Clin. exp. Immunol. (1982) **47**, 351–358.

Alterations of T-cell subsets in primary biliary cirrhosis

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(Accepted for publication 28 August 1981)

SUMMARY

To determine whether abnormalities of immunoregulatory T cells are associated with primary biliary cirrhosis, we characterized peripheral blood mononuclear cells in 16 patients with primary biliary cirrhosis and compared them with 30 normal controls. For this analysis we used monoclonal antibodies to the surface antigens on helper/inducer ($T4^+$) and suppressor ($T8^+$) T cell subsets and to a common T cell antigen ($T3^+$). In contrast to normal persons, patients with primary biliary cirrhosis had reduced percentages of $T3^+$ cells. More importantly, there was a relative decrease in helper/inducer ($T4^+$) cells in 9/16 patients and a decrease in suppressor ($T8^+$) cells in 5/16 patients. Furthermore, clinical studies indicated that patients with a decreased suppressor cell population (increased $T4^+:T8^+$ ratio) had more advanced disease, as reflected by serum bilirubin levels ($P<0{\cdot}05$) and histological changes in the liver ($P<0{\cdot}001$), than those patients with a reduced helper T cell population (decreased $T4^+:T8^+$ ratio). These data suggest that abnormalities of immune responsiveness in primary biliary cirrhosis may have a more complex origin than a uniform alteration in one immunoregulatory T-cell subset and that these immunoregulatory cell changes vary according to the severity of the disease.

INTRODUCTION

Primary biliary cirrhosis (PBC) is a disease of unknown cause which has a variety of autoimmune features. These include production of autoantibodies to mitochondrial, smooth muscle, nuclear and hepatobiliary antigens, pronounced elevation of serum IgM concentration, and increased levels of circulating immune complexes (Klatskin & Kantor, 1972; Doniach *et al.*, 1966; Thomas *et al.*, 1976b; Wands *et al.*, 1978; Thomas, Potter & Sherlock, 1977). In addition to aberrant humoral responses, patients with PBC have abnormal cellular immune responses. Their peripheral blood lymphocytes manifest enhanced *in vitro* cytotoxicity for a variety of target cells (Thomson *et al.*, 1974; Paronetto & Vernace, 1975; Geubel *et al.*, 1976) and increased reactivity when exposed *in vitro* to hepatic, biliary, and extra-hepatic antigens (Miller *et al.*, 1972; McFarlane *et al.*, 1979; McFarlane *et al.*, 1976). Furthermore patients with PBC also may have features of scleroderma, Raynaud's phenomenon, Sjörgren's syndrome, thyroiditis, and renal tubular acidosis, disorders believed to have an autoimmune pathogenesis (Golding, Smith & Williams, 1973). These findings, as well as the presence of intense lymphocytic infiltrates surrounding damaged intrahepatic bile

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0009-9104/82/0200-0353$02.00

ducts in the livers of patients with PBC, support the concept that the bile duct may be a primary target of an auto-destructive immune response (Sherlock & Scheuer, 1973).

In this regard, it is now becoming increasingly evident that a balance between helper/inducer and suppressor T cells plays a crucial role in maintaining immune homeostasis (Reinherz *et al.*, 1979e, 1979f). To determine whether immunoregulatory T-cell subsets are altered in PBC, we characterized peripheral blood lymphocytes from patients with the disease by using recently developed monoclonal antibodies to human T cells (Reinherz *et al.*, 1979a, 1979b, 1979c, 1980a). In addition to a decrease in total T cells, we found significant alterations in T-cell subsets which correlated with clinical activity of the disease.

MATERIALS AND METHODS

Patients. Patients included in this study were derived from the in-patient and out-patient services of the Massachusetts General Hospital. The diagnosis of primary biliary cirrhosis was made on the basis of clinical and biochemical features, diagnostic or compatible histological changes on liver biopsy, and patency of the extrahepatic biliary tree, as determined radiologically or surgically. All were female and had serum antibodies to mitochondria; their mean $\pm$ s.d. (range) age was $50{\cdot}0 \pm 8{\cdot}4$ (26–65) years. At the time of study, none of the patients were receiving therapy known to affect peripheral blood lymphocytes. This study was approved by the Subcommittee on Human Studies of the Committee on Research of the Massachusetts General Hospital.

