Clin. exp. Immunol. (1982) **49**, 105–114.

Aberrant immunoregulatory control of B lymphocyte function in lepromatous leprosy

W. E. BULLOCK, SUSAN WATSON, K. E. NELSON, VICTORIA SCHAUF, S. MAKONKAWKEYOON & R. R. JACOBSON *Department of Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio; Departments of Preventive Medicine and Pediatrics, University of Illinois School of Medicine, Chicago, Illinois; Chiang Mai University, Chiang Mai, Thailand and The United States Public Health Service Hospital, Carville, Louisiana*

(Accepted for publication 12 February 1982)

SUMMARY

The capacity of peripheral blood mononuclear (PBM) cells from patients with leprosy to generate immunoglobulin-secreting cells in response to pokeweed mitogen (PWM) was evaluated by a reverse haemolytic plaque forming cell (PFC) assay. The PFC responses of PBM cells from patients with lepromatous (Lpr) leprosy were significantly higher ($P < 0{\cdot}01$) than those of PBM cells from normal controls and patients with tuberculoid leprosy. Co-culture of T lymphocytes from normal donors with PBM cells from Lpr patients reduced the PFC response of these cells to the normal range. $T4^+$-helper lymphocytes from Lpr donors did not induce supranormal responses to PWM by normal PBM cells enriched for B lymphocytes. $T8^+$-suppressor lymphocytes from normal donors greatly reduced the response of cultures containing normal allogeneic B cells plus $T4^+$ cells. Conversely, when $T8^+$ cells from Lpr donors were cocultured with normal B cells plus $T4^+$ cells, they failed to suppress the response to PWM. In summary, these studies have demonstrated abnormally high PWM-stimulated PFC responses by B lymphocytes from patients with Lpr leprosy. This aberration, in turn, is associated with a loss of regulatory function by $T8^+$-suppressor cells in Lpr patients.

INTRODUCTION

The lepromatous (Lpr) form of leprosy is associated with polyclonal hypergammaglobulinaemia and an increased incidence of autoantibody formation (Turk & Bryceson, 1971; Bullock, 1978). Patients with Lpr leprosy also are reported to have higher isohaemagglutinin titres and higher serum agglutinin titres against *Candida albicans* than either those with tuberculoid (Tbd) leprosy or normal controls (Buck & Hasenclever, 1963). In addition, the antibody response of Lpr patients to immunization with extrinsic antigens at least equals, and may exceed, the responses of healthy controls (Almeida, Brandao & de Lima, 1964; Jha *et al.*, 1971). Moreover, recent studies have demonstrated that there is a profound reduction of the concanavalin A (Con A)-inducible T suppressor lymphocyte activity in patients with Lpr leprosy (Nath *et al.*, 1979; Artz, Jacobson & Bullock, 1980). Thus, there is increasing evidence that T cell-mediated immunoregulatory control over B cell function may be abnormal in these patients.

In the present study we have explored the issue of T cell control over B cell function in Lpr patients by employing a reverse haemolytic plaque forming cell (PFC) assay both to measure the

Correspondence: Ward E. Bullock, MD, Division of Infectious Diseases, Department of Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267, USA.

0009-9104/82/0700-0105$02.00

PFC response by peripheral blood mononuclear (PBM) cells from Lpr patients and controls and to study the regulatory function of T cell subsets.

MATERIALS AND METHODS

Patients and controls. Patients were classified by standard clinical and histological criteria (Ridley & Jopling, 1966) as having Lpr leprosy ($n=9$) or borderline Tbd leprosy ($n=4$). The mean age was $39{\cdot}2 \pm 4{\cdot}9$ years and nine of the 13 were males; all but one were residents of the United States. All were free of other serious disease and erythema nodosum leprosum and none had received corticosteroid drugs. Four of the nine Lpr patients were untreated at the time of study. Three were in the earliest phase of treatment having received anti-leprosy drugs for less than 17 days; two patients had been treated for more than 3 months. Two patients with Tbd disease had received dapsone for 2 months and the others for 4 and 6 months respectively. The mean age of the healthy control group (nine males and three females) was $37{\cdot}6 \pm 4{\cdot}6$ years.

