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Research Article

Peripheral blood and hepatic tissue T- and B-lymphocyte distributions, serum alpha fetoprotein (AFP) concentrations, and hepatic AFP were studied in 46 patients undergoing diagnostic percutaneous liver biopsy. The patients included 26 with alcoholic liver disease, 13 with nonalcoholic hepatitis or cirrhosis, and 7 with either normal histology or minor nonspecific changes. Serum AFP was determined by radioimmunoassay and hepatic tissue AFP by indirect immunofluorescence. Peripheral blood T lymphocytes were identified by the sheep red-cell rosette technique; and B lymphocytes by fluoresceinated anti-immunoglobulin antisera and IgG aggregates. Tissue identification of T lymphocytes was accomplished using an extensively absorbed rabbit antihuman thymocyte antiserum and indirect immunofluorescence; tissue B lymphocytes were identified using pepsin F (ab')2 fragments of rabbit IgG antibodies to human immunoglobulins. T lymphocytes predominanted in hepatic lymphoid infiltrates from patients with alcoholic liver disease (91+/-4%), whereas in patients with chronic active or chronic persistant hepatitis, viral hepatitis, or cryoptogenic cirrhosis proportions of T and B lymphocytic infiltrates were similar (50+/-15%). Hepatic tissue AFP was detected in 9 of 18 patients with alcoholic hepatitis; serum AFP concentration was increased in only 1 of these 9 patients. Tissue AFP was not observed in the remaining biopsy material nor were serum AFP concentrations increased. Peripheral blood T-cell numbers were significantly decreased in patients with alcoholic liver disease (P less than 0.01) and in […]

Localization of T and B Cells and Alpha Fetoprotein in Hepatic Biopsies from Patients with Liver Disease

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ABSTRACT Peripheral blood and hepatic tissue T- and B-lymphocyte distributions, serum alpha fetoprotein (AFP) concentrations, and hepatic AFP were studied in 46 patients undergoing diagnostic percutaneous liver biopsy. The patients included 26 with alcoholic liver disease, 13 with nonalcoholic hepatitis or cirrhosis, and 7 with either normal hepatic histology or minor nonspecific changes. Serum AFP was determined by radioimmunoassay and hepatic tissue AFP by indirect immunofluorescence. Peripheral blood T lymphocytes were identified by the sheep red-cell rosette technique; and B lymphocytes by fluoresceinated anti-immunoglobulin antisera and IgG aggregates. Tissue identification of T lymphocytes was accomplished using an extensively absorbed rabbit antihuman thymocyte antiserum and indirect immunofluorescence; tissue B lymphocytes were identified using pepsin F(ab')$_2$ fragments of rabbit IgG antibodies to human immunoglobulins.

T lymphocytes predominated in hepatic lymphoid infiltrates from patients with alcoholic liver disease (91± 4%), whereas in patients with chronic active or chronic persistant hepatitis, viral hepatitis, or cryptogenic cirrhosis proportions of T and B lymphocytic infiltrates were similar (50±15%).

Hepatic tissue AFP was detected in 9 of 18 patients with alcoholic hepatitis; serum AFP concentration was increased in only 1 of these 9 patients. Tissue AFP was not observed in the remaining biopsy material nor were serum AFP concentrations increased. Peripheral blood T-cell numbers were significantly decreased in patients with alcoholic liver disease ($P < 0.01$) and in nonalcoholic hepatitis or cirrhosis ($P < 0.025$). A close relationship between peripheral blood T-lymphocytopenia and hepatic T-cell infiltrates was observed in patients with alcoholic liver disease; this relationship was less apparent in patients with nonalcoholic hepatitis or cirrhosis.

INTRODUCTION

Diffuse parenchymal liver disease is often accompanied by immune system derangement. Hypergammaglobulinemia (1-4), together with a depression of cell-mediated immunity (5-7), have been consistently observed in acute or chronic hepatitis and hepatic cirrhosis of varying etiology including that due to alcohol. The explanation for elevations in serum immunoglobulins has not been clarified but has been attributed by some (1), to bypass of reticuloendothelial antigen trapping or processing functions in the damaged liver.

Recent studies of peripheral blood T- and B-lymphocyte distributions in several diverse forms of liver disease, including acute viral hepatitis, chronic hepatitis of varying etiology, and alcoholic hepatitis with cirrhosis, have demonstrated a depression in both proportions and absolute numbers of circulating T cells (8, 9). In these studies the depression of peripheral blood T cells appeared to be clearly related to active hepatocellular necrosis and inflammation (9).

Reports from several laboratories (10-12), have indicated that alpha fetoprotein (AFP)[1] may function as an immunosuppressive agent capable of blocking several in vitro parameters of T-cell function such as mitogen stimulation or mixed leukocyte culture. In man, serum AFP levels may be elevated in viral hepatitis, chronic active hepatitis, alcoholic hepatitis, or childhood cirrho-

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[1] *Abbreviations used in this paper:* AFP, alpha fetoprotein; FITC, fluorescein isothiocyanate; MIF, migration inhibitory factor.

sis (13-16). It seemed possible, therefore, that T-cell depletion or dysfunction in active liver disease might be related to elevation of AFP levels. Alternatively the depression of circulating T cells could be linked to multiple hepatic microfoci of inflammation and T-cell homing to such foci. The current study was therefore directed towards an attempt to relate peripheral blood T-cell depletion to T- and B-cell proportions identified in liver biopsies. In addition, the possible influence of AFP on the deficiency of circulating T cells was studied using an immunofluorescence technique to identify hepatic AFP and radioimmunoassay for serum AFP measurements.

The present study has revealed that a majority of the lymphocytes infiltrating hepatic inflammatory foci, particularly in alcoholic hepatitis, are T cells. Indeed, the pattern of T-cell predominance in alcoholic hepatitis was so uniform and repetitive as to raise intriguing questions relating to the pathogenesis of this disease.

METHODS

Patients. The 46 patients studied were hospitalized at the Bernalillo County Medical Center or Albuquerque Veterans Administration Hospital during the course of treatment for active or suspected liver disease and all underwent percutaneous needle biopsy of the liver for diagnostic purposes. The patients included 26 with alcoholic liver disease, 13 with nonalcoholic hepatitis or cirrhosis, and 7 with either normal liver histology or minor nonspecific changes by light microscopy (Table I).

