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$CD4^+$ T cell subsets defined by isoforms of CD45 in primary biliary cirrhosis

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SUMMARY

Primary biliary cirrhosis (PBC) is an autoimmune condition characterized by destruction of the intrahepatic bile ducts. Autoreactive $CD4^+$ T cells have been reported both in the peripheral circulation and in the mononuclear cell infiltrate in the affected portal tracts. In this large study we have used two- and three-colour flow cytometry to determine the phenotypes of the $CD4^+$ T cell subsets in the peripheral blood and liver-infiltrating lymphocytes of PBC patients ($n = 43$), normal controls ($n = 19$) and patients with alcoholic cirrhosis ($n = 15$), according to a novel classification based on the simultaneous expression of different isoforms of CD45. In PBC patients the proportion of peripheral blood $CD4^+$ cells possessing the $CD45RO^{high}RA^-$ 'memory' phenotype was significantly increased, and the $CD45RO^-RA^{high}$ 'naive' population was significantly decreased, compared with the two control groups. No significant differences in peripheral blood $CD4^+$ T cell subsets were seen between patients with pre-cirrhotic and cirrhotic PBC. A similar, but more marked, shift towards the $CD45RO^{high}RA^-$ 'memory' phenotype was seen in the liver-infiltrating $CD4^+$ T cells in PBC patients compared with alcoholic cirrhotics. Cells within the $CD4^+$ memory subpopulation were further subgrouped according to expression of CD45RB, the level of expression of which has been associated with functional differences in the memory subset. In peripheral blood no differences were seen between PBC patients and controls with respect to the proportion of $CD45RO^{high}RB^{high}$ and $CD45RO^{high}RB^{dim}$ memory subsets. A statistically significant difference in the distribution of these memory subsets, with an increased memory-2/memory-1 ratio was observed in the liver-infiltrating $CD4^+$ T cells of PBC patients compared with those from alcoholic cirrhotic patients. The potential implications of this observation are discussed.

Keywords CD45 CD45RB T cells lymphocyte subsets primary biliary cirrhosis

INTRODUCTION

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease characterized by the destruction of the intrahepatic bile ducts and the presence of antimitochondrial autoantibodies specific for the E2 component of the pyruvate dehydrogenase complex [1]. The early stages of the disease are characterized by infiltration of large numbers of inflammatory cells, in particular lymphocytes, into the affected portal tracts. The role played by these lymphocytes in mediating the biliary cell damage characteristic of PBC is still unknown. Several studies have suggested that most of these infiltrating cells are $CD4^+$ and $CD8^+$ T cells, with some B and natural killer (NK) cells [2,3].

Functionally distinct $CD4^+$ T cell subsets have been defined in the human, as well as in the rat and the mouse [4,5], which differ in the cytokines they secrete [6] and their function [7,8]. It has been suggested that the balance between different $CD4^+$ T cell subsets may control self-reactivity in the normal state, and that alteration in this balance may lead to autoimmunity [4,9,10]. These functionally distinct $CD4^+$ T cell subsets can be defined by their surface expression of leucocyte common antigen (LCA, CD45) isoforms [11–13]: CD45RA (high mol. wt isoform), CD45RB, CD45RC and CD45RO (low mol. wt lacking all three exons). Initially a subdivision of $CD4^+$ T cell subsets was made based on the differential expression of the CD45RA and CD45RO isoforms, where $CD45RA^+$ cells comprised the population of naive, unprimed cells, whereas the $CD45RO^+$ cell population represented memory or recently activated cells [7,8]. The transition from naive type to memory/activated cell has been associated with a switch in surface expression from $CD45RA^+$ to $CD45RO^+$ [14], although it is now known that these isoforms may be simultaneously co-expressed.

