Clin. exp. Immunol. (1969) **5**, 499–514.

IMMUNOGLOBULIN-CONTAINING CELLS IN THE LIVER

S. HADZIYANNIS,* TEN FEIZI,† P. J. SCHEUER AND SHEILA SHERLOCK

Departments of Medicine and Pathology, The Royal Free Hospital, London

(*Received* 24 *June* 1969)

SUMMARY

Liver samples from sixty-four patients with hepato-biliary diseases, and from twenty-three with miscellaneous (primarily non-hepatic) conditions, were examined by the fluorescent antibody technique for mononuclear cells containing immunoglobulins G, A, M and D. Immunoglobulin-containing cells ('Ig-cells') were found not only in liver diseases but also in non-hepatic diseases. IgA was the predominant cell type in liver diseases, except in primary biliary cirrhosis where IgM was the main cell type. In primarily non-hepatic diseases when a significant lymphoid infiltration was present, high 'Ig-cell' counts were often observed, IgG being the predominant cell type. IgD cells were found in the livers of five patients with cirrhosis and in one patient with carcinoma of the thyroid. The mean 'Ig-cell' counts of patients with lymphoid infiltration was higher than that of patients without such infiltration. Also, the mean Ig-cell count was higher in the presence than in the absence of piecemeal necrosis.

High serum immunoglobulin levels were not necessarily associated with high 'Ig-cell' counts in the liver. However, when more than one cell per high power field containing a certain immunoglobulin was seen, the respective serum immunoglobulin level was almost always above the normal mean. This suggested that in some patients hepatic 'Ig-cells' may contribute to serum immunoglobulin levels.

INTRODUCTION

Raised serum levels of immunoglobulins G, A and M commonly occur in diseases of the liver (Lee, 1965; McKelvey & Fahey, 1965; Hobbs, 1966; Feizi, 1968). Immunocytochemical studies (Cohen *et al.*, 1960; Paronetto, Rubin & Popper, 1962) have shown that in patients with cirrhosis and various forms of hepatitis mesenchymal cells in the liver may

Correspondence: Professor Sheila Sherlock, Department of Medicine, Royal Free Hospital, London, W.C 1.

* Present address: Evangelismos Hospital, Athens, Greece.

† Present address: The Rockefeller University, New York.

contain, and presumably form, IgG. Primary biliary cirrhosis is associated with high serum [gM levels and IgM containing cells may be found in large numbers in the liver (Paronetto, Schaffner & Popper, 1964). Cells containing, and presumably producing, rheumatoid factors have been observed in the livers of those patients with liver diseases who have rheumatoid factors in the serum (Bonomo, Tursi & Minerva, 1966). γ-Globulin synthesis is believed not to occur in normal liver (Cohen *et al.*, 1960; Miller & Bale, 1954), but this may not be so in the diseased liver.

This paper reports immunocytochemical studies on liver tissue obtained from patients with a variety of liver diseases and with miscellaneous (primarily non-hepatic) diseases. Cells containing IgG, IgA, IgM and IgD have been investigated. The immunocytochemical findings have been correlated with hepatic histology and with the serum levels of immunoglobulins G, A and M.

MATERIALS AND METHODS

Eighty-seven patients were studied, sixty-four with diseases of the liver and biliary system and twenty-three with miscellaneous primarily non-hepatic diseases (referred to as 'non-hepatic') (Table 1). Diagnosis was based on clinical, hepatic histological and biochemical findings.