Peripheral blood T- and B-lymphocyte determinations. Lymphocytes were separated from heparinized venous blood by Ficoll-Hypaque gradient centrifugation at room temperature. No attempt to remove monocytes was made, however, in many instances monocytes present in such preparations were identified using addition of small latex particles easily identified during fluorescence microscopy after monocyte ingestion. T cells were enumerated using the sheep red-cell rosette technique, which employed fresh sheep erythrocytes no more than 7-days-old, initial incubation at 37°C for 30 min, followed by overnight incubation at 4°C in pH 7.4 phosphate-buffered saline but with no added serum (17–19).

B cells were determined by direct immunofluorescence using a fluoresceinated polyvalent anti-Ig rabbit antiserum or summation of cells staining with fluoresceinated anti-IgG + anti-IgA + anti-IgM (19, 20). Since B cells values by this technique may be artificially high due to binding of fluoresceinated antibody reagents to Fc receptors (21), B-cell enumeration was also carried out using pepsin-digested rabbit antihuman $F(ab')_2$ conjugated with fluorescein isothiocyanate (FITC) (21) in addition to aggregates of fluoresceinated IgG and the method described by Dickler and Kunkel (22). Absolute numbers of peripheral blood T and B cells were calculated from simultaneously performed total lymphocyte counts.

Localization of T and B lymphocytes in liver biopsy samples. All liver biopsies were divided into two portions, one being fixed in Bouin's solution for hematoxylin and eosin or Trichrome-Mallory staining, while the second portion was snap frozen in a dry ice bath and cut immediately for immunofluorescent studies.

TABLE I
Patients and Diagnoses

Diagnosis	Patients
Alcoholic liver disease	
Cirrhosis with alcoholic hepatitis	16
Alcoholic hepatitis	2
Cirrhosis	4
Alcoholic fatty liver	4
Nonalcoholic hepatitis or cirrhosis	
Chronic active hepatitis	2
Chronic persistent hepatitis	3
Recurrent acute viral hepatitis	1
Fulminant hepatitis	1
Granulomatous hepatitis	1
Reactive hepatitis	4
Cryptogenic cirrhosis	1
Normal liver or minor nonspecific changes	
Triaditis, cholestasis, and mild fatty changes	1
Carcinomatosis, not involving the liver	1
Fatty changes in diabetic	1
Fatty changes in patient with chronic myelogenous leukemia	1
Coeliac disease	1
Extrahepatic portal hypertension	1
Hypogammaglobulinemia	1
Total	46

Preliminary experiments showed that treatment of sections with acetone did not alter the staining with the various fluoresceinated antibodies. The cryostat sections were, therefore, fixed in cold acetone for 5 min before staining. Cellular detail was much improved using this approach when compared with unfixed sections. The fluoresceinated antisera used in this study were passed through a DEAE-cellulose column (23) before use to avoid nonspecific fluorescence of the tissue sections.

B cells. Initially, B-cell identification in liver tissue was attempted using two parallel methods. First, purified rabbit IgG antibodies to human immunoglobulins (4) were digested with pepsin (enzyme-protein ratio 1:50) at 37°C for 18 h, and the resulting $F(ab')_2$ fragments were labeled with FITC and used to identify tissue cells with surface or cytoplasmic immunoglobulin as well as extracellular deposits of immunoglobulin. The $F(ab')_2$ fragments were employed to avoid reactions with the Fc receptors of tissue-fixed macrophages, polymorphonuclear leukocytes, or lymphocytes (24). Cells with lymphocyte or plasma cell morphology, which showed staining for immunoglobulins using $F(ab')_2$ fragments of rabbit antihuman Ig either on the surface or in the cytoplasm were regarded as belonging to the B-cell line. Secondly, aggregates of fluoresceinated human IgG were prepared by the method of Dickler (25), and were used to identify cells bearing Fc receptors. In blocking experiments, pretreatment of the sections with nonfluoresceinated aggregated IgG abolished the fluorescence with fluoresceinated, aggregated IgG. In several instances, however, biopsy samples showed considerably higher proportions of cells binding aggregated IgG

than cells staining with pepsin-digested antibodies to human Ig. Parallel hematoxylin/eosin, peroxidase, and nonspecific esterase staining (26) of serial sections in such instances showed that a large proportion of the cells among such infiltrates were indeed not only lymphocytes but macrophages and polymorphonuclear leukocytes, both of which are known to have Fc receptors (24) (Fig. 1). Thus binding of aggregated IgG to the cells could not serve as a marker for B cells in the tissues. Estimation of B cells in tissues was therefore entirely based on the staining with the pepsin-digested and FITC-labeled antihuman immunoglobulin antibodies.

T cells. We tried unsuccessfully to adapt the technique of sheep red-cell rosette formation to the problem of identifying T cells in human tissue sections. Methods advocated by others (27, 28), as well as modifications of our own were used to attempt to localize tissue T cells in frozen sections of human lymph node, spleen, and liver. In many instances, sheep red-cell binding to thymic-dependent areas of normal lymph nodes or spleen was recorded; on the

