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T-Lymphocyte Subsets in Liver Tissues of Patients with Primary Biliary Cirrhosis (PBC), Patients with Primary Sclerosing Cholangitis (PSC), and Normal Controls

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T lymphocytes infiltrating hepatic tissues were typed and enumerated in liver biopsies of patients with primary biliary cirrhosis (PBC), patients with primary sclerosing cholangitis (PSC), and normal controls using monoclonal antibodies and the avidin–biotin–immunoperoxidase technique. The peripheral blood mononuclear cells were studied also by flow cytometry. In PBC, T lymphocytes were decreased ($P < 0.001$) in the blood [absolute number was 426 ± 200 (SE) vs 1351 ± 416 in 15 controls], as was the helper/suppressor (T4/T8) ratio (1.0 ± 0.1 vs normal 2.3 ± 0.3). T lymphocytes were the most numerous mononuclear cells infiltrating portal areas of PBC livers: 749 ± 93/5 high-power fields (HPF) in PBC vs 98 ± 15/5 HPF ($P < 0.01$) in controls. The T4/T8 ratios varied from 0.9 to 2.3 (mean, 1.8 ± 0.1) in the portal triads (normal mean, 1.6 ± 0.1), with the T4+ cells accounting for more than 75% of infiltrating T cells. In contrast, the mean T4/T8 ratio in portal triads of PSC was reduced (1.0 ± 0.3) due to a significant increase ($P < 0.001$) in the number of T8+ cells. The T cells around and in the walls of bile ducts in PBC were mostly T8+, and the T4/T8 ratio was 0.8 ± 0.2. No T8+ cells were seen in this location in PSC and normal livers. Few mononuclear cells were present in hepatic lobules. Subtyping of T lymphocytes in liver tissues of patients with PBC and PSC may be helpful in the differential pathologic diagnosis. In patients with advanced PBC, a decrease in T4+ cells in the blood appeared to be accompanied by their accumulation in the portal triads. In contrast, T8+ cells accumulated preferentially around bile ducts.

KEY WORDS: Mononuclear cells in liver tissues; primary biliary cirrhosis; monoclonal antibodies to lymphocytes; avidin–biotin–immunoperoxidase method; T4/T8 ratios in hepatic tissues.

INTRODUCTION

The etiology of primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) remains unknown. Although immunologic phenomena are thought to be important in the pathogenesis of PBC, it is not clear how they relate to the initiation and progression of the disease. The presence of autoantibodies (1, 2) to various hepatobiliary antigens, smooth muscle (ASM), mitochondria (AMA), and nuclear (ANA) antigens, elevations in serum IgM concentration (3), and the high prevalence of circulating immune complexes (4) in these patients all point to aberrant humoral immunity in PBC. In addition, patients with PBC are known to have abnormalities in cellular immunity as evidenced by *in vitro* reactivity of their peripheral blood lymphocytes to liver and biliary antigens (5, 6) and increased cytotoxicity of their lymphocytes for a variety of target cells including hepatocytes (7, 8). Moreover, the histopathologic finding of prominent mononuclear cell infiltrates in the portal tracts and around the bile ducts (9) in patients with PBC suggests that the bile duct injury characteristic of this disease may be mediated by lymphocytes sensitized to an as yet unidentified antigen or antigens (10). Also, PBC is often associated with other autoimmune diseases such as thyroiditis, Sjogren's syndrome, rheumatoid arthritis, and progressive systemic sclerosis (11). Little is known about the immunopathology of PSC.

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Abnormalities in the immunoregulatory T lymphocytes in the circulation of many patients with PBC have been described and consist of a relative decrease either in the helper–inducer (T4+) or in the cytotoxic–suppressor (T8+) lymphocyte populations (12, 13). Importantly, these changes in immunoregulatory cells were reported to vary according to disease severity, in that patients with depressed cytotoxic–suppressor (T8+) cells in the circulation (increased T4/T8 ratio) tended to have more advanced disease (13).

Monoclonal antibodies to lymphocyte surface antigens were used in some of the described studies to discriminate between the two functional subpopulations of lymphocytes (12, 13). While it is now known that each of these subpopulations may be functionally heterogeneous and that, for example, not all lymphocytes with the T4+ phenotype represent helper T cells (14), phenotypic analysis offers a means of identifying different lymphocytes at diseased sites and in relation to other cells in the tissue. We have used monoclonal antibodies to lymphocyte subsets and the avidin–biotin–peroxidase complex (ABC) technique (15) to characterize and enumerate lymphocyte subpopulations in tissue sections of liver biopsies in patients with PBC, patients with PSC, and normal controls. Specifically, we have analyzed the composition of inflammatory infiltrates present in the portal tracts and parenchyma of diseased livers, with particular attention to the cells around the bile ducts, in the hope of identifying the putative effector cell(s) involved in histopathologic changes characteristic of PBC and PSC.