Cell preparation, culture, and PFC assay. PBM cells were obtained from patients and controls for paired study after informed consent, separated on Ficoll-Hypaque (Litton Bionetics, Kensington, Maryland, USA), and washed twice in balanced salt solution (BSS). The cells were cultured for 6–7 days at a final concentration of 10^6/ml in RPMI 1640 medium (GIBCO, New York, USA) supplemented with 0·1% gentamicin, 10% heat-inactivated fetal calf serum (FCS, MA Bioproducts, Walkersville, Maryland) and 0·1 ml of a 1/10 dilution of a stock PWM (GIBCO) solution. Cells cultured without PWM served as a control. At the end of the culture period, the number of PWM-induced immunoglobulin (Ig) secreting cells was assayed by the reverse haemolytic plaque assay technique of Fauci, Whalen & Burch (1980). FPC were counted under a dissecting microscope and the data expressed as PFC/10^6 cells in culture or as PFC/10^6 B cells.

Preparation of B cell enriched populations. B cell enriched preparations were obtained from PBM cells by treatment with anti-T3 monoclonal antibody (Ortho Pharmaceutical Corporation, Raritan, New York) and Low-Tox® rabbit complement (Cedarlane Laboratories Ltd., Hornby, Ontario, Canada). Anti-T3 antibody binds to all human peripheral T lymphocytes (Reinherz & Schlossman, 1980). Cells were mixed with anti-T3 antibody such that for every 10^6 cells in 100 μl of RPMI 1640 medium containing 5% FCS, 5 μg of antibody was present. They were incubated at 4°C for 30 min, washed twice with medium and complement added at a final dilution of 1/10. The cells were incubated for an additional 30 min at 37°C, washed once in RPMI 1640 medium, centrifuged through a FCS 'cushion' to remove effete cells and the entire procedure repeated. The cell preparations were checked for residual T cells by indirect immunofluorescence using T3 antiserum followed by fluorescein isothiocyanate (FITC)-conjugated IgG fraction anti-mouse IgG (MeLoy Laboratories, Springfield, Virginia, USA) and counting 200 cells under a Zeiss fluorescence microscope. In all cases $<4\%$ of the cells were T cells. B cells ranged from 75 to 90% by direct immunofluorescence staining with $F(ab')_2$ FITC-labelled anti-human Igs (Cappel Laboratories Inc., Cochranville, Pennsylvania, USA).

Preparation of $T4^+$ and $T8^+$ T cell subpopulations. PBM cells were enriched for T cells by passage through nylon wool and then treated with either anti-T4 or anti-T8 monoclonal antibody (Ortho Pharmaceutical) such that 10^6 cells in 100 μl of medium were exposed to 5 μg of antibody. The anti-T4 and -T8 antibodies recognize distinct T lymphocyte subpopulations that mediate helper or suppressor functions respectively (Reinherz & Schlossman, 1980). The procedure for preparing $T4^+$ or $T8^+$ lymphocyte subpopulations by lysis of unwanted cells with either anti-T4 or anti-T8 antibody plus complement was the same as that used to prepare B cell enriched populations. By indirect immunofluorescence, the $T4^+$ lymphocyte preparation contained $>95\%$ $T4^+$ cells and $<2\%$ $T8^+$ cells; the $T8^+$ cell preparation contained $>92\%$ $T8^+$ cells and $<2\%$ $T4^+$ cells.

B cells and T cell subsets in blood. One million PBM cells in 100 μl of medium with 0·1% sodium azide were incubated with either 5 μg of anti-T3, anti-T4, anti-T8 antibody, or a 1/10 dilution of FITC-labelled anti-human Ig $F(ab')_2$ for 30 min at 4°C and washed twice with azide-containing medium at 4°C. For determination of T cells and T cell subsets, the cells were further incubated with a 1/40 dilution of FITC-labelled IgG fraction anti-mouse IgG for 30 min at 4°C and again washed

twice. Fluorescent cells were counted using the fluorescence microscope. Blood samples from Tbd patients were insufficient for study.

Statistical methods. Differences between the PFC response of various groups were analysed by the Mann-Whitney test using a two-sided alternative. Differences in absolute counts of blood lymphocyte populations were evaluated by the *t*-test.

RESULTS

PWM-stimulated PFC responses (Fig. 1)

The PWM-induced PFC responses by PBM cells from patients with Lpr leprosy (median 33,000 PFC/10^6 cells in culture) were significantly greater ($P < 0{\cdot}01$) than those mounted by cells from healthy controls (median 7,100 PFC/10^6 cells) and by cells from four patients with Tbd leprosy (median 6,625 PFC/10^6 cells). On the other hand, when PBM cells from Lpr patients were cultured for 6 days in the absence of PWM, the responses (median 215 PFC/10^6 cells) were similar to those of unstimulated cell cultures from normal donors (median 195 PFC/10^6 cells).