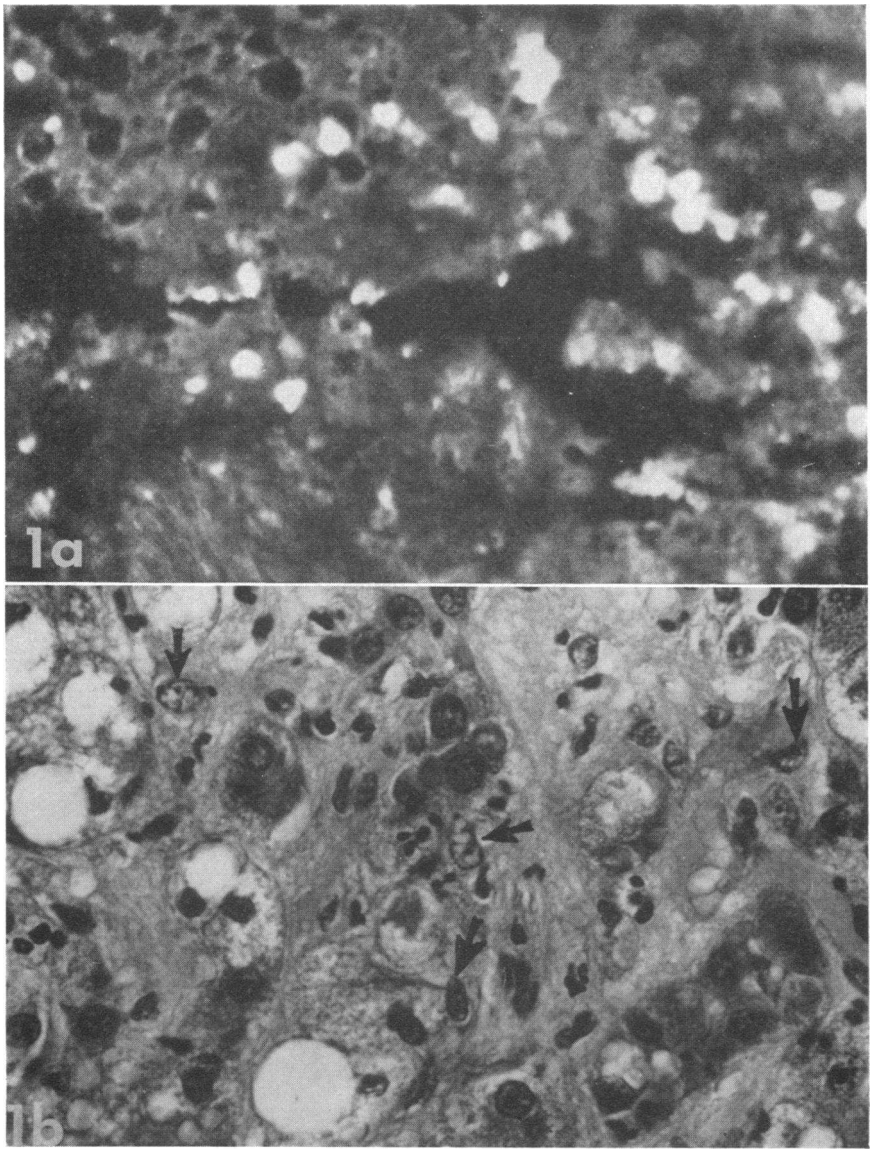

FIGURE 1 (*a*) Immunofluorescence micrograph. Liver section from a patient (M. C.) with alcoholic hepatitis stained with aggregates of FITC-conjugated human IgG showing numerous fluorescent cells with aggregate receptors (× 400). (*b*) Section from the same biopsy stained with hematoxylin and eosin and examined by light microscopy. Polymorphonuclear granulocytes as well as macrophages (arrows) are present in the inflammatory areas being responsible, at least in part for the binding of aggregated IgG (× 400).

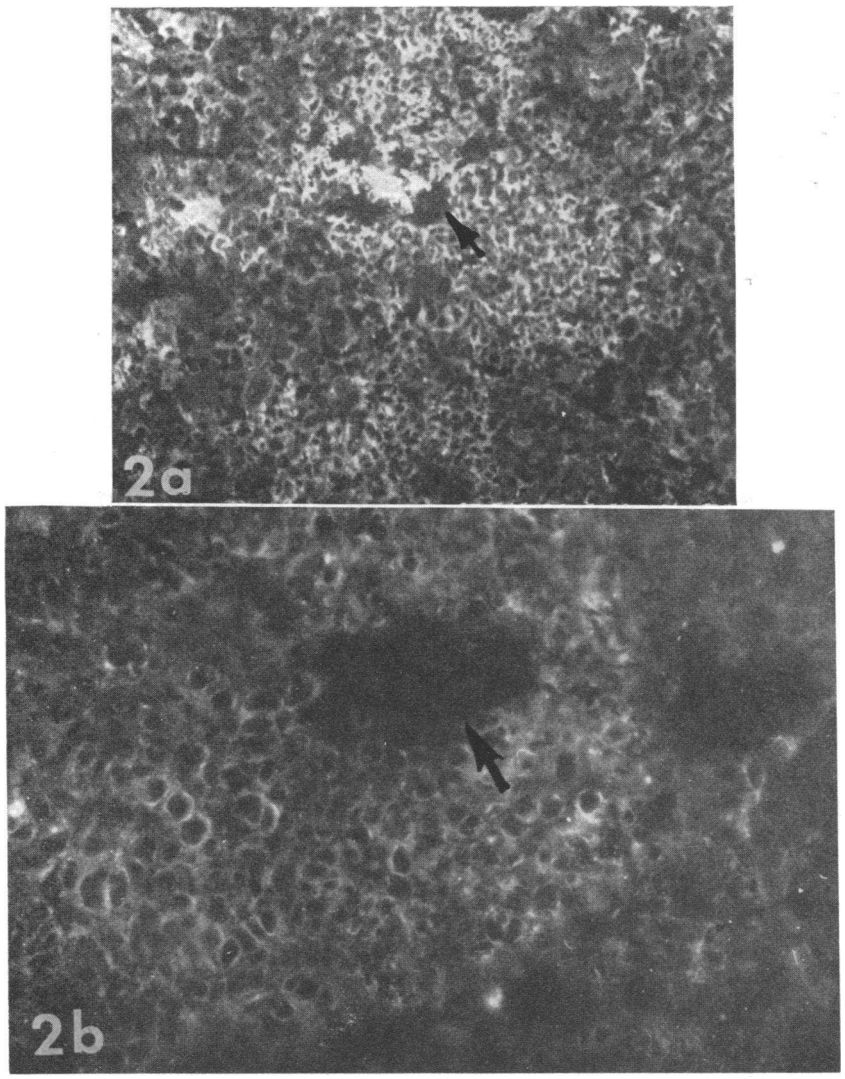

FIGURE 2 Immunofluorescence micrographs. Section from normal human spleen incubated with the extensively absorbed rabbit antihuman thymocyte antiserum and followed by staining with FITC-conjugated goat antirabbit IgG. Membrane staining of lymphocytes is seen mainly in the thymic-dependent periarteriolar area (arrow) while only a few fluorescent cells are seen outside this region (*a*) × 160; (*b*) × 400.

other hand, our results were not reproducible, and therefore this method was not regarded as reliable. Indirect immunofluorescence was therefore used to identify T cells in hepatic tissue. Rabbit antihuman thymocyte antiserum was prepared as previously described (29, 30) using isolated T cells obtained from human thymuses in children undergoing cardiac surgery. Before use, the following absorptions of the rabbit antiserum were performed: Eight serial absorptions each for 30–60 min at 37°C, with B cells (5×10^7 cells/ml) from patients with chronic lymphatic leukemia; three serial absorptions with rhesus-positive red cells (5×10^8/ml) from normal blood donors; two serial absorptions, for 30 min at 37°C, with polymorphonuclear leukocytes and monocytes (15×10^6 cells/ml) isolated during in vitro assays for chemotaxis (31, 32); absorption with an equal volume of insolubilized (33) normal human serum; and finally absorption with an insolubilized homogenate of normal human liver tissue. The liver tissue used for absorption was obtained at autopsy from a subject killed in a traffic accident. Liver tissue frozen and thawed five times was minced with scissors and homogenized in an equal volume of phosphate-buffered saline at high speed for 5 min at 0–4°C, using a Sorvall omnimixer (Ivan Sorvall, Inc., Norwalk, Conn.). Liver homogenate was further disintegrated by sonication in a Bronson sonifier (Bronson Instruments, Inc., Stamford, Conn.) and subsequently centrifuged at 18,000 *g* for 20 min. The supernate (60 mg protein/ml) was insolubilized with glutaraldehyde (33) and used for absorption of an equal volume of antiserum. In some experiments the sonicated liver homogenate was