MATERIALS AND METHODS

Patients and Biopsies. Twelve liver biopsies from 10 females ranging in age from 40 to 55 years with advanced PBC were studied. Two patients had consecutive liver biopsies which were obtained 1 year apart. In addition, biopsies were obtained from six patients with primary sclerosing cholangitis (PSC). None of these patients was treated with immunosuppressive drugs. The diagnosis of PBC was made on the basis of clinical and biochemical factors, characteristic histologic changes on liver biopsy, and/or radiologic data. Table I lists the clinical, histologic, and immunologic features of the PBC and PSC patients studied. The 10 patients with primary biliary cirrhosis whose liver tissues were available for study all had advanced or end-stage disease (Table I). The disease duration (i.e., from appearance of symptomatic disease) ranged from 2 to 10 years, with a mean of 5 years. Histologically, all the tissues examined were at the scarring (III) or cirrhotic (IV) stages (Table I). Two patients were treated with penicillamine and one was treated with prednisone at the time of liver biopsy. Seven of the patients with PBC and four with PSC underwent orthotopic liver transplantation, and thus, large pieces of their original livers were available for study. In addition, liver biopsies were obtained from 11 normal individuals who were biopsied because of medical indications and were found to have no histologic or biochemical evidence of liver disease.

A portion of each biopsy was embedded in Tissue Tek OCT medium immediately after it was obtained. Frozen serial sections were cut in an Ames cryostat and processed as described below.

Monoclonal Antibodies and Reagents. Monoclonal antibodies were purchased from Becton–Dickinson, Inc., Sunnyvale, CA (Leu 2, Leu 3a, Leu 4, and Leu 7). The following monoclonal antibodies were purchased from Ortho Pharmaceutical Corp., Raritan, NJ: OKT1, OKT3, OKT4, OKT8, OKT6, and OKM1. T11, T4, T8, MO2, and B1 were from Coulter Electronics, Inc., Hialeah, FL. Whenever possible, two monoclonal antibodies of the same specificity but from different commercial sources were employed. Because OMK1 antibody is not absolutely specific for macrophages in that it reacts with granulocytes and certain natural killer (NK) cells, we compared liver sections stained with OKM1 to those stained with MO2 in selected cases. Biotinylated anti-mouse IgG and avidin–biotinylated horseradish peroxidase complex were purchased from Vector Laboratories, Inc., Burlingame, CA. The chromogen, 3-amino-9-ethylcarbazole (AEC), and Mayer's hematoxylin were purchased from Sigma, St. Louis, MO. Aqua-mount used for mounting the stained section was obtained from Lerner Labs, Greenwich, CT.

Working dilutions of the primary antisera were determined by titration using sections of human tonsils and cytocentrifuged smears of normal blood lymphocytes.

Staining Procedure. Cryostat sections (4 μm) were cut, air-dried for 12–24 hr at room temperature, and stored at −20°C until staining was performed. Prior to staining, sections were fixed in acetone for 10 min, air-dried, and washed in PBS, pH 7.4. The avidin–biotin–peroxidase complex

Table I. Clinical, Immunologic, and Histologic Features of the Patients with Primary Biliary Cirrhosis and Primary Sclerosing Cholangitis

Patient	Histologic stage	Enceph.	Ascites	Esoph. varices	P.T.	Bilirubin, total/direct (mg/100 ml)	SGOT/ SGPT (IU/L)	Alk. phos. (IU)	γ-globulin (g/100 ml)	AMA (titer)
Primary biliary cirrhosis										
1. BM										
1980	IV	0	−	+	16/12	15/9	188/162	575	1.6	1/1520
1981	IV	0	+	+	16/12	16/21	183/88	674	1.7	1/1520
2. GW	IV	+1	−	−	18/12	Total 46	282/230	1120	2.0	1/100
3. JW	IV	+3	+	+	12.8/NA	41/21	211/82	289	2.2	1/500
4. HW	IV	0	−	−	15/12	9/5.4	89/186	1315	3.4	Neg
5. MB	III	0	−	−	15/12	4.3/4.0	339/189	3420	1.6	1/40
6. VD	III	0	−	−	12/11.7	11/7.2	106/147	477	1.5	1/300
7. LW	III	+1	−	+	13/11.4	11/16	137/NA	675	3.7	1/100
8. FH										
1981	III	+1	−	+	16/11	11.6/8	378/284	1213	2.0	1/40
1982	III	+1	−	+	16/11	15.6/11.0	286/249	1238	2.0	1/40
9. SS	IV	+1	+	−	11.8/11.3	29/32	160/65	2150	1.8	Neg
10. RG	III	+2	−	+	13.1/11.8	5.9/8.8	258/255	480	4.9	1/100
Primary sclerosing cholangitis										
CC		−	−	−	12.3	1.3/0.2	110/87	660	1.4	Neg
MR		+	+	+	14.5	7.9/6.0	62/177	346	1.7	Neg
MB		+	−	+	14.4	13/10.1	288/187	3700	2.0	Neg
DM		+	−	+	14.7	26.4/17.4	100/34	142	2.2	Neg
FL		+	−	+	20.1	14.8/11.7	105/89	912	2.9	Neg
DA		+	+	+	15.5	17.4/2.8	205/85	2170	2.1	Neg

(ABC) technique of Hsu (15) was used for immunostaining with minor modifications. The tissues were overlayed with the appropriately diluted primary antibody and incubated for 15 min in a humidified chamber at room temperature. The sections were then washed in three changes of PBS and incubated with biotinylated anti-mouse IgG for 15 min. After another wash in PBS, avidin–biotinylated–peroxidase complex (ABC) was added for 15 min. The sections were then incubated in 3-amino-9-ethylcarbazole (AEC) with 0.01% hydrogen peroxide for 10 min to develop the color, washed in PBS, counterstained with Mayer's hematoxylin for 10 min, and mounted in Aqua-mount for light microscopy. Negative controls without the primary antibody were included. To check for the presence of endogenous peroxidase activity, tissues were incubated with the substrate alone. Blocking of endogenous avidin-binding activity was performed prior to staining with monoclonal antibodies using avidin and biotin as described by Wood and Warnke (16).