Recent studies have allowed a further, more functional, subdivision in the memory subset [15], in which the memory

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$CD4^+$ 'helper' T cells can be subdivided, in turn, into two subpopulations according to the expression of either high or low levels of another isoform of CD45, CD45RB [16–18]. The $CD4^+$ memory cell subsets identified by the level of expression of the CD45RB exon have been proposed to show the characteristics of the T helper 1 (Th1, producer of IL-2 and interferon-gamma (IFN-γ)) and T helper 2 (Th2, producer of IL-4, IL-5, IL-6 and IL-10) cell subpopulations identified previously in the mouse, and proposed, although not clearly identified as yet in humans [6,19]. Three human $CD4^+$ T cell subsets have thus been identified [4,5,10]: (i) the $CD45RA^{high}RB^{high}RO^-$ $CD4^+$ subset comprising the naive mature peripheral T cells; (ii) the $CD45RA^-RB^{high}RO^{high}$ $CD4^+$ subset comprising memory cells with Th1-like function, producing IFN-γ (memory-1); (iii) the $CD45RA^-RB^{dim}RO^{high}$ $CD4^+$ subset comprising memory cells with Th2-like function, supporting mature B cell responses (memory-2).

The purpose of the present study was to analyse the distribution of the different $CD4^+$ T cell subsets, according to this novel classification, using two- and three-colour flow cytometry in order to determine the simultaneous co-expression of CD45 isoforms on peripheral blood lymphocytes and liver-infiltrating lymphocytes of a large number of PBC patients and appropriate normal and chronic liver disease controls. The aim was to identify any predominant subset in PBC, which might be correlated with the pathogenesis or activity of the disease.

SUBJECTS AND METHODS

Subjects

Peripheral blood $CD4^+$ T cell subsets were studied in 43 patients with biopsy-proven PBC (21 pre-cirrhotics, stage I–III, and 22 cirrhotics, stage IV) attending the out-patient clinics at the Freeman Hospital, and 34 controls. The control group consisted of a chronic liver disease group (15 alcoholic cirrhotic patients (ALD)) and 19 normal subjects. The normal controls were hospital or laboratory staff recruited on a voluntary basis. All subjects gave informed consent. Abbreviated patient details are given in Table 1. None of the patients or controls was receiving immunosuppressive therapy. In 14 patients (PBC $n = 8$, all females, ALD $n = 6$, two females) who underwent orthotopic liver transplantation for end stage liver disease, the liver-infiltrating lymphocytes (LIL) as well as the peripheral blood lymphocytes (PBL) were studied.

Immunological reagents used

The following MoAbs were used for the two- and three-colour flow cytometry analysis: anti-CD45-FITC/anti-CD14-PE (2D1/MoP9; Simultest Leucogate, Becton Dickinson Immunocytometry Systems, Oxford, UK), anti-CD4-PerCP (SK3; Becton Dickinson), anti-CD45RO-LCA-PE (UCHL1; Immunotech S.A., Marseille, France), anti-CD45RA-LCA-FITC (ALB11, Immunotech S.A.), anti-CD45RB-FITC (PD7/26 Dako Als, Glostrup, Denmark). FACS lyse solution (Becton Dickinson) was used in the peripheral blood preparations.

Peripheral blood lymphocytes

Whole blood (3–4 ml) was obtained from each subject and added to a tube containing EDTA. Four tubes for flow cytometry analysis were prepared from each sample using a whole blood lysis protocol. Erythrocytes were lysed using FACS lyse (Becton Dickinson) and the samples were prepared following the manufacturer's recommended procedures. The combinations of MoAbs used were: (i) tube 1, CD45-FITC/CD14-PE; (ii) tube 2, unstained control; (iii) tube 3, CD45RB-FITC/CD45RO-PE/CD4-PerCP; (iv) tube 4, CD45RA-FITC/CD45RO-PE/CD4-PerCP. Percentages of lymphocytes and absolute lymphocyte and $CD4^+$ counts were obtained from concomitant leucocyte counts (Table 1) done by the clinical haematology laboratory of the Freeman Hospital.

Liver-infiltrating lymphocytes

Liver tissue (20 g) was removed from the explant of eight PBC patients and six ALD patients who underwent liver transplantation. Lymphocytes infiltrating the liver were obtained using a previously described method [2,3]. Liver-derived lymphocytes were analysed using the same protocol as peripheral blood cells. Previous studies have shown that enzymatic release of lymphocyte populations for flow cytometry analysis does not alter the original proportions of liver-infiltrating cells [3].

