Reduction in peripheral blood K cells and activated T cells in primary biliary cirrhosis

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SUMMARY Lymphocyte subpopulations were measured in the peripheral blood of 13 patients with primary biliary cirrhosis (PBC), 13 with chronic active hepatitis (CAH), and 16 age- and sex-matched normal subjects. A significant, relative and absolute, reduction in activated T cells and K cells, compared with that in normal subjects, was found in PBC but not in CAH. The reduction in K cells observed in PBC was found to be accompanied by a parallel decrease in lymphocyte-mediated antibody-dependent cytotoxicity.

Various immunological abnormalities have been described in primary biliary cirrhosis (PBC) and chronic active hepatitis (CAH), and cell mediated immune mechanisms have been implicated in the production of the intrahepatic lesions. This is supported by the *in vitro* demonstration of lymphocyte cytotoxicity against isolated hepatocytes in tissue culture (Thomson *et al.*, 1974; Paronetto and Vernace, 1975). The destruction of target cells *in vitro* may be mediated either by direct cytotoxicity by T cells which are sensitised to specific target cell antigens (Cerottini and Brunner, 1974), or by an indirect cytotoxic effect by non-thymus dependent cells which react with IgG coated target cells. This latter antibody-dependent cytotoxicity (ADC) is mediated by so-called killer (K) cells, and the mechanism is thought to be of potential importance in autoimmune diseases (MacLennan, 1972).

In the present study we have attempted to measure various lymphocyte subpopulations in the peripheral blood of patients with primary biliary cirrhosis and chronic active hepatitis, our original premise being that sequestration of a particular subpopulation in the liver might be reflected by a diminution of this population in the circulation.

T cells were detected by rosette formation with sheep erythrocytes (SE) (Jondal *et al.*, 1972) and 'activated' T cells were detected by rosette formation with autologous erythrocytes (Sandilands *et al.*, 1975; Sheldon and Holborow, 1975); B cells were

characterised by demonstrating surface immunoglobulin (SIg) (Brown and Greaves, 1974); and Fc receptor-bearing cells were detected by antibody sensitised chicken erythrocyte (EA) rosette formation (Sandilands *et al.*, 1976). It was assumed that all B cells possess Fc receptors—that is, are EA⁺, SIg⁺ cells.

In a previous publication we have shown that ADC activity measured directly by IgG dependent lysis of human Chang liver cells (Campbell *et al.*, 1973) correlates closely with a population of Fc receptor-bearing, SIg negative (EA⁺, SIg⁻) cells (Sandilands *et al.*, 1976). While this subgroup may not wholly compose K cells, for the purposes of this study the number of EA⁺, SIg⁻ cells was regarded as indicative of the number of K cells.

Methods

Samples of heparinised blood were obtained from 13 patients with HBsAg negative chronic active hepatitis (mean age $\pm$ SD = 55.5 $\pm$ 13.2 years) and 13 with primary biliary cirrhosis (57 $\pm$ 12.7 years), the diagnosis having been confirmed on clinical, biochemical, serological, and histological grounds. Six patients with chronic active hepatitis were receiving combined prednisolone and azathioprine chemotherapy and four patients with primary biliary cirrhosis were receiving azathioprine alone. Control samples were obtained from 16, age- (54.4 $\pm$ 6.8 years) and sex-matched, healthy members of hospital staff.

Lymphocyte suspensions were obtained by centrifugation of whole blood over a Ficoll-metrizoate column (SG = 1.077) as described by Böyum (1968).

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Received for publication 14 June 1977

The lymphocytes were washed thrice in Hepes buffered Earles medium (HEM pH 6.9: Gibco-Laboratories, Glasgow) before final adjustment to 4×10^6 cells/ml. Contaminating monocytes were identified in final rosette preparations by morphology or after phagocytosis of Latex beads.

Heparinised sheep blood was obtained fresh each week and stored at 4°C in Alsever's solution. For use, SE were washed thrice and adjusted to a 0.5% suspension in HEM. Autologous erythrocytes were similarly washed thrice in HEM and adjusted to a 1.5% suspension in HEM.