Enumeration of Cells in Tissue Sections. Mononuclear cells immunostained red–brown with monoclonal antibodies were counted in the portal areas and parenchyma in serially cut sections using a 7 × 7 grid in the ocular at 400× magnification. Cells in five representative high-power fields of each biopsy were enumerated, and results expressed as a mean count ± SE. In 11 cases, large pieces of liver (removed at the time of a transplant) were available for analysis so that larger sections sampled from different areas of removed livers could be studied and compared. To enumerate lymphocyte subpopulations around the bile ducts, the ocular grid was positioned in such a way that an area surrounding the duct and equal to two grid divisions at high power was examined in each instance. Statistical analyses of the cell counts were performed using Student's *t* test.

Analysis of Lymphocyte Subsets in Blood. Cytofluorographic analysis of T-cell populations was performed by indirect immunofluorescence using standard procedures (e.g., Ref. 13). Briefly, mononuclear cells were isolated by centrifugation on Ficoll–Hypaque gradients from the peripheral blood obtained at the time of liver biopsy. Washed cells were incubated on plastic petri dishes for 45 min at 37°C to remove monocytes. The nonadherent cells generally were found to contain less than 1% monocytes as determined by staining of smears with α-naphthyl acetate esterase. The recovered lymphocytes were checked for viability using trypan blue, diluted to an appropriate concentration with medi-

um, and stained with fluoresceinated monoclonal antibodies. The analysis was performed using a FACS IV flow cytometer (Becton–Dickinson, Sunnyvale, CA).

RESULTS

Mononuclear cells (MNC) stained with monoclonal antibodies had a characteristic red reaction product deposited along their periphery and could be easily recognized and enumerated in liver tissues. Control sections incubated with PBS were not stained. Considerable background staining was sometimes present in liver sections stained with OKT4 or Leu 3a antibodies even after blocking for endogenous peroxidase and avidin-binding activities. Large pieces of hepatic tissue were available in 11 cases, and sections could be stained with several antibodies of the same specificity but from different commercial sources. Results obtained with liver sections using the OKT, Leu, and Coulter reagents were comparable. In terms of reactivity with OKM1 and MO2 antibodies, we found a comparable number of positive cells in normal tissues. However, OKM1+ cells were sometimes, but not always, slightly more numerous than MO2+ cells.

A significant increase ($P < 0.001$) in the total number of inflammatory cells was observed in the portal areas of PBC and PSC livers as compared to normal livers (Table II). Despite such differences in the overall number of cells per areas examined, the relative proportions of the various mononuclear cells (MNC) in the portal areas of PBC, PSC, and normal livers were quite similar. Specifically, T lymphocytes (T11+) were the most numerous MNC. Their mean number/5 high-power fields ± SE in the examined portal areas was 749 ± 93 in PBC, 526 ± 110 in PSC, and 98 ± 15 in normal control tissues (Table III). This represents a significant accumulation ($P < 0.001$) of T lymphocytes in portal triads of diseased livers compared to the controls (Figs. 1 and 2a). In contrast, no significant increase in T lymphocytes was observed in the lobules in either of the two diseases studied (Table III). The numbers of OKM1+ cells in the portal infiltrates were higher ($P < 0.01$) in diseased livers (75 ± 18 in PBC; 73 ± 20 in PSC) than in control tissues (7 ± 4). B cells were increased in number ($P < 0.05$) only in the PBC infiltrates (66 ± 19 vs 18 ± 6 in PSC and 10 ± 6 in the controls). Only rare Leu 7+ cells were seen (Table II) but these were stained very intensely.

To determine which T-lymphocyte subsets predominated in the liver tissues, we phenotyped and enumerated the T8+ and T4+ populations in all the livers studied (Fig. 1, Table III). In PBC, T4+ cells (see Figs. 2b and c) were the major component (>75%) of the T lymphocytes infiltrating portal areas, and their number was significantly increased ($P < 0.05$) in comparison to that of the controls. In contrast, the mean number of T8+ cells was not significantly increased in the portal tracts of PBC livers (Fig. 1). The T4/T8 ratio in the portal areas of PBC livers ranged from 0.9 to 2.3 (mean, 1.8 ± 0.1; see Fig. 1) and was not statistically different from that of normal tissues (mean, 1.6 ± 0.1). Cells expressing both the T8 and the T4 antigens were not many in the portal areas as indicated by the fact that the sum of T4+ and T8+ cells was only slightly higher than the number of T11+ cells (787 ± 85 vs 749 ± 93). The portal T4/T8 ratio in PBC differed significantly ($P < 0.01$) from that found in the tissues of patients with PSC (1.0 ± 0.3). The difference between these two diseases was due to a relative increase in the proportion of T8+ cells in the PSC livers ($P < 0.001$; see Fig. 1). The numbers