Kinetics of PFC responses in vitro *(Fig. 2)*

In additional experiments, PFC assays were performed after PBM cells from Lpr patients and controls had been cultured with PWM for 3, 5 and 7 days, respectively. After 3 days in culture, cells from normal donors typically displayed little or no PWM-driven Ig biosynthesis. Conversely, cells from each of four Lpr patients tested mounted responses ranging from 1,520 to 5,010 PFC/10^6 cells. On day 5 of culture, PFC responses by PBM cells from Lpr patients greatly exceeded those of normal cells as they did also on day 7, at which time peak responses were mounted by cells from both normal and Lpr donors; by day 10 the PFC response of cells from either healthy or Lpr donors was decreased considerably (data not shown).

Effect of cell number on PFC response (Fig. 3)

Normal PBM cells achieved optimum PFC responses to PWM stimulation in concentrations ranging from 5×10^5 to 2×10^6 cells/1 ml of culture. However, at a concentration of 2×10^6 cells/1 ml of culture, the PFC response of cells from Lpr donors was decreased in four of five cases (note

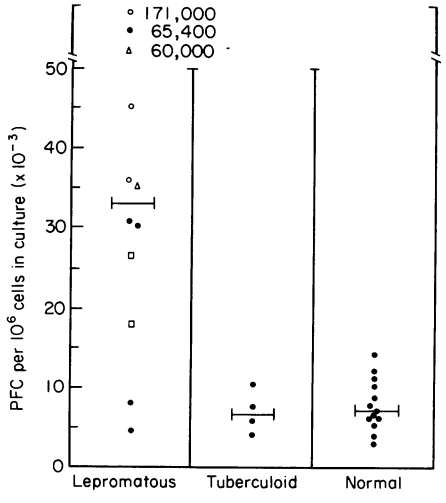

Fig. 1. PWM-stimulated PFC responses of PBM cells from patients with Lpr ($n = 9$) or Tbd ($n = 4$) leprosy and healthy controls ($n = 13$). Two assays, separated by an interval of at least four weeks, were performed on three Lpr patients; in each case the results of both assays are indicated by the paired symbols ○, △, and □, respectively. Horizontal bars indicate the median PFC response for each group.

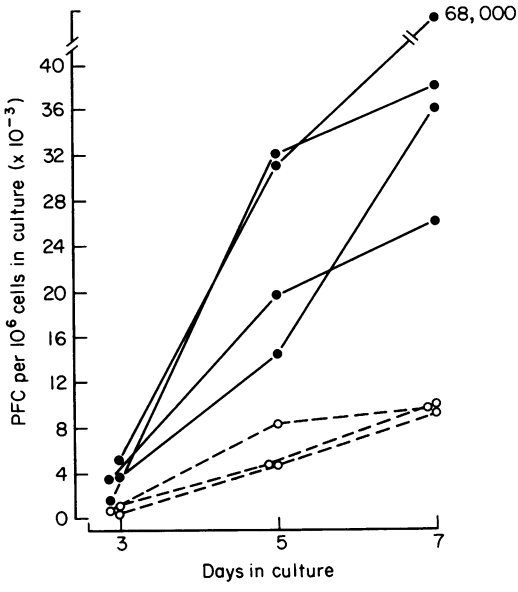

Fig. 2. PFC responses by PBM cells from normal (○) and Lpr donors (●) after 3, 4 and 7 days of stimulation by PWM *in vitro*.

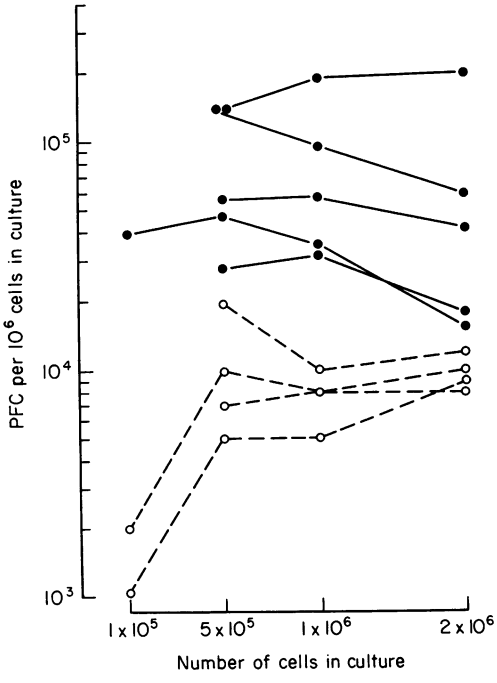

Fig. 3. Effect of cell number on the PFC response of PBM cells from normal (○) and Lpr donors (●) to PWM-stimulation.