Antibody-sensitised chicken E (EA) were prepared by incubating equal volumes of 5% washed chicken E and rabbit anti-chicken erythrocyte serum, diluted 5×10^{-2} , at 37°C for one hour. The EA complexes were then washed twice in HEM and adjusted to a 1% suspension in HEM.

Rosette tests were performed as previously described (Sandilands *et al.*, 1975). At least 200 cells were counted per slide and lymphocytes binding three or more indicator erythrocytes were regarded as rosette-forming cells.

Surface immunoglobulin (SIg) was detected by the indirect immunofluorescence technique described by Brown and Greaves (1974) using rabbit anti-human Ig serum (absorbed with human thymocytes) followed by fluorescein-conjugated goat anti-rabbit Ig serum (Behringwerke AG—Marburg). The SIg detected by this method was stable at 37°C and was predominantly SIg M.

Antibody dependent lymphocyte cytotoxicity (ADC) activity was determined using the ^{51}Cr

release assay as described by Campbell *et al.* (1972). IgG sensitised, ^{51}Cr labelled Chang liver cells were incubated at 37°C for 22 hours with purified PBL at lymphocyte to target cell ratios of 10:1 and 30:1. The percentage net lysis of Chang liver cells was calculated as previously described (Sandilands *et al.*, 1976).

Results

The overall results are summarised in Table 1. The absolute number of PBL/mm³ was significantly reduced in both patient groups ($P = 0.003$). The proportions of the various lymphocyte subpopulations in the CAH patients were similar to those in the controls, but a significant reduction in activated T cells ($P = 0.01$) and K cells ($P = 0.002$) was found in patients with primary biliary cirrhosis. The reduced total PBL count was reflected in a significant reduction in the absolute numbers of the various subpopulations, except for activated T cells in those with chronic active hepatitis and B cells in those with primary biliary cirrhosis. Thus in primary biliary cirrhosis there was a relative and absolute reduction in activated T cells and in K cells. In the four treated patients with this disorder the K cell numbers were 29/mm³ (2.6%) and in the nine untreated patients 116/mm³ (5.8%), both these results being statistically significant ($P = 0.001$) as compared with the controls 273/mm³ (12%). The reduction of activated T cells found in primary biliary cirrhosis was similar in the treated and untreated subgroups. In the chronic active hepatitis

Table 1 Subpopulations of peripheral blood lymphocytes (PBL) in PBC (13 patients) and CAH (13), and age- and sex-matched normal controls (16). Results given as total number in peripheral blood and as % age of total PBL count

		Controls	Chronic active hepatitis	Primary biliary cirrhosis
Total	(No./mm ³)	2571 ± 709	1754 ± 853 (0.003)	1520 ± 762 (0.003)
'T' cells (SE ⁺)	No./mm ³	1951 ± 642	1307 ± 612	1178 ± 527
	%	75 ± 7.1	75 ± 6.9	74 ± 7.4
Activated 'T' cells (SE ⁺)	No.	79 ± 52	59 ± 49	25 ± 13 (0.003)
	%	3.1 ± 2.2	2.8 ± 1.5	1.4 ± 1.1 (0.01)
Fc receptor bearing cells (EA ⁺)	No.	643 ± 237	377 ± 228 (0.002)	327 ± 281 (0.001)
	%	26 ± 9.7	22 ± 8.2	22 ± 11.9
'B' cells (EA ⁺ , SIg ⁺)	No.	353 ± 123	236 ± 145 (0.01)	273 ± 260
	%	14 ± 3.8	13 ± 4.5	18.5 ± 12.3
'K' cells (EA ⁺ , SIg ⁻)	No.	273 ± 14.8	173 ± 172 (0.05)	100 ± 173 (0.003)
	%	12 ± 6.4	10.5 ± 8.4	4.5 ± 5.9 (0.002)

*() P values—significant reduction compared with controls

SE⁺ = lymphocytes forming rosettes with sheep erythrocytes

Auto E⁺ = lymphocytes forming rosettes with autologous erythrocytes

EA⁺ = lymphocytes forming rosettes with IgG sensitised chicken erythrocytes

SIg⁺ = lymphocytes with surface immunoglobulin.

patients there were no significant differences in the subpopulations in relation to therapy.