Table II. Average Percentages of MNC in Liver Infiltrates in Primary Biliary Cirrhosis (PBC), Primary Sclerosing Cholangitis (PSC), and Controls

MNC	PBC ($N = 12$)		PSC ($N = 6$)		Normal ($N = 11$)	
	Portal areas	Lobules	Portal areas	Lobules	Portal areas	Lobules
T lymphocytes	85.7 ± 1.9	75.4 ± 2.5	83.6 ± 2.9	64.0 ± 3.0	90.0 ± 1.8	64.0 ± 2.1
B lymphocytes	6.0 ± 1.3	Rare	2.6 ± 2.9	Rare	3.0 ± 1.2	2.0 ± 0.1
Macrophages	8.6 ± 2.1	21.2 ± 2.8	12.2 ± 2.6	27.3 ± 1.0	6.0 ± 2.1	33.0 ± 1.3
NK (Leu 7+)	Rare	Rare	Rare	Rare	Rare	Rare
Total no. of MNC/5 HPF[a]	885.0 ± 120.0	96.0 ± 13.0	623.0 ± 168.0	55.0 ± 6.0	106.0 ± 18.0	43.0 ± 8.6

[a]The data are presented as means ± SE. The differences between normal livers and livers of patients with PBC and with PSC were significant at $P < 0.001$ for portal areas only.

Table III. Lymphocyte Subpopulations in Liver Tissues and Blood of Patients with Primary Biliary Cirrhosis (PBC), Patients with Primary Sclerosing Cholangitis (PSC), and Controls

Lymphocyte subpopulation	No. of cells/5 HPF[a]		Blood	
	Portal areas	Lobules	%	Absolute no.[a]
PBC	$N = 11$	$N = 7$	$N = 4$	
T11+	749 ± 93	71 ± 10	41 ± 14	426 ± 200
T4+	500 ± 59	36 ± 8	23 ± 7	214 ± 100
T8+	289 ± 33	38 ± 4	21 ± 7	202 ± 100
T4/T8	1.8 ± 0.1	0.97 ± 0.2	1.0	
PSC	$N = 6$	$N = 6$		
T11+	526 ± 110	35 ± 4		
T4+	281 ± 80	17 ± 1.5		
T8+	276 ± 72	19 ± 1.5	ND[b]	
T4/T8	1.0 ± 0.1	0.9 ± 0.1		
Normal	$N = 11$	$N = 6$	$N = 15$	
T11+	98 ± 15	35 ± 2	78 ± 2	1350 ± 109
T4+	59 ± 10	23 ± 1.2	50 ± 10	862 ± 70
T8+	39 ± 6	12 ± 1.3	25 ± 2	406 ± 142
T4/T8	1.6 ± 0.1	2.3 ± 0.6	2.3 ± 0.3	

[a]Mean counts ± SE.
[b]Not done.

and localization of lymphocytes in liver tissues of the three treated and seven untreated patients with PBC were the same.

In four patients with PBC, we were able to quantitate the T-lymphocyte subpopulations in the peripheral blood as well as in liver tissues. As shown in Table III, T lymphocytes were decreased ($P < 0.01$) in the blood (absolute mean number was 426 ± 200 vs 1351 ± 416 in 15 controls), and the T4/T8 ratio was reduced also (1.0 ± 0.1 vs the