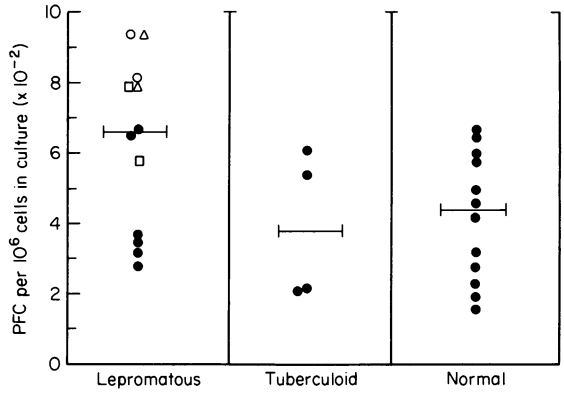

Fig. 4. Day 0 spontaneous PFC activity of cells from patients with Lpr or Tbd leprosy and healthy controls. Two assays, separated by an interval of at least 4 weeks, were performed on three Lpr patients; in each case the results of both assays are indicated by the paired symbols ○, △, and □, respectively. Horizontal bars indicate the median PFC response for each group.

logarithmic scale in Fig. 3). Thus, the Lpr cells appear to have been more susceptible to the adverse effect of cell crowding.

Day 0 spontaneous PFC (Fig. 4)

The observation of high PFC responses to PWM by PBM cells from Lpr patients as early as the 3rd day of culture prompted further investigations to determine if the blood of these individuals contained large numbers of B lymphocytes spontaneously secreting Ig's on day 0. PBM cells were separated over a Ficoll-Hypaque gradient, washed twice, and incubated for 2 hr at 37°C in medium to elute cytophilic Ig. The cells were then assayed for spontaneous Ig secreting PFC by reverse haemolytic plaque assay. The numbers of Ig secreting PFC on day 0 in nine Lpr patients (median 665 PFC/10^6 cells, range 280–940) were not significantly higher ($P > 0{\cdot}1$) than the spontaneous PFC in healthy controls (median 440, range 160–670/10^6 cells).

B cells and T cell subsets in peripheral blood

As shown in Table 1, the mean absolute lymphocyte count in the blood of Lpr patients was lower

Table 1. Numbers of lymphocytes and lymphocyte subsets* in peripheral blood of patients and normal controls

Cell type	Cell donor group (cell count/mm^3) Lepromatous ($n=8$)	Normal ($n=11$)
Total lymphocytes	1,688±253†	2,092±353†
B lymphocytes	209± 47 (12·4)‡	167± 40 (8·0)‡
T lymphocytes ($T3^+$)	796±113 (47·2)§	1,463±286 (69·9)
T helper lymphocytes ($T4^+$)	517± 60 (30·6)§	1,018±178 (48·7)
T suppressor lymphocytes ($T8^+$)	280± 50 (16·6)§	580±121 (27·7)

* See methods section for techniques of quantitation.

† Mean ±s.e.m.

‡ Figures in parentheses indicate percentage of total lymphocyte count.

§ Difference from the normal group is significant at $P < 0{\cdot}01$.

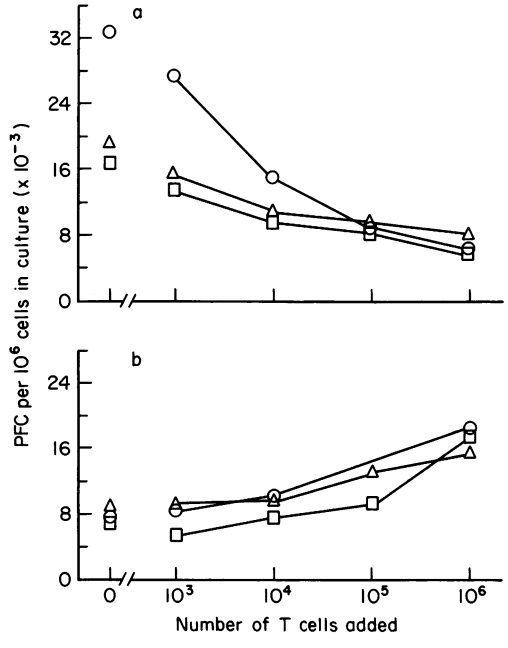

Fig. 5. (a) Summary of three experiments measuring the effect of normal allogeneic T cells on the PWM-stimulated PFC response of PBM cells from Lpr patients. (b) Effect of T cells from Lpr donors on PFC responses of normal PBM cells. In each experiment from 10^3 to 10^6 T cells were co-cultured with 10^6 PBM cells. PFC responses by control cultures containing 2×10^6 PBM cells alone are indicated by separate symbols. The s.e.m. for all points in (a) was 1,176 and 765 in (b) as calculated by randomized block analysis.