insolubilized and used without centrifugation. Absorption with the liver preparations was used to avoid staining of endothelial cells and reticulin fibers. After incubation with anti-T-cell antiserum, the sections were washed and subsequently stained with a fluoresceinated sheep antiserum to rabbit IgG. The absorbed anti-T-cell antiserum appeared to show primary specificity for T cells in frozen sections of normal human spleen and lymph nodes (29). It did not stain follicular lymph node areas nor plasma cells (29). Definite cell membrane staining was clearly evident in periarteriolar regions of human spleen as well as paracortical areas of normal human lymph nodes (Fig. 2). The absorbed anti-T-cell antiserum also stained an average of 65±10% of normal peripheral blood lymphocytes while no staining of chronic lymphatic leukemia cells was observed. Staining with anti-T-cell antiserum was completely abolished by absorption with homogenates or suspensions of human fetal thymocytes.

The degree of lymphocytic infiltration in the hepatic biopsies studied was evaluated both by immunofluorescence and by examination of serial sections stained with hematoxylin and eosin. Each biopsy was thus classified as showing absent, mild, moderate, or severe lymphocytic infiltration. Proportions of T and B cells in liver tissue were estimated by counting the number of the fluorescent cells found in neighboring sections stained with antisera to T cells and immunoglobulins, respectively. In the case of moderate or severe hepatic lymphocytic infiltrates, the relative proportions of T and B cells were estimated by counting 100 or more lymphocytic cells in several microscopic fields. When infiltrates were less extensive, smaller numbers of lymphocytes (15–100 cells) were available for counting.

Cells containing AFP were identified using a goat antiserum to human AFP (kindly provided by Dr. Stewart Sell, University of California at San Diego) and indirect immunofluorescence with fluorescein-labeled rabbit anti-goat IgG. Specificity of fluorescent staining was confirmed by marked reduction of staining after absorption with human cord or amniotic fluid high in AFP content, but not after absorption with normal human serum. The anti-AFP antiserum gave no lines on electrophoresis against normal human serum and a single, clearly defined AFP arc on similar assay against human cord serum or amniotic fluid fractions rich in AFP. Nevertheless, absorption of anti-AFP antiserum with insolubilized normal adult human serum was employed to avoid the highly sensitive indirect immunofluorescent technique revealing possible antibody activity to other serum proteins.

Serum AFP was determined using a double-antibody radioimmunoassay technique employing highly purified AFP and monospecific anti-AFP antiserum. Fetal AFP was isolated from pooled cord serum using a combination of salt fractionation, gel chromatography, and ion-exchange chromatography. Residual contaminating normal adult proteins were removed by adsorption with an agarose column conjugated with rabbit antibodies to normal adult serum proteins. Hyperimmune antiserum to isolated AFP was prepared in rabbits. To assure a monospecific antiserum, the rabbit anti-AFP serum was absorbed with an agarose immunoadsorbent conjugated with pooled normal adult serum. Rabbit antisera produced a single precipitin line in agar when tested against fetal cord serum or sera from patients with known AFP-producing hepatomas, and did not react with normal human serum. High titered goat antirabbit IgG Fc was prepared by injections (every 3 wk), of 2 mg of triple recrystallized papain Fc fragments emulsified in Freund's adjuvant. Rabbit anti-AFP was diluted 1/7,000 in borate saline, pH 7.4, that contained 10% normal rabbit serum. Each test tube received 100 μl of diluted antibody followed by 5–50 μl of the human serum to be tested. The tubes were incubated for 30 min at 37°C, and then 2 ng of AFP, previously labeled with ^{125}I by the chloramine-T method (34) at activities ranging from 500 to 2,000 cpm/ng, were added. The tubes were incubated for 30 min at 37°C, the appropriately diluted goat anti-rabbit IgG Fc serum was then added. Incubation was continued for 30 min at 37°C, and the tubes were then left for 12–18 h at 4°C. Tubes were centrifuged, the supernates removed, and then precipitates washed with borate saline. The precipitate, the pooled supernate, and wash for each tube were counted in a Packard scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). Control tubes included maximum antigen precipitation (40–50%), nonspecific precipitation (1–2%), and maximum inhibition by a fetal cord serum. A World Health Organization AFP reference preparation (72/225) was obtained from the International Agency for Research on Cancer and used to standardize a pool of fetal cord serum. Mean (±1 SD) serum AFP concentration in 30 normal adult subjects was 8±2.5 nU/ml.

TABLE II

Liver Tissue and Peripheral Blood Lymphocyte Subpopulations and AFP in Patients with Liver Disease and Controls

Diagnosis	No. of biopsies	Lymphocytic infiltration*				Liver tissue		Peripheral blood, absolute number		AFP	
		Severe	Moderate	Mild	Absent	T cells	B cells	T cells	B cells	Liver tissue	Serum
						%				No. pos.	*nU/ml*
Alcoholic liver disease	26	9	5	8	4	91±4‡	9±4	865±749§	279±219	9	12±5
Nonalcoholic hepatitis and cryptogenic cirrhosis	13	4	2	7	0	50±15	50±15	1,104±850§	429±472	0	10+3
Normal blood donors	0	—	—	—	—	—	—	1,571±289‖	543±289‖	—	8±2.5¶

* Determined by the sum of fluorescent cells as revealed by the anti-immunoglobulin and anti-T-cell antisera.

‡ The proportion of liver tissue T cells is significantly higher ($P < 0.01$) in alcoholic liver disease than in nonalcoholic hepatitis and cirrhosis.

§ The absolute numbers of the peripheral blood T cells are significantly lower in alcoholic liver disease ($P < 0.001$) and nonalcoholic hepatitis and cirrhosis ($P < 0.025$) than in normal blood donors.

‖ Based on T- and B-cell determinations in 100 normal individuals.

¶ Based on determination of AFP in 30 normal adult individuals.