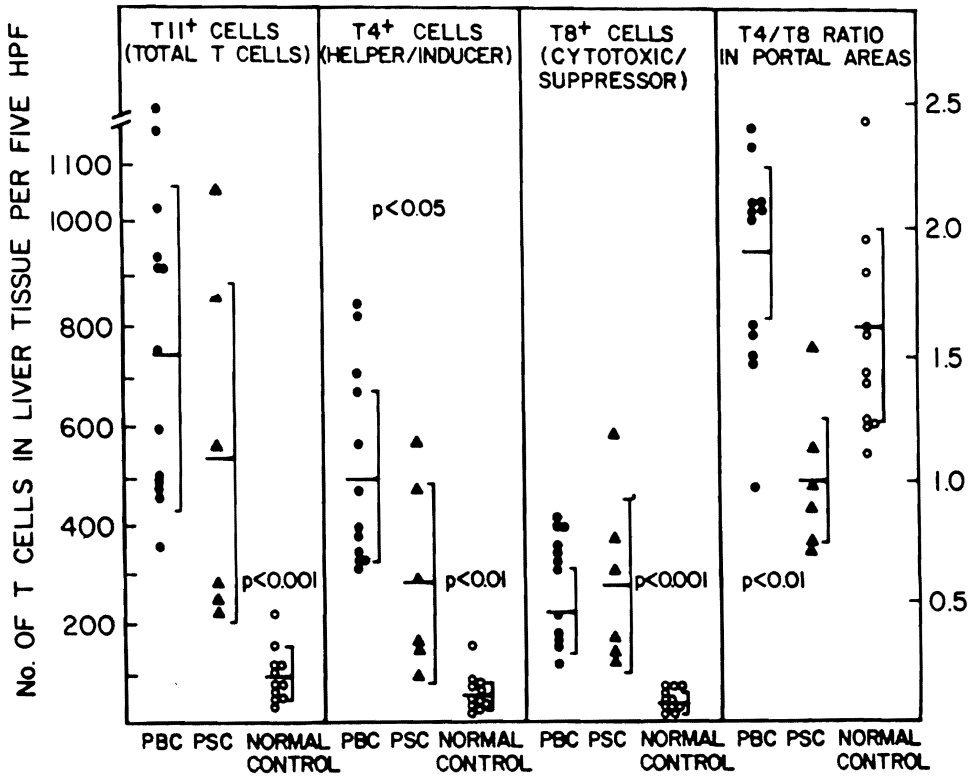

Fig. 1. The numbers of T lymphocytes, T4+ cells, and T8+ cells in portal tracts of livers in patients with primary biliary cirrhosis (●) and sclerosing cholangitis (▲) and in normal livers (○). The horizontal bars indicate means. The vertical lines denote ±1 SD.

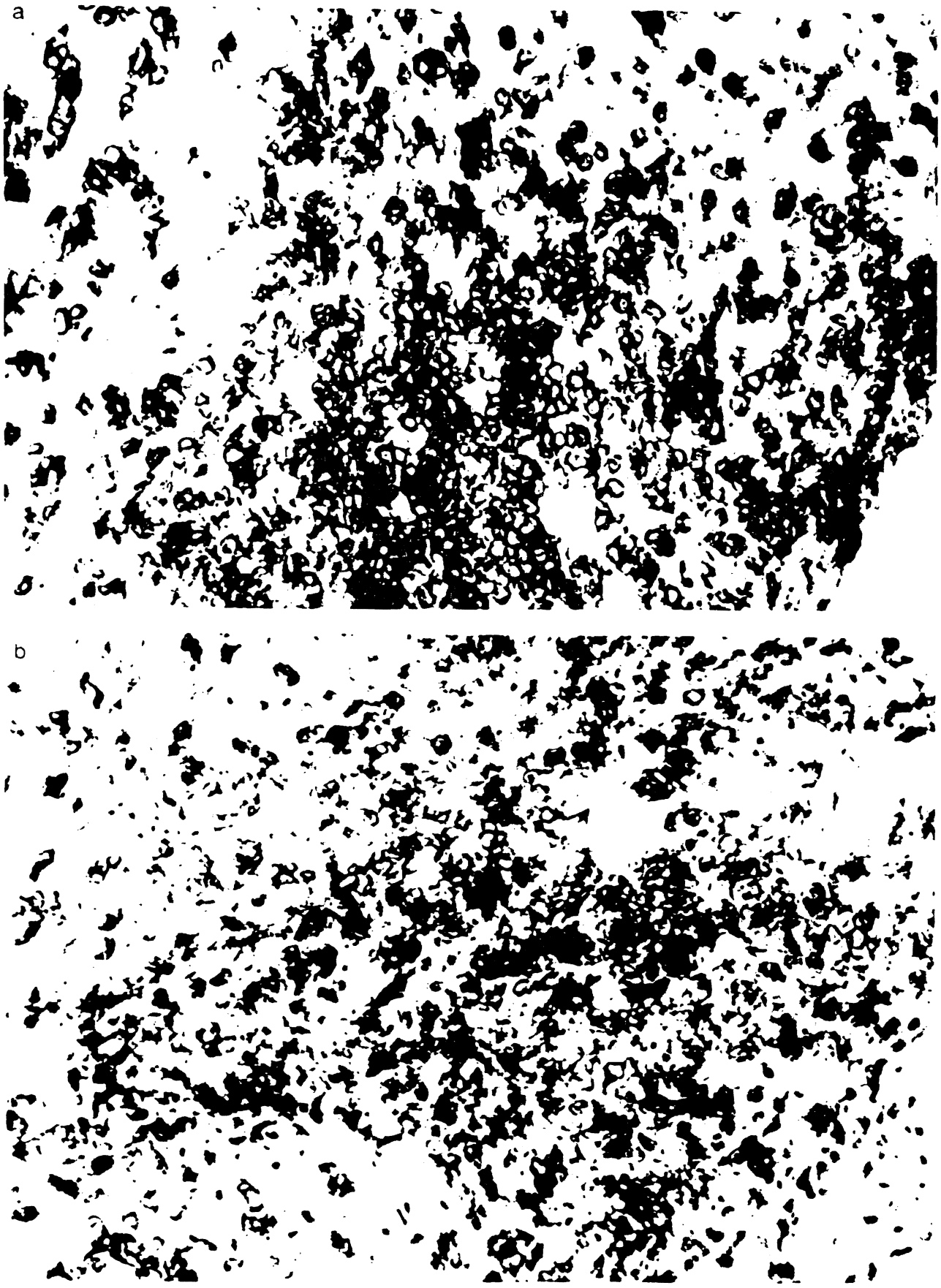

Fig. 2. T lymphocytes in liver tissues of patients with primary biliary cirrhosis. T cells were seen scattered in the septa and infiltrating parenchyma in the areas adjacent to septa. Limiting plates were frequently especially strongly infiltrated. Focal accumulations of T cells could be found in the septa (a) or in liver parenchyma. Most of the T lymphocytes in the portal areas were T4+ (b). The minority had a T8 phenotype (c). The ABC techniques. ×400.