than that of healthy controls whereas the count of B lymphocytes was somewhat higher than normal. The differences however were not significant ($P > 0{\cdot}2$). On the other hand, the mean number of T lymphocytes ($T3^+$) in Lpr blood samples was significantly lower ($P < 0{\cdot}01$) than normal as were both the number of cells in the $T4^+$-helper subpopulation ($P < 0{\cdot}01$) and the $T8^+$-suppressor subpopulation ($P < 0{\cdot}01$).

Effect of normal and Lpr T cells on PFC responses (Fig. 5)

To determine if T cell immunoregulation of B cell function might be aberrant in Lpr patients, graded numbers of T cells from normal or Lpr donors were cultured with 10^6 allogeneic PBM cells from Lpr patients or normal controls. Co-culture of normal T cells with normal PBM cells neither augmented nor decreased the PFC response as compared with the responses of PBM cells alone (data not shown). In contrast, the PWM-stimulated PFC responses of Lpr PBM cells were reduced by culture with as few as 10^3 normal T cells (Fig. 5a). Moreover, 10^5 normal T cells consistently reduced the response of Lpr cells to the normal range. In the converse experiments (Fig. 5b) co-culture of either 10^3, 10^4 or 10^5 Lpr T cells with normal PBM cells exerted little effect upon the PFC response to PWM; at a concentration of 10^6/culture, Lpr T cells appeared to augment the responses of normal PBM cells. These findings therefore indicate that low concentrations of normal allogeneic T cells provided suppressor immunoregulatory control over Lpr PBM cells *in vitro*, whereas similar concentrations of T cells from Lpr donors did not provide an extraordinary degree of helper activity to normal PBL.

Effect of $T4^+$ and $T8^+$ lymphocyte subpopulations on PFC responses

In an attempt to differentiate between a possible deficiency of $T8^+$ suppressor cell function on the one hand and hyperfunction of $T4^+$ helper cells on the other in Lpr patients, $T4^+$ or $T8^+$ cell subpopulations from normals or patients were co-cultured with B enriched cells from normal

Table 2. Effect of normal or lepromatous $T4^+$ and $T8^+$ cells on the PWM-stimulated response by allogeneic B cells

Cells in culture	PFC/10^6 B cells in culture*		
	Experiment 1	Experiment 2	Experiment 3
(A) 10^6 B cell			
Normal	205 ± 5	100 ± 12	210 ± 34
Lepromatous	360 ± 40	80 ± 19	300 ± 16
(B) 10^6 Normal B cells			
+ 10^4 normal $T4^+$ cells	26,500 ± 2,500	18,950 ± 500	37,000 ± 4,000
+ 10^5 normal $T4^+$ cells	29,050 ± 950	23,500 ± 500	ND†
+ 10^6 normal $T4^+$ cells	29,200 ± 3,810	ND	ND
(C) 10^6 Lpr B cells			
+ 10^4 normal $T4^+$ cells	27,950 ± 3,960	20,950 ± 7,050	ND
+ 10^5 normal $T4^+$ cells	40,400 ± 601	24,500 ± 1,500	ND
+ 10^6 normal $T4^+$ cells	29,550 ± 450	ND	ND
(D) 10^6 Normal B cells			
+ 10^4 Lpr $T4^+$ cells	29,200 ± 800	16,300 ± 1,300	32,000 ± 7,000
+ 10^5 Lpr $T4^+$ cells	32,250 ± 2,250	19,750 ± 2,500	ND
+ 10^6 Lpr $T4^+$ cells	28,000 ± 1,000	ND	30,600 ± 600
(E) 10^6 Normal B cells + 10^4 normal $T4^+$ cells			
+ 10^4 normal $T8^+$ cells	20,450 ± 1,450	19,450 ± 550	30,950 ± 7,050
+ 10^5 normal $T8^+$ cells	10,450 ± 450	11,500 ± 500	ND
+ 10^6 normal $T8^+$ cells	8,750 ± 250	5,700 ± 700	16,400 ± 1,600
(F) 10^6 Normal B cells + 10^4 normal $T4^+$ cells			
+ 10^4 Lpr $T8^+$ cells	29,900 ± 4,914	20,700 ± 700	36,350 ± 3,350
+ 10^5 Lpr $T8^+$ cells	31,450 ± 552	22,450 ± 500	31,850 ± 850
+ 10^6 Lpr $T8^+$ cells	31,950 ± 2,056	23,700 ± 700	35,500 ± 3,500

