD, C., FADAI-GHOTBY, M., LESAVRE, P. & BACH, J.F. (1977) Education of human lymphocytes against mouse cells: specific recognition of H-2 antigens. *Eur. J. Immunol.* **7,** 81.

CEROTTINI, J.C. & BRUNNER, K.T. (1972) *In vitro* assay of target cell lysis by sensitized lymphocytes. In *In Vitro Methods in Cell-mediated Immunity* (ed. by B.R. Bloom and P.R. Glade,) Academic Press, London.

CHARPENTIER, B., CARNAUD, C. & BACH, J.F. (1979) Selective depression of the xenogeneic cell-

mediated lympholysis in systemic lupus erythematosus. *J. clin. Invest.* **64,** 351.

CONVIT, J., PINARDI, M.E. & ROJAS, F.A. (1971) Some considerations regarding the immunology of leprosy. *Int. J. Lepr.* **39,** 556.

DAVIDSON, W.F. (1977) Cellular requirements for the induction of cytotoxic T cells *in vitro*. *Immunol. Rev.* **35,** 111.

DWYER, J.M., BULLOCK, W.E. & FIELDS, J.P. (1973) Disturbance of the blood T/B lymphocyte ratio in lepromatous leprosy: clinical and immunologic correlations. *N. Engl. J. Med.* **288,** 1036.

GODAL, T., MYRVANG, B. STANFORD, J.L. & SAMUEL, D.R. (1974) Recent advances in the immunology of leprosy with special reference to new approaches in immunoprophylaxis. *Bull. Inst. Pasteur,* **72,** 273.

HAN, S.H., WEISER, R.S. & KAU, S.T. (1971) Prolonged survival of skin allografts in leprosy patients. Int. J. Lepr. **39,** 1.

HERSCOWITCH, H.B. & MCKILLIP, T.W. (1974) A simple method for liquid scintillation counting of ^{125}Iodine and ^{51}Chromium used in antigen binding and cytotoxicity studies. *J. Immunol. Meth.* **4,** 253.

KANTOR, F.S. (1975) Infection, anergy and cell-mediated immunity *N. Engl. J. Med.* **292,** 629.

KATZ, S.E., DE BETZ, B.H. & ZARAS, N. (1971) Production of macrophage inhibitory factor by patients with leprosy. *Arch. Dermatol.* **103,** 358.

KLIMPEL, G.R. & HENNEY, C.S. (1978) BCG-induced suppressor cells. I. Demonstration of a macrophage-like suppressor cell that inhibits cytotoxic T cell generation *in vitro*. *J. Immunol.* **2,** 563.

KWOK-CHOY, L., KAY, J. & WONG, M. (1979) Separation of functionally distinct subpopulations of *Corynebacterium parvum* activated macrophages with predominantly stimulatory or suppressive effect on cell-mediated cytotoxic T cell response. *Cell. Immunol.* **42,** 28.

LIM, S.D., JACOBSON, R.R., PARK, B.H. & GOOD, R.A. (1975) Leprosy XII. Quantitative analysis of thymus-derived lymphocyte response to phytohemagglutinin in leprosy. *Int. J. Lepr.* **43,** 95.

MURAHATA, R. & ZIGELBOIM, J. (1979) Inhibition of memory cell-s mediated cytotoxic T cell response by systemic administration of *Corynebacterium parvum*. *Cell. Immunol.* **42,** 289.

NATH, I., CURTIS, J., BHUTANI, L.K. & TALWAR, G.P. (1974) Reduction of subpopulation of T lymphocytes in lepromatous leprosy. *Clin. exp. Immunol.* **18,** 81.

NELSON, D.S., NELSON, M., THURSTON, J.M., WATERS, M.F.R. & PEARSON, J.M.E. (1971) Hamagglutinin-induced lymphocyte transformation in leprosy. *Clin. exp. Immunol.* **9,** 33.

PEARSON, T.W., ROELANTS, G.E. LUNDIN, L.B. & MAYOR-WITHEY, K.S. (1978) Immune depression in trypanosome-infected mice. I. *Eur. J. Immunol.* **8,** 723.

REA, T.H. & LEVAN, N.E. (1976) Variations in DNCB responsivity in untreated leprosy: evidence of a beneficial role of anergy. *Int. J. Lepr.* **48,** 120.

REA, T.H., QUISMORIO, F.P., HARDING, B., NIES, K.M., DI SAIA, LEVAN, N.E. & FRIOU, G.J. (1976) Immunologic responses in patients with lepromatous leprosy. *Arch. Dermatol.* **112,** 791.

RIDLEY, D.S. & JOPLING, W.H. (1966) Classification of leprosy according to immunity. A five-group system. *Int. J. Lepr.* **34,** 255.

RIDLEY, D.S. & WALTERS, M.F.R. (1969) Significance of variations within the lepromatous group. *Lepr. Rev.* **40,** 143.

ROELANTS, G.E., PEARSON, T.W. TYRER, H.W. MAYOR-WITHEY, K.S. & LUNDIN, L.B. (1979) Immune depression of trypanosome infected mice. II. Characterization of the spleen cell types involved. *Eur. J. Immunol.* **9,** 195.

ROJAS-ESPINOSA, O., MENDEZ-CASOLUENGO, M. & VILLANUEVA, G. (1976) Antibody-mediated immunity in CFW mice infected with *Mycobacterium lepraemurium*. *Clin. exp. immunol.* **25,** 281.

SAHA, K., WHITTINGHAM, S., RAY, D., MITTAL, M.M. & BEOHAR, P.C. (1979) Impairment of Jones-Mote hypersensitivity and specific antibody response against depolymerized flagellin in lepromatous leprosy. *Scand. J. Immunol.* **10,** 31.

SALAZAR-MALLEN, M., ZAMORA, A.C. & MONTES, J.M. (1971) Estudios sober la immunidad de la lepra. Alergia, **18,** 185.

SENGUPTA, U., GHEI, S.K., VENKATESAN, K. & BHARADWAG, V.P. (1979) *In vivo* effect of DDS on phytohemagglutinin (PHA) Induced lymphocyte transformation cultures in normal healthy volunteers. *Int. J. Lepr.* **47,** 167.

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

SHEPARD, C.C. (1968) Immunologic suppression in leprosy and relation to lepromatous disease. Editorial. *Int. J. Lepr.* **36,** 87.

TALWAR, G.P., KRISHMAN, A.D. & MEHRA, V.L. (1972) Evaluation of cell-mediated immune responses in untreated cases of leprosy. *Clin. exp. Immunol.* **12,** 195.

TURK, J.L. & BRYCESON, A.D.M. (1971) Immunological phenomena in leprosy and related diseases. *Adv. Immunol.* **13,** 209.

ULRICH, M., DE SALAS, B. & CONVIT, J. (1972) Lymphocyte transformation with mitogens in leprosy. *Int. J. Lepr.* **40,** 4.

WAISON, S.R., SLIJIVIC, V.S. & BROWN, I.N. (1975) Defect of macrophage function in the antibody response to SRBC in systemic *Mycobacterium Lepraemurium* infection. *Nature,* **26,** 206.

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