TABLE 1. Diagnosis, sex, age and specimens (serum and liver) in eighty-seven patients

	No. patients studied	Sex		Mean age (range)	No. sera tested	Liver specimens*		
		Female	Male			A†	W†	N†
Acute hepatitis	5	3	2	42(13–67)	3	5	0	0
Active chronic hepatitis	4	1	3	32(13–55)	4	0	4	0
Cryptogenic cirrhosis	8	2	6	41(17–58)	7	3	7	0
Alcoholic cirrhosis	6	1	5	51(39–70)	6	4	1	1
Primary biliary cirrhosis	7	6	1	54(40–67)	7	2	2	3
Secondary biliary cirrhosis	5	3	2	27(2–50)	5	2	3	0
Large duct biliary obstruction	14	9	5	49(23–77)	10	1	14	0
Cholelithiasis	6	4	2	57(51–63)	4	0	6	0
Miscellaneous liver diseases	9	4	5	54(29–69)	6	4	5	0
Miscellaneous other diseases 'non-hepatic'	23	9	14	63(15–84)	9	19	4	0

* Ninety specimens obtained from eight-seven patients.
† A, Autopsy; W, operative wedge biopsy; N, needle biopsy.

Ninety liver specimens were obtained. Forty-six were operative wedge biopsies, four were Menghini needle biopsies and forty were samples obtained at autopsy. In sixty-one cases serum samples for immunoglobulin levels were obtained 1–7 days before the liver material.

Acute hepatitis (five patients)

Four patients suffered from virus hepatitis. The fifth patient had received Fluothane

(halothane) anaesthesia three times, for neurosurgical procedures. In four patients the disease was fulminant and liver histology showed massive hepatic necrosis. In the fifth patient, who in addition to virus hepatitis had chronic renal failure and septicaemia, the hepatic histology did not show such severe changes. All patients died and the liver specimens studied were obtained at autopsy.

Active chronic hepatitis (four patients)

The diagnostic criteria were as described by Sherlock (1968) and included a chronic hepatitis with high serum γ-globulin levels, heavy lymphocyte infiltration of the liver with liver-cell necrosis and rosette formation. In all, the disease had progressed to cirrhosis with portal hypertension. Two patients had previously received corticosteroids and one was currently taking these drugs. Operative wedge biopsies were obtained at the time of surgery which had been undertaken for relief of portal hypertension in three patients and in the fourth for duodenal ulcer. In all cases the liver histology disclosed that, despite the apparently compensated clinical state, the disease was still active since piecemeal necrosis and lymphoid infiltration were present.

Cryptogenic cirrhosis (eight patients)

These were patients with no history of alcoholism and no ascertainable cause for their cirrhosis. One patient had a decompensated cirrhosis and died in liver failure following oesophageal bleeding; the other seven patients were compensated. Six patients were being operated on for surgical relief of portal hypertension and one for cholecystectomy. In addition to operative wedge biopsies, follow-up autopsy liver samples were obtained from two patients who died of post-operative infection.

In five the cirrhosis was macronodular and in three of mixed micro- and macronodular type (Sherlock, 1968). In four patients piecemeal necrosis and lymphoid infiltration were present.

Alcoholic cirrhosis (six patients)

One patient also suffered from diabetes mellitus; in another hepatoma had developed. Autopsy liver samples were obtained from four patients who died in hepatic failure precipitated by infection (two cases) or by oesophageal bleeding (one case). The other two patients had well compensated cirrhosis and an operative wedge biopsy was obtained from one of them at the time of surgery for relief of portal hypertension. A needle biopsy was obtained from the other.

Primary biliary cirrhosis (seven patients)

In all cases mitochondrial antibodies (Doniach *et al.*, 1966) were present in the sera and in five, laparotomy confirmed the absence of main bile duct obstruction. The duration of illness ranged from 6 months to 10 years. In five cases the liver histology was that of a late stage (stages 3–4) of primary biliary cirrhosis and in the other two of an earlier one (stages 1–2) (Scheuer, 1967). Lymphoid aggregates were present in six of the samples studied. No liver granulomas were seen.

Two biopsies were operative, three were needle biopsies less than 1 cm long, and two were post-mortem samples obtained by needle aspiration and consisting of many long cylinders of liver tissue.

Secondary biliary cirrhosis (five patients)

In two, biliary cirrhosis was secondary to congenital biliary atresia, in two others to calculus biliary disease with benign strictures and in one it was associated with long-standing ulcerative colitis. One of the patients with biliary atresia was receiving prednisolone. One of the patients with calculus biliary disease had severe cholangitis with abscess formation. In this case granulomata were repeatedly seen in biopsies of the liver but their cause remained unknown. Of the liver specimens studied three were operative biopsies and two autopsy samples.