* Mean ± s.e.m.; † ND = Not done.

allogeneic donors (Table 2). Cultures containing only a B cell enriched population from either normal or Lpr donors, produced few plaques (A). However, co-culture of normal allogeneic $T4^+$ cells with 10^6 normal B enriched cells resulted in a vigorous PFC response to PWM (B). Co-culture of normal $T4^+$ cells with 10^6 B enriched lymphocytes from the Lpr donors also resulted in PFC responses equivalent to the controls (C). Moreover, co-culture of Lpr $T4^+$ cells with normal B enriched cells did not drive the response to supranormal levels under the experimental conditions employed (D).

In Table 2, E and F, are summarized the results obtained when $T8^+$ cells from normal donors or from Lpr patients were co-cultured with a mixture of 10^6 B enriched cells and 10^4 $T4^+$ cells from normal allogeneic donors. As the number of normal $T8^+$ cells in co-culture was increased from 10^4 to 10^6, the PFC response was suppressed markedly. Conversely, however, all concentrations of $T8^+$ cells from Lpr patients failed to suppress the response of the normal B cell plus $T4^+$ cell mixture. It should be noted that the results of these studies performed with co-cultures of allogeneic cells do not differ from those obtained with entirely syngeneic cell mixtures; $T8^+$ cells from Lpr donors did not suppress the PFC response of an autologous B cell plus $T4^+$ cell mixture (Bullock & Watson, unpublished observations).

DISCUSSION

Heretofore, it has been generally assumed that the polyclonal hypergammaglobulinaemia and variable degrees of autoantibody formation observed in Lpr leprosy are stimulated non-specifically

by the chronic presence of *M. leprae* in host tissues. However, the 'adjuvant' hypothesis clearly is overly simplistic as demonstrated by the present finding of abnormal T cell immunoregulatory control over B cell function in a sample of patients with Lpr leprosy. Thus, the number of PBM cells from Lpr donors that respond to PWM stimulation *in vitro* by Ig secretion was much higher than normal despite the fact that the absolute numbers of B cells in the peripheral blood were not significantly elevated. Moreover, the abnormally high Ig-secretory response by these cells could be controlled easily by co-culture with relatively small numbers of normal T lymphocytes. Of even greater significance was the finding that PBM cells from Lpr patients bearing the $T8^+$ surface antigen were incapable of functioning in the suppressor mode under the experimental conditions employed.

The reason for the failure of $T8^+$ cells from Lpr donors to exert normal suppressor activity is unknown but currently is under investigation. Conceivably, the functional deficit could stem from the loss of an auxiliary cell population *in vivo* that may be necessary to permit expression of efferent-acting $T8^+$ suppressor cells. For example, macrophages provide auxiliary cell function and clearly can potentiate the activity of suppressor T cells (Stobo, 1977). In Lpr patients, whose tissues contain extensive granulomatous infiltrates composed predominantly of macrophages, a subpopulation of macrophages necessary for T suppressor cell activation may be sequestered within inflammatory foci or altered functionally by interaction with *M. leprae*. Subsequent diminution of macrophage auxiliary function therefore might limit development of the functional properties of T cells that nevertheless may be capable of expressing T8 cell surface antigens. Indeed, Yachie *et al.* (1981) have shown that $T8^+$ cells in human cord blood, although phenotypically identical to adult $T8^+$ cells, differ functionally by exerting less suppressor activity on B cell differentiation than do adult cells.

It seems unlikely that Lpr B cells themselves were defective in their response to T suppressor cell signals since the high PWM-driven PFC responses of these cells were easily controlled by co-culture with normal T cells.

It should be noted that although the present study provides strong evidence against the thesis that the elevated responses to PWM stimulation by B cells from Lpr patients were secondary to hyperactive function by a $T4^+$ helper cell population, we can not exclude this possibility entirely. Thus, within the co-culture system that we employed (Table 2), it is conceivable that B cells could have achieved a maximum threshold of response thereby precluding detection of supranormal helper activity.

The loss of suppressor T cell function in Lpr patients bears some similarity to the suppressor dysfunction that has been observed in active systemic lupus erythematosus (SLE) (Bresnihan & Jasin, 1977). In SLE Morimoto, Reinherz & Schlossman (1980) have reported that the absolute number of $T8^+$ cells in the blood of patients is decreased, commonly in association with the presence of serum antibodies that are selectively cytotoxic for these cells (Morimoto *et al.*, 1979). Thus, actual destruction of suppressor cells may contribute to the immunoregulatory dysfunction associated with SLE.