Analysis of simultaneous assessment of membrane determinants by flow cytometry

Acquisition and analysis for two- and three-colour immunofluorescent procedures was carried out using a FACScan flow cytometer (Becton Dickinson) and Lysis II software; 10^4 cells were counted per sample. The flow cytometry parameters were set up following the manufacturer's procedures for three-colour analysis using PerCP.

Table 1. General characteristics of primary biliary chirrosis (PBC) patients and controls

	Normals	PBC-pre-cirrhotics	PBC-cirrhotics	ALD
Number of subjects	19	21	22	15
Mean age (range) (years)	53 (37–75)	58 (44–71)	57 (37–72)	49 (39–72)
Female/male ratio	19/0	20/1	22/0	13/2
Per cent lymphocytes in leucocytes	32·66 (1·72)*	30·70 (1·96)	26·50 (2·20)	24·05 (2·78)
No. of lymphocytes ($\times 10^3/l$)	2244 (127)	1887 (138)	1541 (175)	1820 (250)
Per cent $CD4^+$ T cells	49·77 (1·65)	47·25 (1·8)	46·54 (1·78)	50·16 (2·49)
No. of $CD4^+$ T cells	1111 (70)	873 (63)	713 (79)	949 (145)

* Mean ± s.e.m.

ALD, Alcoholic cirrhotics.

Analysis of membrane determinant co-expression using triple-colour combinations (FITC = Fl-1, PE = Fl-2, PerCP = Fl-3) was done as previously described [20,21]. Samples were gated on forward and side scatter to select the lymphocyte population for analysis, backgated using CD45/CD14 to confirm the purity of the gate, and gated again on Fl-2 and Fl-3 to select $CD4^+$ ($PerCP^+$, Fl-3) lymphocytes. The analysis was done using single histogram plots of Fl-1 and Fl-2 and dot plot graphs of Fl-1 *versus* Fl-2 of the $CD4^+$ lymphocytes. The histograms allowed us to separate three subpopulations for each of the CD45 isoforms we were studying according to fluorescence intensity: high, intermediate or dim, and low (Fig. 1a–c). By transferring this information to the dot plots it was possible to define and quantify subpopulations within the $CD4^+$ lymphocytes according to their reciprocal or simultaneous expression of CD45RO/RA or CD45RO/RB (Fig. 1d–g). Results for the $CD4^+$ subsets defined by the CD45 isoforms are given as a percentage of the main $CD4^+$ population, and results of the memory subsets defined by the CD45RB isoform are given as a percentage of the total memory ($CD4^+CD45RO^{high}$) subset.

Storage and statistical analysis

Results for the lymphocyte subsets are expressed as mean ± s.e.m. All the results were stored and analysed using the MINITAB statistical software. Descriptive statistics were obtained for all the $CD4^+$ T cell subsets identified in each of the groups studied, and *t*-tests were done to compare results between the groups.

RESULTS

$CD4^+$ $CD45RO^+$, $CD4^+$ $CD45RA^+$ single-positive populations in PBL

No differences were seen between PBC patients and normal controls or chronic liver disease patients, with respect to the percentage of lymphocytes in the peripheral blood leucocyte population, or the percentage of $CD4^+$ cells in the PBL population. The percentage of lymphocytes in the peripheral blood leucocyte population was significantly lower in cirrhotic ALD patients than in normal subjects, as has been previously noted [22].

CD45RA and CD45RO were expressed reciprocally and had a continuous distribution among PBL (Fig. 1b–e). The total $CD45RO^+$ population consisted of $CD45RO^{high}$ cells and $CD45RO^{dim}$ cells; similarly, the total $CD45RA^+$ population consisted of $CD45RA^{high}$ and $CD45RA^{dim}$ cells.