Isolation of lymphocytes. Peripheral blood mononuclear cells were isolated from heparinized venous blood by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, New Jersey) density centrifugation.

Monoclonal antibodies. In this study we used recently described murine monoclonal antibodies to human peripheral blood T cells, the production and characterization of which were described in prior reports (Reinherz *et al.*, 1979a; 1979b; 1979c; 1980a). The antibodies were obtained in the form of ascitic fluid from mice injected with hybridoma cells. Anti-T3 reacts with 100% of peripheral E rosette-positive T cells and approximately 10% of thymocytes. Anti-T4, which defines the helper/inducer T-cell subset, reacts with 60% of peripheral T cells, while anti-T8, which identifies suppressor/cytotoxic T cells, reacts with 20–30% of peripheral T cells. In addition, a monoclonal antibody (anti-Il) that defines a non-polymorphic region of human Ia-like antigens, was used (Reinherz *et al.*, 1979d).

Analysis of lymphocyte populations. Cytofluorographic analysis of T cell populations was performed by indirect immunofluorescence, as previously described (Reinherz *et al.*, 1979a; 1979b). In brief, aliquots of lymphocytes were incubated first with the respective monoclonal antibodies, washed, and stained with fluorescein labelled goat anti-mouse IgG (Meloy Laboratories, Springfield, Virginia). Stained cells were enumerated on a Fluorescence Activated Cell Sorter (FACS-1, Becton Dickinson, Sunnyvale, California). Background fluorescence reactivity was determined with a control ascitic fluid obtained from mice injected with non-secreting hybridoma clones.

RESULTS

To determine whether patients with primary biliary cirrhosis had alterations in immunoregulatory T-cell subsets, we characterized their peripheral circulating lymphocytes with monoclonal antibodies and indirect immunofluorescence. In our control groups of normal persons, the mean $\pm$ s.e.m. percentage of peripheral lymphocytes that were T cells was $67 \pm 3\%$, as determined by reactivity with anti-T3; $41 \pm 2\%$ were helper T cells reactive with anti-T4; and a smaller percentage, $22 \pm 1\%$, reactive with anti-T8, were suppressor T cells. In addition, only a small percentage of cells, $10 \pm 4\%$, were reactive with anti-Il. The Ia^+ population includes all B cells and a fraction of monocytes. In contrast, the resting T cell population is normally unreactive with anti-Il.

Lymphocytes from patients with primary biliary cirrhosis were abnormal in almost all cases. In

Table 1. Cell surface characteristics of T cells in patients with PBC and normal controls

PBC patients	Number of mononuclear* cells/ml blood	% Reactivity with monoclonal antibodies				
		anti-T3	anti-T4	anti-T8	$T4^+:T8^+$ ratio	anti-Il
Group 1	1467	68	45	35	1·3	22
($T4^+:T8^+$ ratio < 1·9)	1222	23	19	16	1·1	7
	1830	21	20	17	1·2	26
	1550	18	9	13	0·7	10
	nd†	60	28	42	0·7	13
	435	19	16	20	0·8	33
	nd†	50	32	29	1·1	32
	1834	40	18	20	0·9	24
	1200	34	26	18	1·4	16
	2450	30	27	15	1·8	18
	2210	10	6	6	1·0	25
Mean ± s.e.m.		34 ± 6**	22 ± 3**	21 ± 3	1·1 ± 0·1‡	21 ± 3
Group 2	1035	70	43	14	3·1	7
($T4^+:T8^+$ ratio > 2·5)	967	48	40	15	2·6	29
	543	75	68	15	4·5	7
	1652	31	31	6	5·2	20
	835	15	14	5	2·8	13
Mean ± s.e.m.		48 ± 11‡	39 ± 9	11 ± 2**	3·6 ± 0·5§	15 ± 4
Normal controls (30)	1000–3000++	67 ± 3	41 ± 2	22 ± 1	1·9 ± 0·2	10 ± 4

* The figures indicate the number of mononuclear cells obtained after Ficoll hypaque separation; † not done; ‡ $P < 0·05$; § $P < 0·01$, ** $P < 0·001$ statistical significance compared to value for normal controls, (Student's *t*-test); ++ normal range.