In Lpr patients on the other hand, we have not observed a selective reduction of $T8^+$ cells in the peripheral blood; instead, the $T8^+$ and $T4^+$ cells appear to be reduced proportionately. This evidence plus the failure of $T8^+$ cells in high concentration to overcome the deficient suppressor function *in vitro*, both indicate that this abnormality did not result from *in vivo* destruction of $T8^+$ cells by cytotoxic antibody. It seems more likely that the sera of Lpr patients may contain non-cytotoxic factors that could abrogate T suppressor cell function. Indeed, precedent for this hypothesis is provided by other workers who have demonstrated autoantibodies of the IgG class in the sera of SLE patients that interfere with suppressor cell activity by a noncytotoxic mechanism (Twomey, Laughter & Steinberg, 1977; Sagawa & Abdou, 1979). Currently, our laboratory is assaying Lpr sera to determine if autoantibodies reactive to $T8^+$ suppressor cells, in fact, are present.

It is of interest that the day 0, spontaneous Ig secretory activity of PBM cells from SLE patients is often higher than that of normal cells (Blaese, Grayson & Steinberg, 1980). If these cells are then stimulated *in vitro* by PWM for 7 days, the PFC response is below that of normal cells (Fauci *et al.*, 1978). Thus, B cells from SLE patients may already be 'preactivated' *in vivo*, perhaps secondary to

loss of suppressor cell activity. In contrast to these findings in SLE, PBM cells from Lpr patients did not display significant elevations of spontaneous PFC activity on day 0. Therefore, the degree of suppressor cell dysfunction among Lpr patients appears to be less severe. Hence, the abnormality of immunoregulation is best demonstrated in the reverse haemolytic plaque assay system under conditions of intense blastogenic stimulation by a potent mitogen.

The present study is not necessarily in conflict with the findings of Mehra *et al.* (1980) who reported that exposure of PBM cells from Lpr patients to a preparation of *Dharmendra lepromin* induced a T cell subset to mediate non-specific suppression of the blastogenic response to Con A by allogeneic PBM cells from normal donors. Although we have demonstrated a non-specific diminution of suppressor regulatory function by lymphocytes bearing the T8 surface antigen, nevertheless, it is possible that the non-specific suppressor activity reported by Mehra *et al.* (1980) may be mediated by a small subpopulation of T cells that can be triggered to suppressor activity by exposure to the antigens of *M. leprae in vitro*.

In contrast with the present report, many of the Lpr patients studied by Mehra *et al.* (1980) had received substantial amounts of anti-leprosy therapy. Therefore, at the time of the study, the PBM cells of these patients may have recovered the capacity to mediate suppressor function similar in intensity to that which can be induced in PBM cells from healthy donors and patients with tuberculoid leprosy by *in vitro* exposure to mycobacterial antigens (Bjune, 1979; Touw, Stoner & Belehu, 1980; Bahr, Rook & Stanford, 1981).

The demonstration of aberrant suppressor T cell function in Lpr leprosy reveals yet another facet of the complex disturbances of immunity associated with the extraordinarily chronic interactions between *M. leprae* and the human host. It remains to be determined how consistently this defect will be observed among patients with the Lpr forms of leprosy and whether it is primary or secondary to the pathophysiological mechanisms of the disease. Underscoring the need for answers to these questions is the observation that the PWM-stimulated PFC response of cells from two Lpr patients were normal in the present study; one patient had received anti-leprosy therapy for more than 1 year and the other was an untreated, 21 year old male. Clearly, it will be necessary to conduct longitudinal studies of the PFC responses by lymphocytes from Lpr patients at serial intervals throughout the course of anti-leprosy therapy. While difficult, such investigations are essential to improvement in our understanding of the immunopathogenesis of hypergammaglobulinemia and autoantibody formation in leprosy and possibly in other chronic infections as well.

The authors thank Mrs Barbara Huber for excellent assistance in the manuscript preparation and Dr Carlotta Hill for referral of patients. Supported by NIH Grant AI 16308.

REFERENCES

ALMEIDA DE, J.O., BRANDAO, H. & DE LIMA, E.G. (1964) Enhanced serologic response of lepromatous patients to antityphoid vaccination. *Int. J. Lepr.* **32,** 292.

ARTZ, R.P., JACOBSON, R.R. & BULLOCK, W.E. (1980) Decreased suppressor cell activity in disseminated granulomatous infections. *Clin. exp. Immunol.* **41,** 343.