The percentage of $CD4^+$ T cells expressing CD45RA was significantly lower in the PBC patient group compared with both normal controls and ALD patients (data not shown). No significant difference was seen between cirrhotic and pre-cirrhotic PBC patients. Conversely, the percentage of $CD4^+CD45RO^+$ cells was significantly higher in the PBC patient group than in controls. Again, no significant differences were seen between the PBC patient subgroups. As a result, the $CD4^+CD45RO^+/CD4^+CD45RA^+$ ratio was significantly increased in the PBC patients ($3{\cdot}27 \pm 0{\cdot}59$ in the pre-cirrhotic group and $3{\cdot}47 \pm 0{\cdot}44$ in the cirrhotics) compared with normal ($1{\cdot}33 \pm 0{\cdot}11$, $P \leqslant 0{\cdot}01$) and disease ($1{\cdot}69 \pm 0{\cdot}17$, $P \leqslant 0{\cdot}01$) controls. No significant differences were seen between cirrhotic and pre-cirrhotic PBC patients, and between normal and ALD controls. Supporting these results, the fluorescence intensity (analysed as relative median channel fluorescence) for CD45RO in $CD4^+$ cells was significantly increased in PBC patients (pre-cirrhotics $462 \pm 71{\cdot}7$, cirrhotics $514 \pm 65{\cdot}4$) compared with controls (normals $187 \pm 42{\cdot}6$, ALD $180 \pm 26{\cdot}2$, $P < 0{\cdot}005$), being 2·5 times greater in the PBC patient group; while the fluorescence intensity for CD45RA in $CD4^+$ cells was lower in PBC patients (pre-cirrhotics $7{\cdot}2 \pm 2{\cdot}8$, cirrhotics $8{\cdot}9 \pm 2{\cdot}4$) compared with normal subjects ($15{\cdot}2 \pm 3{\cdot}1$). However, this difference did not attain statistical significance.

Subsets defined by simultaneous expression of determinants in PBL

The simultaneous study of the expression of CD45RA and CD45RO on $CD4^+$ T cells allows a more precise definition of both naive ($CD45RA^{high}RO^-$), and memory ($CD45RA^-RO^{high}$) subpopulations. In the present study, the analysis of $CD4^+$ T cell subsets using three-colour flow cytometry allowed subdivision using this classification. The results were similar to those found using single isoform expression, that is to say a decreased naive and an increased memory subpopulation in PBC patients (both cirrhotic and pre-cirrhotic) compared with both control groups (Fig. 2a,b). Again no differences were seen between pre-cirrhotic and cirrhotic PBC patients, and between normal controls and patients with ALD.

The $CD4^+$ $CD45RA^-RO^{high}/CD45RA^{high}RO^-$ ratio was significantly higher for the PBC patient group than for both control groups, especially for the PBC pre-cirrhotic subgroup, in which the mean ratio was six times greater than the mean for normal controls and four times greater than the mean for ALD patients. There was a small, but not statistically significant, difference between pre-cirrhotic and cirrhotic PBC patients. Similarly, the difference between normal subjects and alcoholic cirrhotics was not significant (Fig. 2c).

No difference was found in the subset that co-expresses both isoforms at an intermediate level ($CD45RA^{dim}RO^{dim}$) between any of the groups compared (data not shown).

Subanalysis of the $CD4^+$ $CD45RO^{high}RA^-$ memory subpopulation according to CD45RB isoform expression in PBL

Within the $CD4^+CD45RO^{high}RA^-$ memory population further phenotypical subdivision was attempted according to the level of expression of CD45RB isoform. There was no significant difference in the relative distribution of these two subsets within the $CD4^+$ memory population in PBC patient groups compared with normal and disease controls. This is reflected in the analysis of the $CD4^+$ memory-2/memory-1 ratio, which was not significantly different in PBL between the groups: $4{\cdot}93(\pm 0{\cdot}82)$ and $3{\cdot}92(\pm 0{\cdot}61)$ for pre-cirrhotic and cirrhotic PBC patients, respectively, $4{\cdot}24(\pm 0{\cdot}54)$ for normal controls and $4{\cdot}6(\pm 0{\cdot}62)$ for ALD patients.