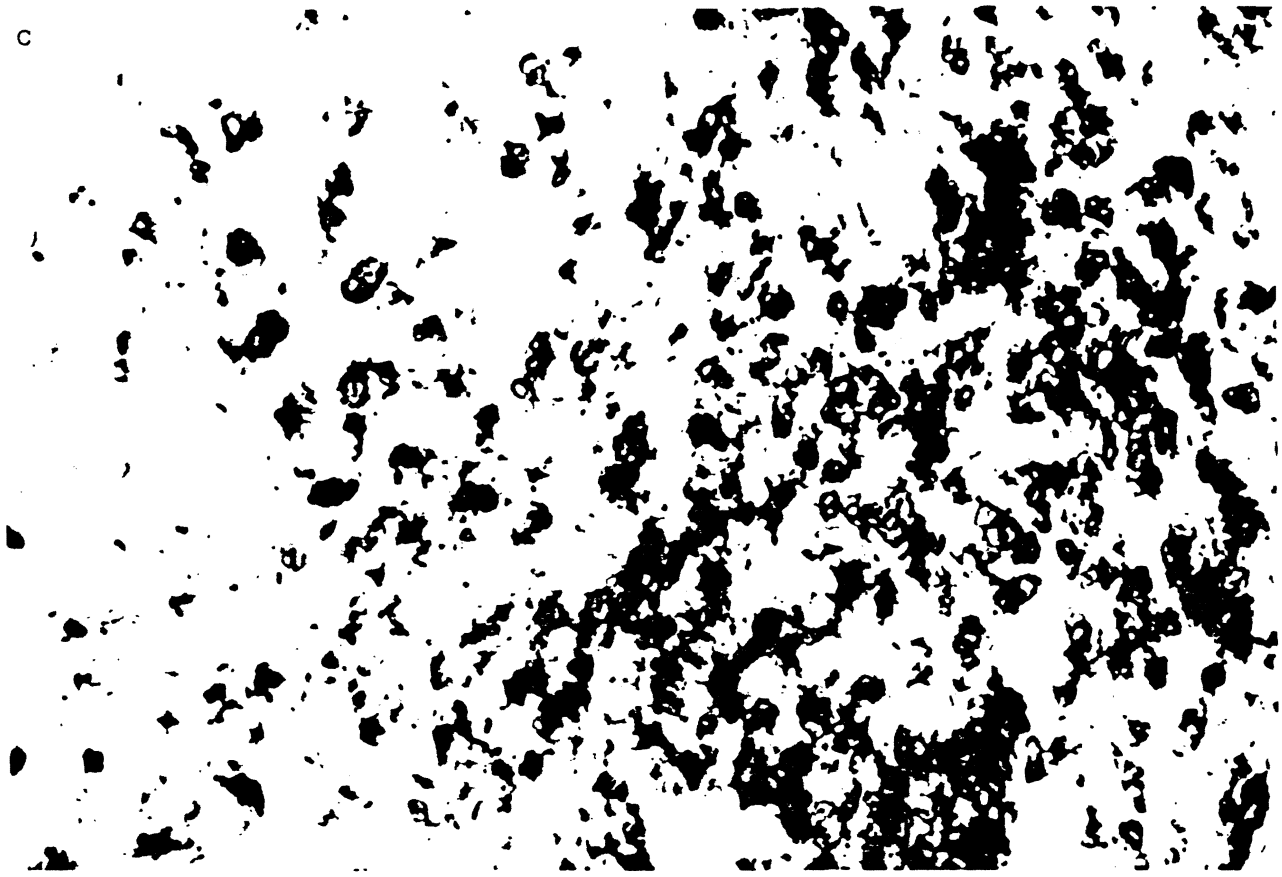

Fig. 2. Continued.

normal ratio of 2.3 ± 0.3). The T4/T8 ratio in hepatic lobules of PBC was similar to that in the peripheral blood. Few infiltrating MNC were present in lobules, and in comparison to normal controls, there appeared to be a preferential increase in T8+ cells in the liver parenchyma of diseased livers.

Because destruction of the bile ducts is a characteristic feature of PBC, we subtyped and enumerated T lymphocytes around and in the wall of the bile ducts in six of our cases. The T cells in the walls and around bile ducts were predominantly of the T8 phenotype (Fig. 3). The T4/T8 ratio in these areas was 0.79 ± 0.2, compared with the ratio of 1.8 ± 0.2 for the portal triads in the same liver specimens (Table IV). This indicates that T8+ (suppressor–cytotoxic) cells accumulated preferentially around and in the walls of bile ducts in PBC. In contrast, only rare T lymphocytes could be seen in the bile duct walls in PSC. Among 35 bile ducts studied in normal livers, 7 had occasional T lymphocytes in their walls, and these were all subtyped as T4+ cells.

Thus, a decrease in T4+ cells in the peripheral blood and their concomitant increase in the portal areas of livers were observed in patients with PBC. Also, a selective enrichment of T8+ (suppressor–cytotoxic) cells around bile ducts and in the areas of "piecemeal" necrosis was a characteristic feature of PBC.