BAHR, G.M., ROOK, G.A. & STANFORD, J.L. (1981) Inhibition of the proliferative response of peripheral blood lymphocytes to mycobacterial or fungal antigens by co-stimulation with antigens from various mycobacterial species. *Immunology*, **44,** 593.

BJUNE, G. (1979) *In vitro* lymphocyte stimulation in leprosy; simultaneous stimulation with *Mycobacterium leprae* antigens and phytohemagglutinin. *Clin. exp. Immunol.* **36,** 479.

BLAESE, M., GRAYSON, A. & STEINBERG, A.D. (1980) Increased immunoglobulin-secreting cells in the blood of patients with active systemic lupus erythematosus. *Am. J. Med.* **69,** 345.

BRESNIHAN, B. & JASIN, H.E. (1977) Suppressor function of peripheral blood mononuclear cells in normal individuals and in patients with systemic lupus erythematosus. *J. clin. Invest.* **59,** 106.

BUCK, A.A. & HASENCLEVER, H.F. (1963) The influence of leprosy on delayed-type skin reactions and serum agglutination titers to *Candida albicans*. *Am. J. Hyg.* **77,** 305.

BULLOCK, W.E. (1978) Leprosy: a model of immunological perturbation in chronic infection. *J. Infec. Dis.* **137,** 341.

FAUCI, A.S., STEINBERG, A.D., HAYNES, B.F. & WHALEN, G. (1978) Immunoregulatory aberrations in systemic lupus erythematosus. *J. Immunol.* **121,** 1473.

FAUCI, A.S., WHALEN, G. & BURCH, C. (1980) Activation of human B lymphocytes. XIV. Cellular requirements, interactions, and immunoregulation

of pokeweed mitogen-induced total-immunoglobulin producing plaque-forming cells in peripheral blood. *Cell. Immunol.* **54,** 230.

JHA, P., BALAKRISHNAN, K., TALWAR, G.P. & BHUTANI, L.K. (1971) Status of humoral immune responses in leprosy. *Int. J. Lepr.* **39,** 20.

MEHRA, V., MASON, L.H., ROTHMAN, W., REINHERZ, E., SCHLOSSMAN, S.F. & BLOOM, B.R. (1980) Delineation of a human T cell subset responsible for lepromin-induced suppression in leprosy patients. *J. Immunol.* **125,** 1182.

MORIMOTO, C., REINHERZ, E.L., ABE, T., HOMMA, M. & SCHLOSSMAN, S.F. (1979) Characteristics of anti-T cell antibodies in SLE: evidence for selective reactivity with normal suppressor cells defined by monoclonal antibodies. *Clin. Immunol. Immunopathol.* **16,** 474.

MORIMOTO, C., REINHERZ, E.L. & SCHLOSSMAN, S.F. (1980) Alterations in immunoregulatory T cell subsets in active systemic lupus erythematosus. *J. clin. Invest.* **66,** 1171.

NATH, I., NARAYANAN, R.B., MEHRA, N.K., SHARMA, A.K. & GUPTA, M.D. (1979) Concanavalin A induced suppressor activity in human leprosy. *J. clin. Lab. Immunol.* **2,** 319.

REINHERZ, E.L. & SCHLOSSMAN, S.F. (1980) The differentiation and function of human T lymphocytes. *Cell,* **19,** 821.

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

SAGAWA, A. & ABDOU, N.I. (1979) Suppressor-cell antibody in systemic lupus erythematosus: possible mechanism for suppressor cell dysfunction. *J. clin. Invest.* **63,** 536.

STOBO, J.P. (1977) Immunosuppression in man: suppression by macrophages can be mediated by interactions with regulatory T cells. *J. Immunol.* **119,** 918.

TOUW, J., STONER, G.L. & BELEHU, A. (1980) Effect of *Mycobacterium leprae* on lymphocyte proliferation: suppression of mitogen and antigen responses of human peripheral blood mononuclear cells. *Clin. exp. Immunol.* **41,** 397.

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

TWOMEY, J.J., LAUGHTER, A.H. & STEINBERG, A.D. (1978) A serum inhibitor of immune regulation in patients with systemic lupus erythematosus. *J. clin. Invest.* **62,** 713.

YACHIE, A., MIYAWAKI, T., NAGAOKI, T., YOKOI, T., MUKAI, M., UWADANA, N. & TANIGUCHI, N. (1981) Regulation of B cell differentiation by T cell subsets defined with monoclonal OKT4 and OKT8 antibodies in human cord blood. *J. Immunol.* **127,** 1314.