13 of 16 cases, the percentage of T cells ($T3^+$) was decreased. More importantly, there was an abnormal ratio of $T4^+$ to $T8^+$ cells in 15 of 16 cases. Two distinct patterns of immunoregulatory T cell abnormalities were observed (Table 1), based on $T4^+:T8^+$ ratios. The majority of patients had a decrease in their helper/inducer $T4^+$ subset (Fig. 1) and thus a diminished $T4^+:T8^+$ ratio (Table 1, group 1). Ten of 11 cases within group 1 had a $T4^+:T8^+$ ratio less than 1·5, with a mean ± s.e.m. ratio of 1·1 ± 0·1, in comparison to the $T4^+:T8^+$ ratio in normals of 1·9 ± 0·2 ($P < 0·05$). The decrease in $T4^+$ cells was absolute, since the number of lymphocytes harvested from these patients'

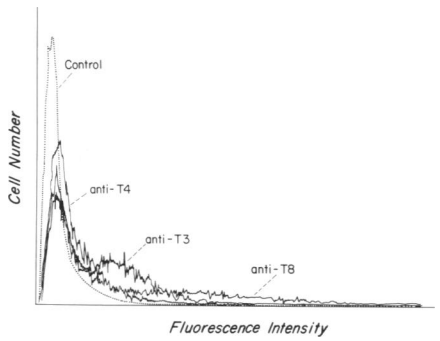

Fig. 1. Fluorescence activated cell sorter profile of lymphocytes from a PBC patient with low $T4^+$ (helper/inducer) cells. The control represents background fluorescence determined by using ascitic fluid from a non-producing hybridoma clone.

Table 2. Correlation between alterations in T-cell subsets and clinical features in patients with PBC

Clinical/immunological feature	PBC-group 1 (↓ helper $T4^+$ cells)	PBC-group 2 (↓ suppressor $T8^+$ cells)	*P*
SGOT (iu/l)*	127±78	111±32	n.s.
Bilirubin (mg/100 ml)*	1·3±0·7	4·9±5·6	<0·05‡
Alkaline phosphatase (iu)*	194±85	328±212	n.s.
Globulin (g/100 ml)*	4·0±0·9	5·0±1·4	n.s.
Histologic stage†			
≤II	7/8 (88%)	1/4 (25%)	<0·001§
≥III	1/8 (12%)	3/4 (75%)	
Autoimmune features**	3/11 (27%)	1/5 (20%)	n.s.

* Mean±s.d.; † Among those for whom histological data available; ‡ Student's *t*-test; § Chi square analysis; ** Thyroditis, Sjögren's syndrome or nephrotic syndrome.

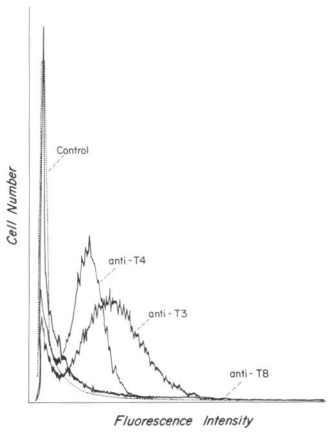

Fig. 2. Fluorescence activated cell sorter profile of lymphocytes from a PBC patient with low $T8^+$ (suppressor/cytotoxic) cells.

peripheral blood was not greater than the number of cells obtained from normals (Table 1). Moreover, in the same group there was an increase in Ia^+ cells. This was apparently due to an increased B cell and/or monocyte population, because lymphocytes from the E-rosetting fraction of peripheral blood did not express Ia antigens (data not shown).

In contrast to individuals in group 1, five other patients with PBC (group 2) had an increased $T4^+:T8^+$ ratio, with a mean±s.e.m. ratio of 3·6±0·5 ($P<0·01$). As shown in Table 1, this was due to a specific decrease in the $T8^+$ suppressor population rather than due to an increase in the $T4^+$ helper subset (Fig. 2). Finally, it should be noted that one individual (patient 10, Table 1, group 1) had no abnormality in $T4^+:T8^+$ ratio although all her T cell populations were decreased in the peripheral blood (Fig. 3).