Analysis of liver-infiltrating lymphocytes

The results of the analysis of $CD4^+$ T helper cell subpopulations in LIL and peripheral blood, done the same day, on eight PBC patients and six ALD patients undergoing liver transplantation are shown in Fig. 3. All the changes observed in the distribution of the $CD4^+$ subsets in PBL between PBC patients compared with normal controls were more

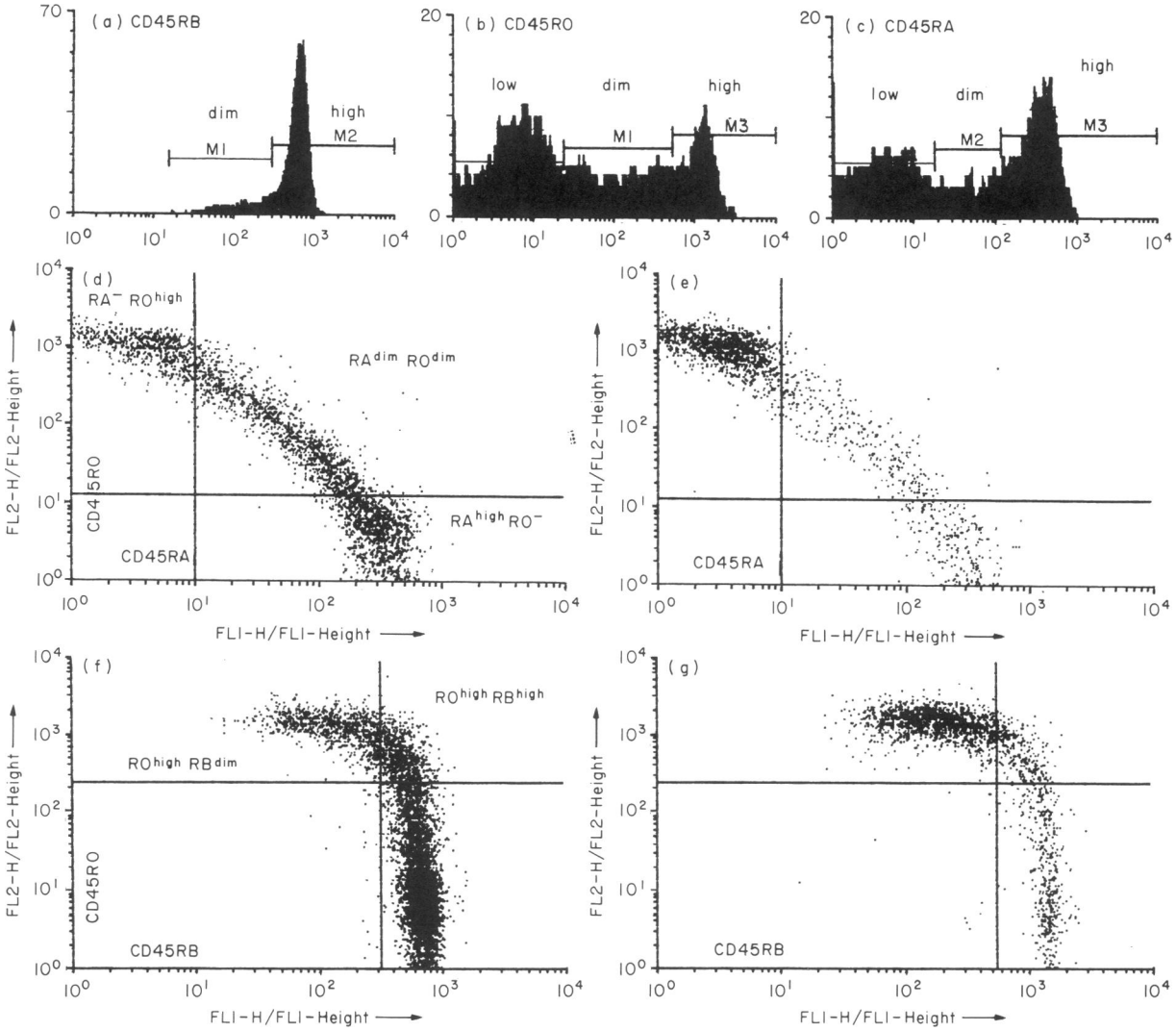

Fig. 1. (a) Representative flow cytometric profile of single-colour immunofluorescence of CD45RB. (b) RO and (c) RA on $CD4^+$ T cells. As these histogram plots show, there is a continuous distribution of antigen density on the cell surface, shown by the range of intensity levels detected. Two subpopulations can be identified in the histogram plot of CD45RB according to its level of expression: $CD45RB^{high}$ and $CD45RB^{dim}$. In the case of the CD45RA and CD45RO, three subpopulations can be identified: negative, dim and high. (d) Two-colour immunofluorescence plot of CD45RA (Fl-1) *versus* CD45RO (Fl-2) expression on a normal subject; it shows the reciprocal expression of these two markers and allows the identification of three subsets: $CD45RA^{high}RO^-$ (naive subset), $CD45RA^{dim}RO^{dim}$, $CD45RA^-RO^{high}$ (memory subset). (e) Dot plot of CD45RA *versus* CD45RO expression on a primary biliary cirrhosis (PBC) patient, showing an increase in the $CD45RO^{high}RA^-$ subset compared with the normal. (f) Two-colour immunofluorescence plot of CD45RB (Fl-1) *versus* CD45RO (Fl-2) expression on a normal subject, which allows the identification of two subpopulations within the memory ($CD45RO^{high}$) subset: $CD45RB^{high}$ (Th1-like) and $CD45RB^{dim}$ (Th2-like). (g) Dot plot histogram of CD45RB *versus* RO in a PBC patient, showing the redistribution of subsets.