Large duct biliary obstruction (*without cirrhosis*) (fourteen patients)

Biliary obstruction was due to calculus biliary disease (six cases), benign structure (three), bile duct carcinoma (two) and pancreatic carcinoma (three).

The duration of jaundice varied from 15 days to 2 years and in some patients it was intermittent. Evidence of recent biliary infection was present in four cases but in only one of them was cholangitis seen histologically with polymorphs in bile duct walls and lumens. Liver histology revealed the usual changes seen in large duct biliary obstruction. All liver samples studied were operative wedge biopsies except one which was an autopsy specimen. From one patient two operative wedge biopsies were obtained on different occasions.

Cholelithiasis (*without cholestasis*) (six patients)

All were anicteric at the time of cholecystectomy. Operative cholangiograms confirmed the absence of main bile duct obstruction. Three of the patients had a history of cholecystitis, no signs of infection were present at the time of operation. In three patients liver histology was normal and in the three others some portal tract oedema and inflammation was seen.

Miscellaneous liver diseases (nine patients)

These patients were suffering from the following diseases: metastatic carcinoma of the liver (three cases), reticulum-cell sarcoma (one), fibroma (one), hydatid cyst of the liver (two), chronic liver abscess of unknown aetiology (one) and liver granulomas of unknown aetiology (one). Five were operative wedge biopsies and four were autopsy samples.

Miscellaneous 'non-hepatic' diseases (twenty-three patients)

Seven were casualties and the information available was limited. The age, sex, diagnosis and liver histology are shown in Table 2. Six had evidence of infection at the time of death. Nineteen specimens were obtained at autopsy and four were operative.

Light microscopy

In addition to routine histology of all liver specimens studied, some specific observations were made for correlation with immunocytochemical findings. For this purpose, in addition to paraffin sections, cryostat sections consecutive to those examined immunochemically were used. When needle biopsies were examined, the same specimen was used both for frozen and paraffin sections according to the method described by Raia & Scheuer (1968). The sections were stained with haematoxylin and eosin and by Gordon and Sweet's method for reticulin fibres. The presence of lymphoid aggregates was recorded, and piecemeal

TABLE 2. Diagnosis, age, sex, liver specimens and histology in the liver of twenty-three patients with miscellaneous diseases

	Diagnosis	Sex and age		Specimen	Liver histology
1.	Bronchopneumonia, chronic bronchitis	M	66	A*	Venous congestion
2.	Bronchopneumonia, chronic bronchitis	M	85	A	Slight portal inflammation
3.	Bronchopneumonia, chronic bronchitis	M	80	A	Venous congestion and fat
4.	Cerebral haemorrhage, chronic bronchitis	F	75	A	Venous congestion
5.	Cerebral haemorrhage	F	84	A	Venous congestion
6.	Myocardial infarction	M	59	A	Venous congestion and fat
7.	Myocardial infarction	M	60	A	No changes
8.	Myocardial infarction	M	62	A	Shock liver
9.	Myocardial infarction, pericarditis	F	68	A	No changes
10.	Myocardial infarction, hypercholesterolaemia	M	47	A	Venous congestion
11.	Myocardial infarction, mild diabetes mellitus	M	75	A	Portal fibrosis and inflammation, much fat
12.	Diabetic coma, ileocolitis	M	80	A	Portal fibrosis and inflammation, loose lymphoid aggregates, fat
13.	Pulmonary embolism, ischaemic vascular disease	M	71	A	Venous congestion
14.	Carcinoma of bronchus	F	70	A	Venous congestion, slight portal inflammation. No metastases
15.	Carcinoma of thyroid, congestive heart failure	F	83	A	Portal fibrosis, mononuclear cell infiltration in portal tracts and sinusoids. No metastases
16.	Cancer of stomach	F	65	A	Fat. Slight portal inflammation. No metastases
17.	Carcinoma of pancreas	F	57	B*	No changes
18.	Extrahepatic portal hypertension (old thrombosis). Septicaemia	M	69	A	Dilated portal and central veins, fat
19.	Extrahepatic portal hypertension	M	15	B	Dilated veins and periductal fibrosis
20.	Duodenal ulcer	M	53	B	Fat
21.	Incisional hernia	M	59	B	Mild portal inflammation, fat
22.	Multiple traumatic injuries	F	31	A	Mild portal inflammation, fat
23.	Multiple traumatic injuries	F	60	A	Mild portal inflammation, venous congestion, fat