DISCUSSION

In this study, we have quantitated and subtyped mononuclear cells (MNC) in the inflammatory infiltrates present in liver tissues obtained from patients with primary biliary cirrhosis (PBC), patients with primary sclerosing cholangitis (PSC), and normal controls. PBC is characterized by the presence of mononuclear inflammatory cells in the portal tracts and especially in and around the walls of bile ducts (9, 17). It has been postulated that the infiltrating

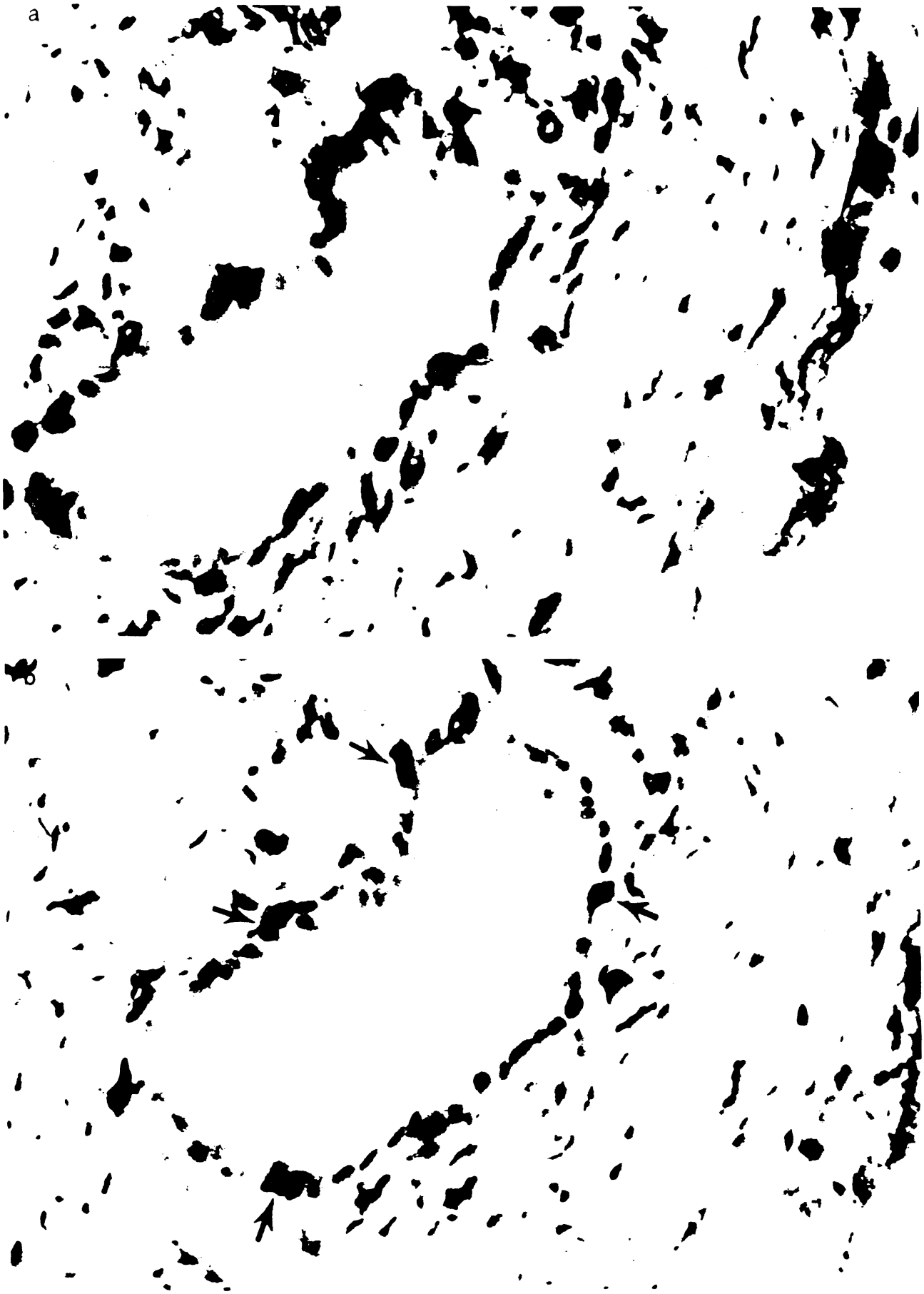

Fig. 3. T lymphocytes around the bile ducts in the liver biopsy of a patient with primary biliary cirrhosis (a). The T8+ lymphocytes (b) are more numerous in this area than T4+ lymphocytes (c); see arrows. ×640.

Fig. 3. Continued.

lymphocytes sensitized to autoantigens mediate the destruction of bile ducts and thus represent an immunologic effector mechanism that may play a central role in the pathogenesis of PBC (10).

The availability of monoclonal antibodies to surface antigens on MNC has made it possible to characterize and enumerate these cells in liver tissues using immunohistopathologic techniques (15). The study of lymphocyte subpopulations at the site of tissue injury offers an advantage of determining a relationship between the histologic changes and the type of inflammatory infiltrate present. Such relationships might provide insights into possible pathogenetic mechanisms operative in the course of a disease and thought to be due, at least in part, to a derangement in immunoregulation.