pronounced in the analysis of LIL from PBC patients. That is, a predominant T helper memory subpopulation ($CD4^+CD45RA^-RO^{high}$) with a markedly decreased naive subpopulation ($CD4^+CD45RA^{high}RO^-$). As a consequence there was a significant increase in the $CD4^+$ memory/naive ratio in the LIL compared with the corresponding one in the PBL of the same patient and compared with the means obtained in PBL for all the groups of patients and controls.

The changes observed in the distribution of the liver-infiltrating $CD4^+$ T helper subsets of the ALD patients studied showed the same trends as in PBC patients, i.e. an increase in the $CD4^+$ memory subpopulation and $CD4^+$ memory/naive ratio, and a decrease in the $CD4^+$ naive subset in the tissue $CD4^+$ cells compared with the PBL of the same patient and with the means obtained for ALD and normals in the peripheral blood analysis. In spite of this, the memory/naive ratio in LIL was still significantly increased in PBC patients compared with the ALD patients.

With respect to the $CD4^+$ memory subset distribution according to the expression of the CD45RB isoform, there

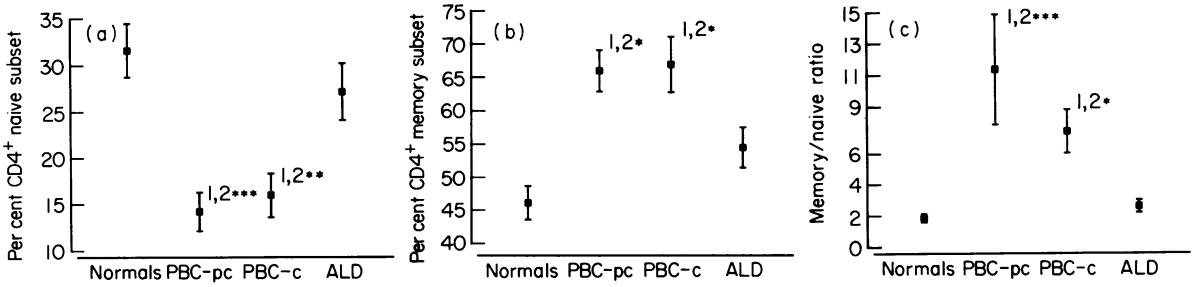

Fig. 2. Distribution of $CD4^+$ T cell subsets defined by simultaneous expression of CD45RA and CD45RO isoforms. (a) Mean percentage of $CD4^+$ naive cells ($CD45RA^{high}RO^-$) in peripheral blood lymphocytes (PBL), decreased in primary biliary cirrhosis (PBC) patients compared with controls. (b) Mean percentage of $CD4^+$ memory subset ($CD45RA^-RO^{high}$) in PBL, clearly increased in PBC patients compared with normals and ALD patients. (c) Ratio of the memory and naive subsets defined by co-expression of isoforms, again increased in PBC patients. [1]Statistically significant compared with normals, $P < 0.001$; [2]statistically significant compared with ALD patients; $*P < 0.05$; $**P < 0.005$; $***P < 0.001$.