Prior studies indicated that T3 antigen was expressed on all mature peripheral T cells while the T4 and T8 antigens were present on helper and suppressor subsets respectively (Reinherz *et al.*, 1980a). However, in some of the present cases we observed that the sum of the percentage of cells reactive with anti-T4 and anti-T8 clearly exceeded the percent of cells reactive with anti-T3 (cases 3, 6, 10). This finding would suggest that a circulating population co-expressing the T3, T4 and T8 antigens may exist in some individuals. Alternatively some $T4^+$ or $T8^+$ T cells may not express the T3 antigen. Since such populations of cells are found normally only within the thymocyte compartment (Reinherz *et al.*, 1980a; Bhan *et al.*, 1980), the significance of circulating cells of this

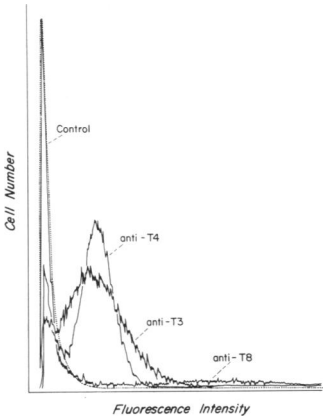

Fig. 3. Fluorescence activated cell sorter profile of lymphocytes from a PBC patient with a normal T cell subset pattern.

phenotype is not clear at the present time but could reflect a rapid T cell turnover. Nevertheless, the present data suggest that two major abnormal patterns of T-cell subset distributions exist in the peripheral blood of patients with primary biliary cirrhosis. On the one hand, patients may present with a specific decrease in the inducer population (Fig. 1, Table 1, group 1) or on the other hand a specific decrease in the suppressor subset (Fig. 2, Table 1, group 2).

Having identified two different abnormal patterns of T-cell subsets in PBC, we attempted to determine whether these alterations in T-cell subsets were related to clinical or other laboratory indicators of disease activity (Fig. 3). As shown in Table 2, there were two striking distinctions between patients in group 1 and group 2 with regard to biochemical features and histologic stage of liver disease (Sherlock & Scheuer, 1973). Firstly, patients in group 2 had a significantly higher level of serum bilirubin than patients in group 1. Secondly, group 2 patients had more advanced histologic liver changes than patients in group 1. In contrast, no differences were noted between these two groups with respect to transaminase or alkaline phosphatase levels, total serum globulin fractions, or the presence of clinically defined autoimmune features.

DISCUSSION

In the present study, we analysed T lymphocyte subpopulations in patients with primary biliary cirrhosis by means of a series of monoclonal antibodies. In addition to anti-T4 and anti-T8, two subset-specific monoclonals, we employed anti-T3, a monoclonal antibody reactive with 100% of peripheral T cells and a monoclonal anti-Ia antibody. In 12 of 16 patients there was a decrease in the total number of T cells as defined by reactivity with anti-T3. This is consistent with prior studies of E-rosetting cells, which also showed a decrease in T lymphocytes (Thomas *et al.*, 1976a), although there are conflicting reports (Salerno *et al.*, 1976). Perhaps more importantly there were major abnormalities in the number of either the $T4^+$ or $T8^+$ cell subset. Ten of 16 patients had a decreased inducer:suppressor ratio secondary to a selective diminution of helper $T4^+$ cells while five of 16 PBC patients had an increased $T4^+$:$T8^+$ ratio resulting from relative diminution of suppressor $T8^+$ cells. Furthermore, correlative clinical studies indicated that those patients with a decreased number of circulating suppressor cells had more severe hyperbilirubinemia and advanced histological changes on liver biopsy in comparison with those patients with a reduced $T4^+$ inducer population.

The above results indicate that the nature of immunoregulatory T-cell subset abnormalities in primary biliary cirrhosis is complex. In one group of patients the balance between inducer and suppressor cells is clearly tilted in favour of the suppressor cell subset. This diminution in $T4^+$ cells

could explain the inability of some patients with PBC to mount a delayed hypersensitivity reaction (Fox *et al.*, 1969), develop an adequate antibody response to hemocyanin (Fox, Dudley & Sherlock, 1973), or proliferate maximally to mitogenic stimuli (Fox *et al.*, 1973). It is possible that the diminution in $T4^+$ cells within the peripheral circulation is related directly to immunologic events taking place in the liver. Thus, $T4^+$ cells, normally responsible for delayed type hypersensitivity events, might be reacting within the liver parenchyma against intrahepatic bile ducts. In addition, they might serve to recruit other cells responsible for immunologic injury in the portal tract areas. Theoretically, then, as a consequence of such intrahepatic recruitment, the size of the $T4^+$ population in the peripheral blood would diminish. The intense lymphocytic infiltration and granuloma formation frequently present around damaged bile ducts in this disease are both consistent with this hypothesis.