* A, Autopsy specimen; B, operative biopsy.

necrosis (patchy degeneration of liver cells) was graded from zero to +++. Finally, the over-all infiltration of connective tissue by all types of mononuclear cells was classified into three groups: (i) absent or insignificant, (ii) significant, and (iii) significant with lymphoid aggregates. The mononuclear cell infiltration was regarded as insignificant when no

plasma cells at all were seen and the number of other mononuclear cells had been recorded as zero or +.

Immuno-histochemical methods

Liver specimens were immediately snap-frozen in a mixture of hexane and dry ice with the exception of eight operative samples which were kept at 4°C for 1–36 hr prior to snap-freezing. Autopsies were performed 12 hr to 3 days after death (cadaver storage at 4°C). The frozen specimens were cut in a cryostat at −20°C. Eight adjacent sections, 5 μ thick, were used; six for immunofluorescent stains and two for ordinary histological stains. To assess the effects of storage on the fluorescence staining of immunoglobulin containing cells, additional cryostat sections were obtained from six operative biopsies after 36 hr of storage at 4°C. No change was observed after such storage.

The following specific antisera were used: Fluorescein-conjugated horse antisera against human IgG, IgA, IgM, albumin and β_{1C} (Hyland Laboratories products); rabbit anti-human IgD (unconjugated) donated by Dr D. S. Rowe and fluorescein-conjugated anti-rabbit globulin (Netherlands Red Cross Laboratories product).

Unfixed frozen sections were initially dried at room temperature under a fan for 30 min. They were then washed for 10 min in phosphate buffered saline, pH 7·1, and treated thereafter for 30 min with fluorescent antisera to human IgG, IgA, IgM, albumin and β_{1C}. For the demonstration of IgD, the rabbit anti-human IgD was initially applied for 30 min on the washed sections; the sections were then washed twice for 10 min in phosphate buffered saline and treated for 30 min with fluorescent anti-rabbit globulin. All sections were finally washed twice for 10 min in phosphate buffered saline, covered with phosphate buffered glycerol mounting medium and examined with a Zetopan Reichert fluorescence microscope.

The stained sections were examined for the presence of mononuclear cells showing bright green cytoplasmic fluorescence and an attempt was made at quantitating them: (a) in the portal and septal connective tissue, and (b) in the liver parenchyma. These areas were sequentially scanned (visually) under high power (magnification ×250) and the immunoglobulin containing cells ('Ig-cells') were counted; the number of cells seen was expressed as cells per high power field (h.p.f.). A minimum of twenty high power fields were scanned per section and the 'Ig-cell' count represented the mean of twenty or more fields. Reproduceability was such that duplicate counts did not vary by more than 20%. This method of quantitation is different from that used by Cohen *et al.* (1960) and Paronetto *et al.* (1962, 1964), who expressed their results as the maximum number of cells seen under high power fields (average of three to five fields) and graded them from ± to +++. We have compared the two methods of quantitation and have found a good correlation between them; on the basis of the correlation curve obtained, we have deduced the following equivalents: up to 0·5 cells/h.p.f. = ±; 0·5–2 cells/h.p.f. = +; 2–4 cells/h.p.f. = ++; 4–6 cells/h.p.f. = +++ and more than 6 cells/h.p.f. = ++++. Counts of less than 0·5 cells/h.p.f. are referred to as occasional cells.