was an expansion of the memory-2 subset in the $CD4^+$ tissue lymphocytes of the PBC patients studied, with a consequent decrease in the memory-1 subset, evident in the $CD4^+$ memory-2/memory-1 subsets ratio, which was significantly elevated in PBC patients ($P = 0.02$) (Fig. 3). No difference was observed in the distribution of the memory subsets 1 and 2 in the LIL of ALD patients compared with their peripheral blood distribution, or compared with the mean obtained for all the ALD in the peripheral blood analysis.

DISCUSSION

$CD4^+$ T cells are present in the portal tracts in PBC from the early stages of the disease. Their role is unclear, nor is their correlation with the distribution of $CD4^+$ T cell subsets in peripheral blood understood. We set up the present study to identify significant differences in peripheral blood and liver-infiltrating $CD4^+$ T cell subsets, according to a novel classification, which may play a role in the pathogenesis of PBC or be related to disease activity. Two-colour and three-colour flow cytometry were used to quantify simultaneous expression of phenotypical and functional subset markers (CD45 isoforms) on $CD4^+$ T helper cells in the blood and liver lymphocytes of PBC patients and controls (normal subjects and ALD). These markers were used to subdivide this heterogeneous population. The analysis showed a clear difference in the frequency of the $CD4^+$ subsets defined by the CD45 isoforms in PBC patients compared with controls.

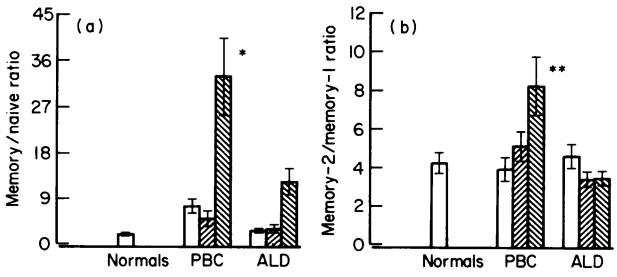

Fig. 3. Distribution of $CD4^+$ T cell subsets defined by the expression of CD45RA, CD45RO and CD45RB isoforms of the leucocyte common antigen (LCA) in liver-infiltrating lymphocytes (LIL) and peripheral blood lymphocytes (PBL) of eight primary biliary cirrhosis (PBC) patients and six ALD, who underwent orthotopic liver transplantation, comparing them with the means obtained for normals, cirrhotic PBC patients and ALD patients in PBL. (a) Memory/naive ratio ($CD45RA^-RO^{high}/CD45RA^{high}RO^-$). (b) Ratio of memory-2 over memory-1 subsets ($CD45RO^{high}RB^{dim}/CD45RO^{high}RB^{high}$) within the memory subpopulation ($CD45RA^-RO^{high}$). $*P < 0.05$; $**P = 0.02$ compared with LIL of ALD patients. □, Means of PBL study; ▨, concomitant PBL analysis; ▧, LIL.

Previous studies, done in limited numbers of PBC patients [2,3] and dividing T cell subsets according to the binding of only one MoAb to an isoform of the CD45 molecule (CD45RA or CD45RO), showed that in PBC there was a tendency towards a reduction in the $CD4^+$ T helper naive subpopulation, with a reciprocal increase in the memory/activated subpopulation. Our study, including a larger number of patients, confirmed those results. Similar changes were observed in both pre-cirrhotic and cirrhotic PBC patients, suggesting that they are not related to the histological stage of the disease. Our findings suggest that changes in the T cell subsets observed in PBL may be related to the etiology or pathogenesis of PBC and not secondary to the fibrotic changes in the liver, because they were not observed in the chronic liver disease control group.