RESULTS

In all, liver biopsies from 46 patients were studied; in 32 of these subjects, peripheral blood T- and B-cell determinations were performed simultaneously. Peripheral blood T-cell numbers were significantly decreased in patients with alcoholic liver disease ($P < 0.01$) and nonalcoholic hepatitis or cirrhosis ($P < 0.025$) when compared to normal control subjects (Table II). Mean B-cell numbers in patients with alcoholic liver disease were decreased compared to normal controls, but the difference was not significant. B-cell numbers in patients with nonalcoholic hepatitis or cirrhosis did not differ from control values (Table II). In 35 of the 46 biopsies studied, lymphocytic infiltrations were present, as revealed by the immunofluorescence technique and these were graded as severe in 13, moderate in 7, and mild in 15 biopsies (Table II).

A striking uniformity was noted in the distribution of tissue lymphocyte subpopulations among the 22 patients with alcoholic liver disease whose biopsies showed lymphocytic infiltration. These included 17 patients with alcoholic hepatitis, 3 with alcoholic cirrhosis, but no alcoholic hepatitis, and 2 with simple fatty change. A majority (91±4%) of the lymphocytes were T cells showing a rim or surface-staining pattern as revealed by the immunofluorescence technique (Fig. 3*a* and *b*,

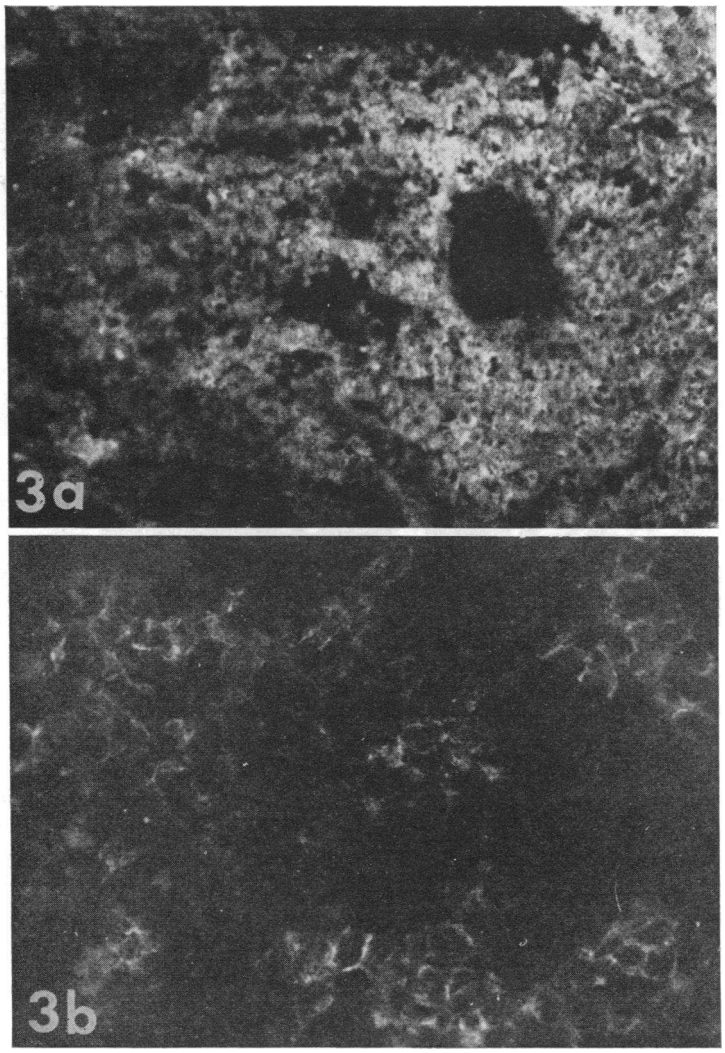

FIGURE 3 Immunofluorescence micrographs. (*a*) Liver section from a patient (C. M.) with alcoholic hepatitis stained with anti-T-cell antiserum showing marked periportal T-cell infiltration (× 160). (*b*) Higher magnification of a less infiltrated area of the same section showing the rim or membrane-staining pattern of the T cells (× 400).

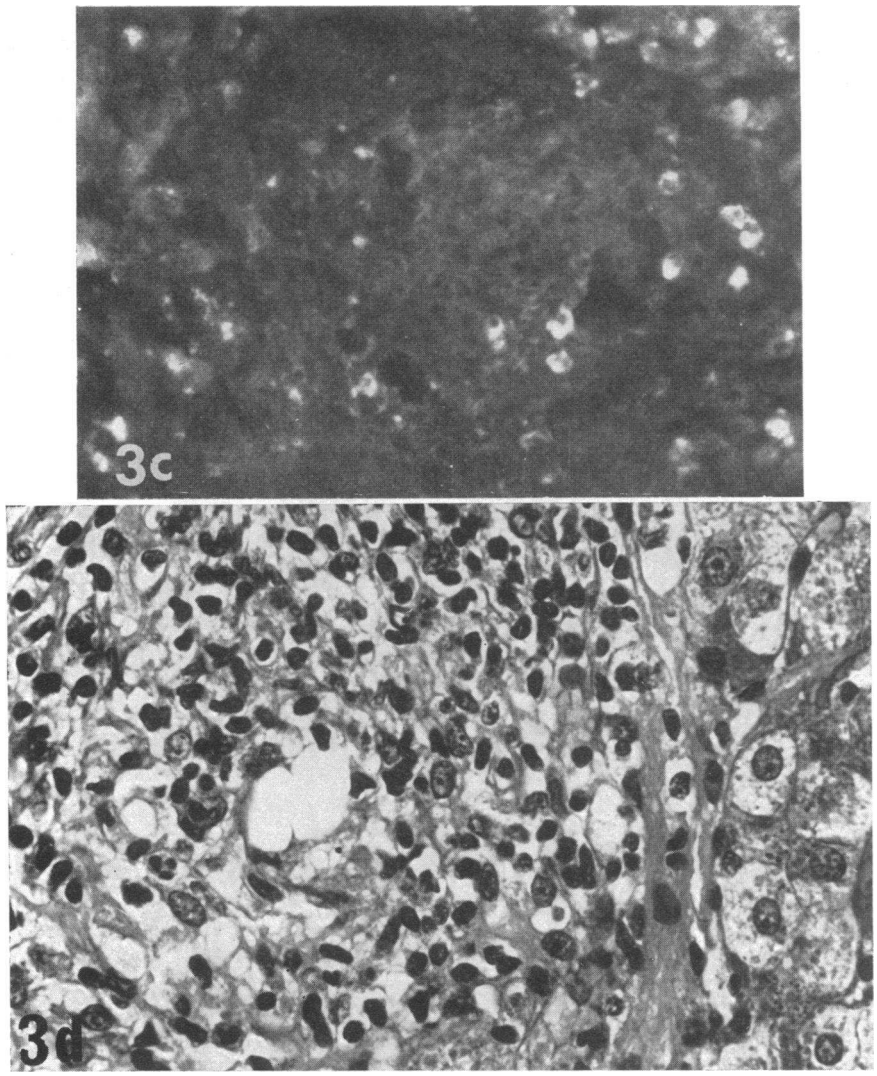

FIGURE 3 (*c*) Section from the same biopsy stained with pepsin-digested and FITC-conjugated rabbit antihuman immunoglobulins. Only a few fluorescent cells (B cells) are observed (× 160). (*d*) Light micrograph of section from the same biopsy stained with hematoxylin and eosin. The periportal area is heavily infiltrated with lymphocytes (× 400).