The immunocytochemical observations and the assessment of the specific histological parameters which were correlated with the cell counts, were performed separately by two independent observers who at the time of examination had no knowledge of the diagnosis.

Serum immunoglobulin determinations.

Serum immunoglobulin levels were determined by the radial immunodiffusion technique

(Mancini, Carbonara & Heremans, 1965; Fahey & McKelvey, 1965), using Hyland immunoplates. The normal range was that found in sixty-eight healthy persons—mean age 31 years (range 17–50)—consisting of blood donors and members of the Royal Free Hospital staff. As immunoglobulin standards three sera (series 7010A002), provided by Dr Shanbrom, Hyland Laboratories, were used. With these standards the normal range for IgG was 530–1400 mg/100 ml (mean 920), IgA 52–540 mg/100 ml (mean 229) and IgM 37–215 mg/100 ml (mean 80). These values are in rather better agreement with the normal range in other published series (Fahey & McKelvey, 1965; Stiehm & Fudenberg, 1966) than those obtained with an earlier set of standards (Feizi, 1968).

RESULTS

Immunocytochemical observations

Mononuclear cells containing IgG, IgA and IgM, collectively termed 'Ig-cells', were seen in the liver in all groups. IgD-cells were seen only in six specimens. Positive staining of mesenchymal cells with fluorescent anti-albumin was only occasionally found. Fluorescent staining with anti-β_{1C} was negative in all specimens.

The 'Ig-cells' were morphologically similar to plasma cells or to other mononuclear cells with less abundant cytoplasm; positively stained cells in the sinusoids usually had the appearance of Kupffer cells.

The immunoglobulin containing cells were located in portal tracts and fibrous septa. These cells, with a few exceptions, were absent or sparse in sinusoids. The cell counts given in the 'Results' section, unless otherwise stated, refer to those in portal and septal areas.

Acute hepatitis. In three of the five specimens more than 0·5 'Ig-cells'/h.p.f. were seen. In two, cells were also present in the liver parenchyma (0·8 and 1 cell/h.p.f.). IgA-cells were rather more numerous than those containing IgG; IgM-cells were scanty (Fig. 1). Very occasional cells containing albumin were observed in two specimens.

Active chronic hepatitis. In three of the four cases, despite the presence of a significant lymphoid infiltration, 'Ig-cells' were absent or occasional. The fourth patient had mainly IgA-cells and occasional cells containing IgG and IgM (Fig. 1). The liver histology in this patient was similar to that of the others but he was the only one of the group who had never received corticosteroids.

Cryptogenic cirrhosis. 'Ig-cell' counts greater than 0·5/h.p.f. were seen in six of the eight cases studied (Fig. 1). In three, the cell count was greater than 2/h.p.f. and in one of these clusters of IgG- and IgA-cells were observed. IgA-cells were the predominant type seen in five patients. The two post-mortem liver samples had a predominance of IgG-cells. One of these was a second sample (a_2) in a patient from whom an operative biopsy (a_1) had been studied 7 weeks earlier. Whereas a change in 'Ig-cell' pattern was found in this case, no difference was observed between the operative and the post-mortem liver specimen (taken 18 days apart) in a second case (b) (Fig. 1). Both patients had died with post-operative infections.

Alcoholic cirrhosis. Many 'Ig-cells', often in clusters, were seen in five of the six specimens examined; they were not seen in the patient with hepatoma. IgA-cells were predominant in four cases and IgG in one (Figs. 1, 2a and b). This latter patient had diabetes mellitus and staphylococcal septicaemia, in addition to alcoholic cirrhosis. In one patient IgD-cells were seen in large numbers, 6/h.p.f.