Using two-colour immunofluorescence analysis of $CD4^+$ gated cells, which allows us to analyse simultaneously the co-expression of two isoforms of the CD45, we have shown that under this stricter classification, there are significant differences, greater than the ones reported previously using single-colour staining, in the distribution of $CD4^+$ T cell subsets between PBC patients and normal and disease controls.

In this study the reduction in the naive subpopulation ($CD4^+CD45RA^{high}RO^-$) and the increase in the memory subset ($CD4^+CD45RA^-RO^{high}$) were significantly greater in the LIL studied in PBC patients. In the case of the ALD LIL there was also a decrease in the naive and an increase in the memory population compared with the result obtained in the concomitant analysis of PBL. However, in the ALD subjects studied the effect was not as marked as in the PBC patients. In normal livers few lymphocytes if any can be seen, making it impossible to study them with flow cytometry; but immunohistochemical studies have shown that the population of a few scattered lymphocytes present in portal tracts and intralobular parenchyma in normal livers contains both $CD45RA^+$ and $CD45RO^+$ cells [23].

An altered profile of T cells with a decreased naive ($CD45RA^+$) subset and an increased memory/activated subpopulation (CD45RO+) has been reported previously in many autoimmune diseases [24], including multiple sclerosis, myasthenia gravis [25] and rheumatoid arthritis (RA) [17,18,26]. In most of the cases the abnormal distribution of $CD4^+$ T cell subsets has been noted in tissue-infiltrating lymphocytes without any change in PBL. In the few cases in which a significantly abnormal distribution was observed in peripheral blood, the changes in the subpopulations in tissue lymphocytes were always more marked, as in this study. The presence of an abnormal distribution of $CD4^+$ T cell subsets in PBL in PBC could be related to the finding of autoreactive T cells in the peripheral blood of patients with the disease [27].

The analysis of dual expression of isoforms also allows a further functional subdivision of the memory subset, based on the expression of the CD45RB isoform by the $CD45RO^{high}$ subset, as already mentioned [4,10,15,18]. The distribution of the memory population in memory-1 and memory-2 subsets was similar for the PBC patient groups, pre-cirrhotic and cirrhotic, and for normal controls and ALD patients in PBL. However, the study of LIL obtained from livers of PBC patients showed a clear expansion of the $CD45RA^-RO^{high}RB^{dim}$ subpopulation (memory-2) in the $CD4^+$ memory subset, with a significant decrease in the memory-1 subset. As mentioned earlier, this was the only difference observed in the distribution of $CD4^+$ subsets in LIL between PBC patients and ALD, and it must be considered significant because it is completely independent of the general expansion of the $CD4^+$ memory subset, which was observed for tissue-infiltrating lymphocytes of both PBC and ALD patients. Results obtained with lymphocytes from the synovia of RA patients and other inflammatory joint disease patients have found a similar distribution with a real expansion of this subset (memory-2) [17,18]. This finding could be related to the pathogenesis of PBC, because the same subpopulation of $CD4^+$ T cells isolated from synovial T cells in RA patients has been shown to be constituted by cells that are potent helpers for B cell immunoglobulin production and to have a lower capacity to down-regulate B cells [18].

The relation of a predominance of memory $CD4^+$ helper cells in the peripheral circulation and liver infiltrates, such as the one found in our study, with the pathogenesis or activity of PBC is yet uncertain, but it has been proposed that inappropriate helper signals from $CD4^+$ T cells to B cells or cytotoxic T cells are probably one of the critical elements in the breakdown of self tolerance [16]. In different autoimmune diseases the same kind of overwhelming accumulation of memory-type T cells in the tissue lesions has been described. Under these circumstances, an important feature of the memory T cells is that they are functionally potent and require less of an activation signal for stimulation than naive cells [28], so lower affinity interactions or lower amounts of antigen can stimulate them, which may explain the generation of self-reactive T cells. The peripheral blood and liver T helper cells of PBC patients exhibit a phenotype that may be important in the initiation and/or perpetuation of PBC, as has been proposed for other diseases like RA [18].

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