Table II). Lymphocytes and plasma cells showing peripheral or cytoplasmic localization of immunoglobulin were seen in much lower proportions (9±4%) in the biopsies from patients with alcoholic liver disease (Fig. 3*c*, Table II). No exceptions to the uniformity of this pattern of the hepatic lymphoid infiltrates (Fig. 3*d*) in patients with alcoholic liver disease were recorded, regardless of the severity of the lymphocytic infiltrations.

Biopsies from nine patients with alcoholic liver disease (all with alcoholic hepatitis) showed single cells or nests of cells containing AFP located mostly in the peripheral parts of the inflammatory lesions. There was a high degree of variability in the number of AFP-containing cells among these nine patients. However, there was a positive correlation with the activity of liver inflammation as revealed by the extent of the lymphocytic infiltrations. Thus, lymphocyte infiltration in these nine biopsies was severe in seven and moderate in two. The AFP-positive cells were morphologically similar to hepatocytes and they showed an even distribution of AFP in their cytoplasm (Fig. 4). Significant serum AFP elevation (29 nU/ml) was detected in only one of the nine patients in whom AFP was identified in hepatic tissue.

Table III shows data in the 32 patients in whom both tissue and peripheral blood was examined for lymphocyte subpopulation distribution at the same time. 13 of the 17 patients with alcoholic liver disease had signifi-

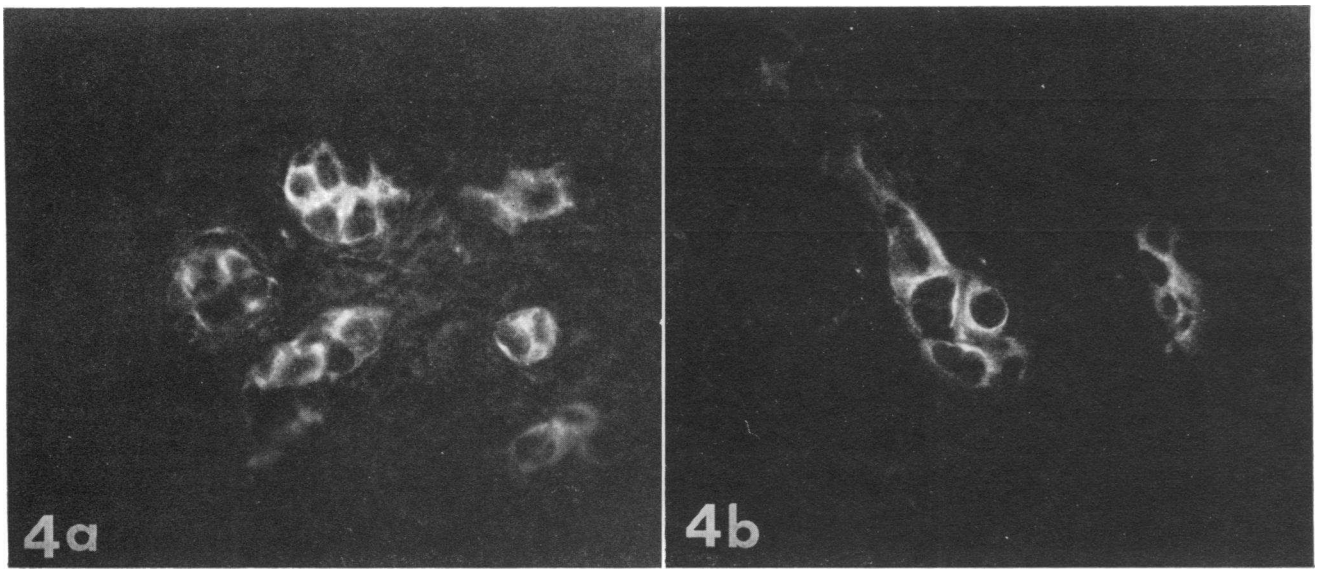

FIGURE 4 Immunofluorescence micrographs showing cells containing AFP in the liver of two patients, L. C. (*a*) and M. C. (*b*) with alcoholic cirrhosis and hepatitis. These cells were located mainly in the peripheral parts of hepatic inflammatory lesions near areas of liver cell regeneration (× 400).

cantly decreased peripheral blood T-cell numbers. In the four patients with normal peripheral blood T-cell numbers, lymphocytic infiltration was absent or mild in two and moderate or severe in two patients. Thus, although the majority of patients studied showed a relationship between peripheral blood T-cell depression and lymphocytic infiltration of the liver with a predominance of T cells, this relationship was not universal.

No clear relationship between peripheral blood T-cell depletion and demonstrable hepatocyte AFP staining was present. Although peripheral blood T-cell numbers were decreased in the four patients whose liver showed the presence of AFP, T lymphocytopenia was also present in a further nine patients in whom tissue AFP deposits were lacking. Similarly, no direct correlation between serum AFP levels and peripheral blood T-cell numbers was apparent in the subjects with liver disease.

By contrast with the findings in hepatic tissue from patients with alcoholic liver disease, patients with nonalcoholic hepatitis or cirrhosis showed a significantly lower proportion of tissue T cells, 50±15%, and a higher proportion of B cells, 50±15% (Tables II and III), in the lymphocytic infiltrates. In nonalcoholic hepatitis with severe lymphocytic infiltration absolute numbers of tissue B cells were higher than in alcoholic hepatitis. Examples of heavy B-cell infiltration in recurrent viral and chronic active hepatitis are shown in Fig. 5*a* and *b*. In some instances immunoglobulins were also traced as granular or lumpy deposits located either extracellularly or within phagocytic cells including Kupffer cells; however, this distribution of Ig was readily differentiated from B-cell staining by clear differences in tissue patterns. None of the biopsies studied in this group of 13 patients with nonalcoholic liver disease contained AFP by immunofluorescence; moreover, no significant elevation of serum AFP was detected (Table II). In 10 of the 13 patients, peripheral blood T- and B-cell numbers were measured at the time the biopsies were obtained. Peripheral blood T cells were depressed in 6 of the 10 subjects (Table III). The correlation of peripheral blood T-cell numbers with the degree of hepatic lymphocytic infiltration or proportions of T cells present in these infiltrates was poor compared to the findings in patients with alcoholic liver disease (Table III). Peripheral blood T-cell numbers were measured in five of the seven patients with normal or mild nonspecific changes without lymphocytic infiltrates on liver biopsy. T-cell numbers were normal in three and decreased in two of these subjects. One of the latter patients (H. S., Table III), had disseminated adenocarcinoma from an unknown primary site, but without liver involvement by tumor, the other patient with decreased peripheral blood T cells (J. B., Table III) had extrahepatic portal hypertension from portal vein thrombosis.