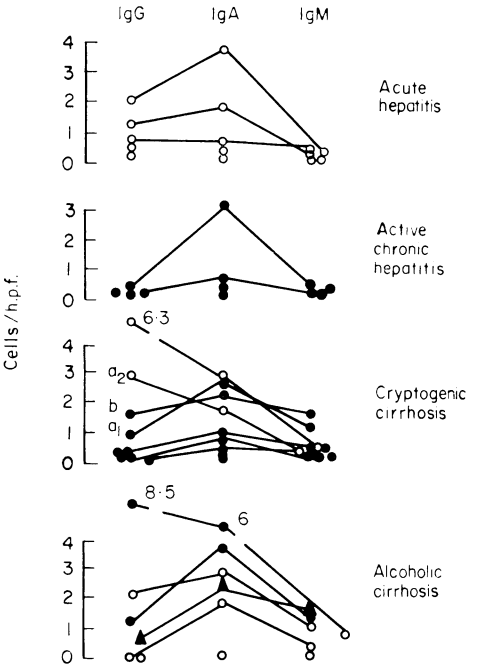

FIG. 1. Immunoglobulin containing cells in the liver of patients with hepatitis and cirrhosis. Cell counts in excess of 0·5/h.p.f. are joined by lines for the demonstration of the immunoglobulin pattern in each patient. ○, Autopsy; ●, biopsy; ▲, needle biopsy.

Primary biliary cirrhosis. Ig-cell counts above 0·5/h.p.f. were obtained in six of the seven patients in this group. IgM-cells were seen in all cases and in contrast to other diagnostic groups, they were the predominant cell type in five (Fig. 2d). IgA-cells were more abundant than those containing IgG and in one case they were the predominant cell type. IgG-cells were a minority in six of the seven cases (Fig. 3). IgD-cells were seen in two cases in counts of 0·5 and 2 cells/h.p.f.

Secondary biliary cirrhosis. In four of the five patients 'Ig-cell' counts above 0·5/h.p.f. were obtained, IgA-cells being the predominant type (Fig. 3). The highest cell counts were in the two patients with calculous biliary disease, in one of whom there was severe cholangitis and liver granulomata. In addition to clusters of IgG and IgA-cells, occasional IgD-cells were present in this patient (Fig. 2c). In the patient with associated colitis, 1·3 IgD-cells/h.p.f. were seen.

Large duct biliary obstruction. 'Ig-cells' were seen in only three of the fourteen patients. In one of these (the patient with pancreatic carcinoma), IgG-cells were present, whereas the other two had IgG and IgA counts with a predominance of IgA (Fig. 3). Of these latter two patients, one had a benign stricture with cholangitis and the other a common bile duct stone. Cholangitis was present in three other patients with scanty or no 'Ig-cells'. Occasional 'Ig-cells' were seen in the patient with two biopsies. The 'Ig-cell' counts did not correlate with the duration or severity of jaundice.

Cholelithiasis (without cholestasis): In all specimens, 'Ig-cells' were scanty or absent (Fig. 3).