Numerous recent studies indicate the existence of abnormalities in the numbers of T lymphocytes and their function as well as in the distribution of T-lymphocyte subsets in the circulation of patients with PBC (12, 13, 18–20). The T lymphocytopenia seen in many of these patients (12, 20) has been reported to be due variously to decreases in the circulating T4+ cells (12) or T8+ cells (13). Whichever is the case, an altered T4/T8 ratio appears to be a consistent feature in the peripheral blood of patients with PBC. There is some evidence that the increased T4/T8 ratio is due primarily to a decrease in T8+ cells which tends to occur in patients with more advanced stages of the disease, as reflected by elevated serum bilirubin levels and grades III and IV histologic changes (13). This suggests that in advanced PBC, circulating T lymphocytes may (a) be deficient in numbers as well as in their suppressive activities and (b) be redistributed, with a preferential migration of T8+ cells to the site of tissue damage within the liver. In fact, a decreased suppressor activity in the blood of patients with PBC has been reported previously (21). Our results of a reduction in circulating T lymphocytes and a preponderance of T4+ cells within the portal tracts of livers with PBC are consistent with some of the

Table IV. Lymphocyte Subpopulations Around and in the Walls of the Bile Ducts and in the Portal Areas of Six Patients with PBC

MNC	Number/8 bile ducts[a]	Number/5 HPF in portal areas[a]
T lymphocytes		
T11+	41 ± 16	771 ± 160
T4+	18 ± 6	506 ± 102
T8+	21 ± 11	294 ± 45
T4/T8	0.79 ± 0.2	1.8 ± 0.2
B lymphocytes	None	81 ± 26
Macrophages	None	54 ± 12

[a]Data presented as means ± SE. In one patient, there were no T8+ cells and only T4+ cells were present around the two bile ducts examined. HPF, high-power field; see Materials and Methods.

previously published data (13, 21). On the other hand, Montano *et al.* (22) and Pape *et al.* (23) reported increased proportions of T8+ lymphocytes in portal tracts and parenchyma of livers from patients with PBC. When we examined the nature of the infiltrates surrounding the bile ducts and in the areas of "piecemeal" necrosis (24), a distinct enrichment in T8+ cells was observed (Table IV and Fig. 3), indicating their preferential accumulation at those tissue sites where disease activity appears to be histopathologically most important. It is not possible on the basis of phenotypic analysis alone (14) to determine whether these T lymphocytes are performing a cytotoxic function. Still, their presence in the areas of active tissue injury suggests that they might be involved in a cytotoxic rather than suppressor activity.

It has been suggested by Eggink *et al.* that the accumulation of numerous T4+ cells in the portal tracts in PBC may be related to the granulomatous destructive cholangitis which characterizes this disease or to the presence of plasma cells in this area (25). While we did not specifically enumerate plasma cells in our sections, we did look at B lymphocytes and found only a few such cells in the portal tracts of livers with PBC. Also, granulomas were not observed in any of our sections, yet, accumulations of T4+ cells and their overall enrichment in portal areas of PBC livers were a constant and reproducible finding. It could be argued that some of these T4+ cells may not be T lymphocytes. Immunoelectron microscopic analysis of mucosal lymphocytes in human small intestine showed that a small number of T4+ cells had ultrastructural characteristics of macrophages (26). It remains to be determined if some T4+ cells in human liver tissues also express macrophage-like features.

We would like to emphasize that all of our patients had advanced PBC (stages III and IV). Possibly, the nature of lymphocytic infiltrates in the liver of patients with PBC changes as the disease progresses. Additional studies are needed to determine how changes in the immunoregulatory cells in the liver relate to the severity of the disease. Our data suggest that the nature of MNC infiltrate may vary from area to area within the organ itself. In this context, it is most important to study large biopsy specimens or biopsies from different areas of diseased livers, as tissue sampling may introduce an important error in attempts to subtype lymphocytes in smaller tissue samples. The differences in numbers of T4+ cells in the portal areas between our and two other recent studies (22, 23) may be explained in terms of tissue sampling. Both Montano *et al.* (22) and Pape *et al.* (23) used needle biopsies, and the former group emphasizes that most of their sections contained only one identifiable portal tract. As 11 of the patients in this study underwent liver transplantation, large pieces of tissue were available to us for typing of MNC infiltrates.

In contrast to PBC, in primary sclerosing cholangitis, the MNC infiltrate in the portal areas is enriched in T8+ cells. As a result, the tissue TH/TS ratio was significantly lower than that found in normal or PBC livers. In addition, there were no T8+ cells observed in the walls of bile ducts in the cases we studied. This preferential accumulation of T8+ cells in portal areas of PSC may facilitate the difficult differential histopathologic diagnosis of PBC from PSC, as standard histologic observations alone may be inconclusive (27).

In summary, we have shown that subtyping of T lymphocytes in liver tissues obtained from patients with PBC and PSC provides additional insight into events culminating in bile duct destruction which characterize each disease and may be helpful in the differential histopathologic diagnosis of these two diseases. Finally, we suspect that the T lymphocytes accumulating in the portal areas, and especially T8+ cells which are enriched in and around bile duct walls and in the areas of piecemeal necrosis (24), may participate in the tissue damage which is characteristic of PBC.

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