DISCUSSION

The present study has utilized immunofluorescence techniques to identify the types of lymphocytic cells present in hepatic tissue obtained from patients with a variety of acute or chronic liver diseases.

The unsuccessful attempts reported here using sheep

TABLE III

Liver Tissue and Peripheral Blood Lymphocyte Subpopulations in Patients with Liver Disease

Diagnosis	Patient	Lymphocytes in liver tissue			Lymphocytes in peripheral blood				
		Lymphocytic infiltration	T cells	B cells	T cells	B cells	T cells	B cells	Absolute lymphocyte count
			%	%	*n*	*n*	%	%	
Alcoholic liver disease									
Cirrhosis with alcoholic hepatitis	C. G.	Severe	90	10	283	192	28	19	1,012
	A. H.	Severe	90	10	285	ND*	12	ND	3,220
	A. G.	Severe	90	10	701	454	51	33	1,375
	G. B.	Severe	90	10	475	80	66	12	720
	M. C.	Severe	95	5	403	ND	32	ND	1,260
	A. P.	Moderate	90	10	3,080	ND	77	ND	4,000
	L. S.	Moderate	90	10	178	ND	12	ND	1,480
	J. A.	Mild	90	10	1,282	612	67	32	1,914
	M. T.	Mild	100	—	955	294	65	20	1,470
	G. D.	Mild	90	10	540	64	50	6	1,080
Alcoholic hepatitis	B. H.	Severe	90	10	1,463	630	57	25	2,520
	M. S.	Moderate	85	15	263	27	39	4	675
Cirrhosis	I. G.	Mild	90	10	582	96	73	12	798
	D. M.	Mild	80	20	981	471	75	36	1,300
	R. T.	Mild	90	10	505	151	32	8	1,890
	J. D.	Absent	—	—	1,998	ND	74	ND	2,700
Alcoholic fatty liver	L. Sa.	Mild	90	10	738	258	60	21	1,230
Nonalcoholic hepatitis or cirrhosis									
Chronic active hepatitis	W. B.	Severe	50	50	801	243	33	10	2,430
	S. M.	Moderate	30	70	442	44	60	10	736
Chronic persistent hepatitis	C. M.	Severe	60	40	239	227	20	19	1,196
	M. P.	Mild	70	30	3,027	1,466	64	31	4,730
	R. C.	Mild	60	40	1,844	968	40	21	4,611
Recurrent acute viral hepatitis	M. B.	Severe	50	50	1,593	292	71	13	2,244
Reactive hepatitis	S. M.	Mild	60	40	889	225	83	21	1,077
	C. H.	Mild	60	40	386	187	55	28	702
	M. F.	Mild	30	70	1,135	ND	48	ND	2,365
Cryptogenic cirrhosis	W. L.	Mild	30	70	684	154	80	18	855
Normal liver or minor nonspecific changes									
Mild fatty changes	R. M.	Absent	—	—	1,069	ND	39	ND	2,740
Cancer	H. S.	Absent	—	—	876	ND	73	ND	1,200
Fatty changes in diabetic	L. T.	Absent	—	—	1,956	ND	65	ND	3,010
Coeliac disease	H. F.	Absent	—	—	1,503	120	86	8	1,748
Extrahepatic portal hypertension	J. B.	Absent	—	—	347	130	56	29	620

* Not determined.

erythrocytes to localize T cells in tissues have also been noted by other workers (35). Moreover, on several occasions, we confirmed the reports of Staber et al. (36), that hepatocytes themselves were capable of binding sheep red cells. For these reasons, the indirect membrane staining of T cells with the extensively absorbed rabbit anti-T-cell antiserum was used in the present study. The antiserum, when absorbed with B cells, monocytes, granulocytes, erythrocytes, whole human serum, and liver homogenate, provided dim staining and therefore, if anything, tended to underestimate proportions of T cells in tissues. However, no overlap with B cells or other nonlymphocyte membrane antigens was apparent, and this reagent appeared to provide reliable estimates of tissue T-cell localization.

The finding that fluorescent aggregates of IgG could not be used as a reliable estimate for tissue B cells alone was not unexpected since Fc receptors binding aggregates are present in a variety of cell types which may be present in inflammatory hepatic foci. Furthermore, reports by Tønder et al. (37), indicate that malignant cells may also contain Fc receptors. Thus, aggre-