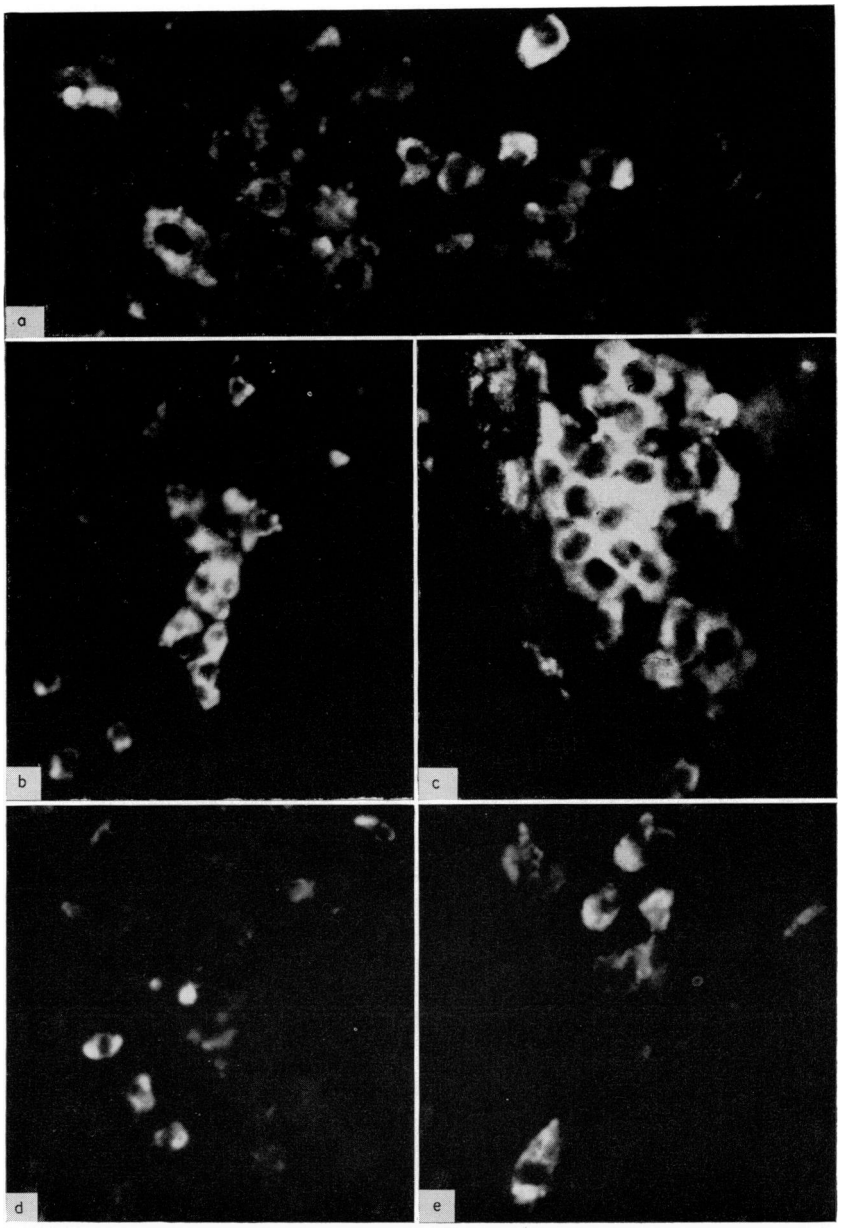

FIG. 2. Photomicrographs showing antibody containing cells in the liver. (a) IgG-cells in a patient with alcoholic cirrhosis; × 510. (b) IgA-cell cluster in a patient with alcoholic cirrhosis; × 320. (c) IgA-cell cluster in a patient with secondary biliary cirrhosis associated with cholangitis and liver granulomas; × 510. (d) IgM-cells in a patient with primary biliary cirrhosis: × 320. (e) IgM-cells in a patient with carcinoma of the thyroid; × 510.

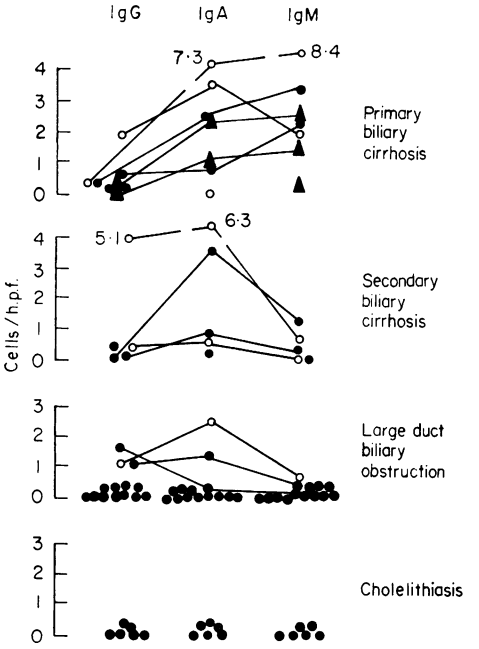

FIG. 3. Immunoglobulin containing cells in the liver of patients with primary biliary cirrhosis, secondary biliary cirrhosis and other biliary diseases. Cell counts in excess of 0·5/h.p.f. have been joined by lines as in Fig. 1. ○, Autopsy; ●, biopsy; ▲, needle biopsy.