It must be emphasized, however, that a second group of patients with PBC had a relative diminution in the suppressor T cell population. In fact, most patients with primary biliary cirrhosis show decreased suppressor cell function at some point in their disease as judged by *in vitro* analysis of mitogen induced or spontaneous suppressor cell activity (Dienstag, Weak & Wands, 1978; James *et al.*, 1980). This may be responsible for the characteristic elevation of serum IgM, circulating immune complexes, and autoantibodies reported in this disease. It is possible that periodic loss or diminution in $T8^+$ suppressor cells could trigger or potentiate additional hepatic disease by allowing unchecked proliferation and/or function of T cell or B cell clones reactive with hepatocytes and biliary elements. Alternatively, rather than causing disease, loss of suppressor cells may represent migration of $T8^+$ cells from the periphery in response to hepatic disease. The observation that PBC patients with decreased inducer cells have less severe clinical disease and less advanced disease stage than patients with a diminished suppressor cell population would support this view.

A diminished suppressor cell number is not unique to patients with advanced PBC. A similar T cell defect has been reported in other autoimmune diseases such as systemic lupus erythematosus, acute graft versus host disease, and the acute phase of multiple sclerosis (Reinherz *et al.*, 1979e, 1980b; Morimoto *et al.*, 1980). In systemic lupus erythematosus, the occurrence of autoantibodies to suppressor T cells may account, at least in part, for the decrease in the suppressor cell population (Morimoto *et al.*, 1980). It is conceivable that a diminution in either the $T4^+$ or $T8^+$ population in PBC could result from production of a similar autoantibody. In this regard, it has now become apparent that some antigens, similar to the QA1 antigen in the murine system, are shared by a subpopulation of $T4^+$ inducer cells and $T8^+$ suppressor cells (Morimoto *et al.*, 1981). To date, these antigens have been defined by autoantibodies from patients with juvenile rheumatoid arthritis (JRA). The JRA^+T4^+ population appears to represent the inducer subset which activates the $T8^+$ suppressor population. It is also possible that the decreased suppressor cell activity measured by functional assays in early stages of PBC (Dienstag *et al.*, 1978; James *et al.*, 1980) are due to a diminution in the JRA^+T4^+ subset which is required to initiate suppressor cell function.

The findings in the present study, for the most part, are in agreement with the results reported by Routhier *et al.* (1980). However, these authors observed depression of both circulating T4 and T8 populations in patients with late stage PBC. In the present study, we observed this pattern in only two patients. The differences between the two studies may have resulted from differences in patient selection or, alternatively, may indicate that there is a spectrum of immunoregulatory T-cell subset changes in PBC.

It has been suggested that PBC resembles chronic graft versus host disease (Epstein, Thomas & Sherlock, 1980; Routhier *et al.*, 1980). In both diseases there are lymphocyte associated injury of bile ducts in the liver and severe disturbances in humoral and cellular immunity. Given the present observation that the inducer/suppressor ratio is decreased in the peripheral blood of many PBC patients and the previous reports showing similar abnormalities in inducer/suppressor ratios in some patients with chronic graft versus host disease (Reinherz *et al.*, 1979e), these results can be interpreted to support this hypothesis.

The present study demonstrates clearly that patients with PBC have a decrease in either inducer or suppressor T-cell subsets. It appears from our findings that in the early stages of the disease there is a selective diminution of the inducer T cell population whereas in the later stages of disease, a relative decrease in suppressor cells occurs. Alternatively, it is also plausible, that there is no

uniform abnormality of T-cell subsets in PBC but that most PBC patients have diminished T inducer cells while others have a selective loss in suppressor cells. In this regard, serial analysis will be of critical importance in determining whether there is a general progression in these T-cell subset alterations. Further characterization of lymphocytes in both blood and liver should provide considerable insight into the pathogenesis of PBC.

This work was supported in part by grants AM 25553, AI 12069, CA 19589, HL 18646 and contract HB-9-2919 from the National Institutes of Health, United States Public Health Service. We thank Michele Angelo for preparation of the manuscript.

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