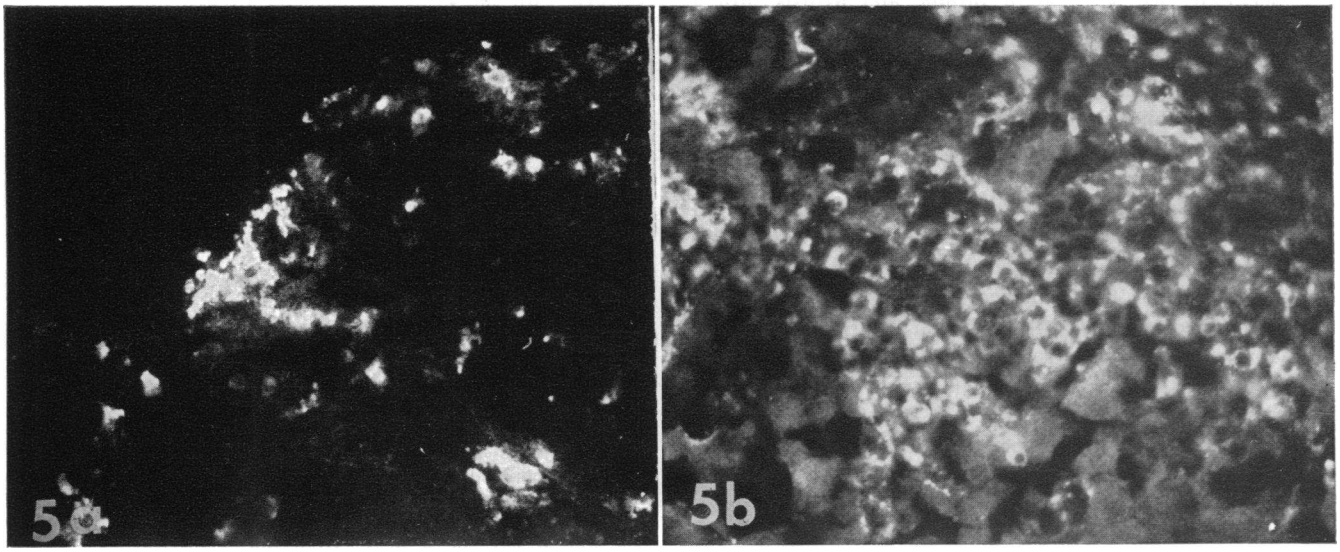

FIGURE 5 Immunofluorescence micrograph of liver tissue sections stained with FITC-conjugated antihuman Ig. (*a*) Section from a patient (M. B.) with recurrent, viral hepatitis showing a dense accumulation of B cells in the portal triad and scattered cells in the surrounding areas (× 160). (*b*) Severe infiltration of B cells in the liver of a patient (W. B.) with chronic active hepatitis (× 250).

gate binding to tissue-fixed Fc receptors is not a useful method for identification of B cells. The ideal technique for such tissue B-cell identification would be immunofluorescence utilizing specific anti-B-cell antisera. Such reagents have been successfully used in the mouse (38) but are not yet generally available for work with human tissues.

Our results indicate that lymphocytic infiltration of the liver may accompany most forms of diffuse hepatocellular injury including that attributed to alcohol, and that T lymphocytes are a prominent component of such infiltrations. The data obtained in the present study are consistent with the possibility that peripheral blood T lymphocytopenia previously demonstrated in liver disease of varying etiology (8, 9), may be due in part to sequestering of portions of the T-cell pool in microfoci of hepatic inflammation. In the present study, there was a correlation between peripheral T lymphocytopenia and degree of hepatic T-cell infiltrate amongst the patients with alcoholic liver disease. This relationship was less apparent in patients with nonalcoholic hepatitis or cirrhosis. However, it should be emphasized that such comparisons are extremely limited by the fact that total hepatic T-cell numbers cannot be accurately estimated by the current method involving examination of one or two microfoci of inflammation within a single percutaneous biopsy sample.

It is also possible that the peripheral blood T lymphocytopenia as measured by the sheep red-cell rosette technique in various forms of active liver disease is unrelated to any inflammatory homing of T cells to the liver. Recent studies by Chisari and Edgington (39), have indicated that cellular or membrane-associated levels of cyclic AMP may be directly related to the numerical values recorded for the sheep red-cell rosette method of quantitating T cells. Lymphocyte-associated cyclic AMP, when increased, is capable of elevating apparent proportions of rosette-forming T cells (40). Thus, low peripheral blood T cells measured by the sheep red-cell rosette technique in various forms of active liver disease may reflect depleted lymphocyte membrane cyclic AMP in addition to the possible trapping of T cells in the hepatic lesions. Studies of lymphocytic membrane cyclic nucleotides in patients with liver disease seem clearly indicated and are currently in progress.

The striking finding of marked T-cell predominance within the lymphocytic infiltrates, particularly in patients with alcoholic hepatitis was of considerable interest. This predominance occurred regardless of the severity of the lymphocytic infiltrations. It suggests that perhaps some of the end results of alcoholic hepatic inflammatory reactions may be mediated in part by T lymphocytes or their products such as potentially toxic lymphokines. Recent studies by Johnson and Ziff (41), have provided evidence that lymphokines, presumably products of T cells, can stimulate fibroblastic synthesis of collagen. Moreover, the studies of Chen and Leevy (42), have shown that ethanol is capable of inducing

increased incorporation of proline and hydroxyproline into collagen using liver biopsy tissue from patients with alcoholic hepatitis or cirrhosis, but not in such tissue from individuals with normal or fatty liver. Additional evidence implicating immunologic factors in the perpetuation of alcoholic liver disease comes from recent studies in which elaboration of migration inhibitory factor (MIF) from peripheral blood mononuclear cells in response to normal human liver (43) or extracts of alcoholic hyaline (44) was detected in patients with alcoholic hepatitis. Thus, further study of the possible role of immunologic tissue damage in both alcoholic and nonalcoholic hepatitis seems clearly indicated and might be directed towards measurement of relative hepatic arterial-venous differences in T-lymphocyte products such as MIF, lymphocytotoxins, or other lymphokines.

A striking finding in the present study was the identification of AFP-containing cells by immunofluorescence in biopsies from 9 of 18 patients with alcoholic hepatitis. AFP was not detected in control biopsies, in nonalcoholic hepatitis, nor in alcoholic liver disease without hepatitis. Positive immunofluorescence was localized to clusters of hepatocytes, and sometimes single cells, many of which appeared to be near areas of liver cell regeneration. These findings are quite similar to those previously reported in the liver of patients with hepatoma by other workers (45, 46). Our findings appear to be the first clear documentation of cellular localization of AFP in the liver of patients with alcoholic hepatitis. However, cell localization of AFP does not constitute definitive evidence that such cells synthesize this material as stressed by previous workers using affinity labeling of cellular AFP with tritiated estrogens (47).

The animal studies of Murgita and Tomasi (11, 12), provided clear evidence that AFP caused immunosuppressive effects in vitro. These authors suggested that AFP synthesis might also be related to immunodepression in patients with liver disease. It is intriguing to think that, occurring as it does in regenerating liver tissue, AFP may induce a local immunosuppressive effect; the inflammation of alcoholic hepatitis may thus be muted or somehow turned off at the very source. In the present study, no quantitative correlation between serum AFP and peripheral blood T lymphocytopenia was apparent. However, the work of Murgita and Tomasi (11, 12), has indicated that AFP is immunosuppressive in vitro at very low concentrations. Thus significant immunosuppressive effects at the local site of AFP production might occur in the absence of elevated serum concentrations. However, no evidence of an immunosuppressive effect of AFP in vivo has so far been provided.

No assays of T-cell function such as MIF, mixed leukocyte culture, or mitogen stimulation were attempted with the group of patients studied here. More work clearly needs to be done to correlate possible functional T-cell impairment in subjects with active liver disease with circulating plasma or tissue-fixed AFP. Moreover, in light of the well-established association between cirrhosis and the later development of hepatoma, careful follow-up of patients showing tissue-fixed AFP is clearly indicated since such findings may be relevant to the later development of this tumor.

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