The results of ADC testing are summarised in Table 2. As in previously reported findings, a good

Table 2 Antibody dependent cytotoxicity (ADC) in patients with PBC and CAH and in normal controls

Group and number of subjects:	% Net lysis of Chang cells at lymphocyte/target cell ratios of			
	10/1		30/1	
Normal controls (12):	16.8	(0.76)*	32	(0.82)
Primary biliary cirrhosis				
All patients (8)	4.4†	(0.89)	19‡	(0.86)
No treatment (3)	8.3		31	
Treatment (5)	2.0		9.8	
Chronic active hepatitis				
All patients (8)	11.1	(0.95)	28.1	(0.79)
No treatment (5)	10.0		29.0	
Treatment (3)	11.8		27.6	

* In parentheses, *r* value for correlation with K cell count.

All these were statistically significant $P < 0.01$ > 0.001.

† $P < 0.01$ } Significant reduction compared with normal subjects,
‡ $P < 0.05$

correlation was observed in all groups between ADC and PBL K cell counts. In patients with primary biliary cirrhosis a significant reduction in ADC activity was found overall, this being so in both the treated and untreated patients. A number of patients (four with primary biliary cirrhosis and two with chronic active hepatitis) had no detectable K cells and correspondingly no demonstrable ADC activity.

Discussion

The nature of the lymphocytic infiltrate thought to mediate the immune reaction directed against hepatocytes in chronic active hepatitis and bile duct epithelium in primary biliary cirrhosis remains to be clarified. Recently Cochrane *et al.* (1976) have shown that PBL from patients with chronic active hepatitis are capable of destroying isolated rabbit hepatocytes *in vitro*; and, furthermore, lymphocyte depletion studies suggested that this cytotoxic effect was not caused by T lymphocytes. Hopf *et al.* (1976) using an immunofluorescence technique demonstrated tissue fixed IgG autoantibody to liver cells in HBsAg negative chronic active hepatitis, an observation which would provide a basis for antibody dependent lymphocytotoxicity, thought to be mediated by K cells.

The characteristics of the K cell remain uncertain. However, in an earlier study we have shown that a population of lymphocytes with Fc receptors but lacking surface immunoglobulin contains the K cell population (Sandilands *et al.*, 1976). These earlier observations are confirmed in the present study

(Table 2), and show that ADC is a function of lymphocytes within the EA⁺, SIg⁻ subgroup.

The absolute number of PBL was significantly reduced in both groups of patients studied, but, with the exception of activated T cells and K cells, in the primary biliary cirrhosis group, the various relative proportions of the lymphocyte subpopulations were similar to that of the controls. This total reduction in the PBL count could not be attributed solely to an effect of chemotherapy. It may represent non-specific sequestration of lymphocytes in general, either in the liver or possibly in the spleen. The reduction in total T cells in both chronic active hepatitis and primary biliary cirrhosis is similar to the report of De Horatius *et al.* (1974) and this finding might, in part, explain the reported *in vivo* depression of cell-mediated immunity found in patients with chronic liver disease (Berenyi *et al.*, 1974; Sodomann *et al.*, 1974).

The most interesting finding was a significant reduction in activated T cell and K cell counts in patients with primary biliary cirrhosis. While the reduction in activated T cells was not influenced by azathioprine treatment, the reduction in K cells was even more profound in the treated subgroup. The speculation that these findings in primary biliary cirrhosis might reflect preferential sequestration of K cells and activated T cells in the liver is supported by the recent findings of Sanchez-Tapias *et al.* (1977) who reported a significant increase in the proportion of activated T cells in the livers of patients with primary biliary cirrhosis but to a lesser degree in patients with chronic active hepatitis.

A further possibility is that the decrease in K cells may be partly caused by the binding of immune complexes to the Fc receptors of these cells. The majority of patients with primary biliary cirrhosis have rheumatoid factors (RF) in their serum. However, no correlation could be demonstrated between the presence or titre of RF and the K cell count. In another study (unpublished data), a significant reduction of EA rosettes was produced by some sera from patients with primary biliary cirrhosis and chronic active hepatitis when normal PBL were preincubated with the sera before rosette testing. However, no serum blocking of activated T cell receptors was found. Thus, some of the reduction in peripheral blood K cell counts might be caused by Fc receptor blocking by serum factors, possibly immune complexes. This possibility merits further study.

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