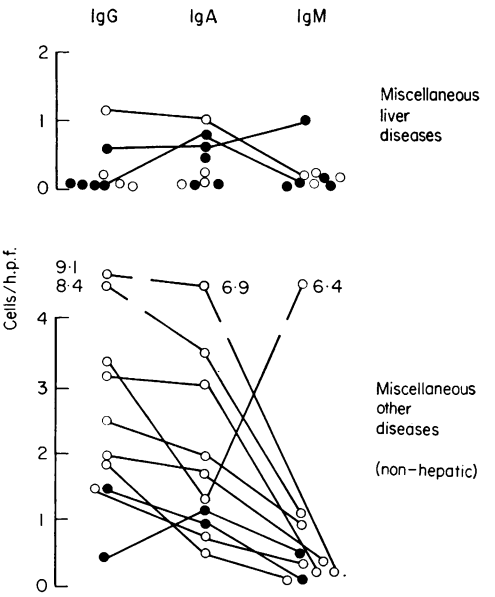

FIG. 4. Immunoglobulin containing cells in the liver of patients with miscellaneous hepatic and miscellaneous 'non-hepatic' diseases. Cell counts in excess of 0·5/h.p.f. are joined by lines as in Fig. 1. ○, Autopsy; ●, biopsy.

Miscellaneous liver diseases. Low 'Ig-cell' counts (0·5–1·2/h.p.f.) were obtained in three of the nine patients: a case of metastatic carcinoma, a fibroma of the liver and a case with liver granulomas of unknown aetiology (Fig. 4). In these three patients, 'Ig-cell' counts of 0·5–0·7/h.p.f. were also obtained in sinusoids.

Miscellaneous 'non-hepatic' diseases. Ten of the twenty-three cases had 'Ig-cell' counts above 0·5/h.p.f. and the cell patterns in these ten patients are shown in Fig. 4.

TABLE 3. Incidence of immunoglobulin containing cells in eighty-seven patients studied

Diagnosis	No. of patients	Immunoglobulin containing cells*				
		Absent or <0·5	0·5—2	>2	Total positive (>0·5)	Predominant cell
Cirrhosis (all types except primary biliary)	23	6	7	10 (44%)	17 (70%)	IgA (84%)†
Primary biliary cirrhosis	7	1	1	5 (71%)	6 (86%)	IgM (83%)
Other hepatobiliary diseases	34	25	7	2 (6%)	9 (26%)	IgA (66%)
Miscellaneous 'non-hepatic' diseases	23	13	5	5 (22%)	10 (43%)	IgG (60%)

* Mean number of cells per high power field (×250).
† Incidence among total positive.

Their diagnoses were as follows: bronchopneumonia on chronic bronchitis; cerebral haemorrhage; myocardial infarction and mild diabetes; diabetes and ileocolitis; carcinoma of thyroid and congestive heart failure; cancer of stomach; extrahepatic portal hypertension; incisional hernia and multiple traumatic injuries (two cases). IgG-cells were by far the commonest cell type. A very high IgM-cell count (Fig. 2e) was, however, obtained in the patient with carcinoma of thyroid and congestive heart failure, who in addition had IgD-cells (2·2/h.p.f.). This was the group of patients with the highest parenchymal Ig-cell counts varying from 0·5 to 3·3 in six cases.

Though there was no evidence of primary liver disease in the twenty-three patients in this group, an inflammatory portal infiltrate (non-specific reactive change) was present in nine of them, seven of whom were among those with high Ig-cell counts. There was no correlation between the immunocytochemical findings and the following factors: age, sex, diagnosis, presence of infection, duration of the agonal period and time interval from death to autopsy.

Summary of the immunocytochemical findings. Apart from the small group of patients with cholelithiasis (without cholestasis) in which Ig-cells were scanty or absent, 'positive' counts (i.e. Ig-cell counts above 0·5/h.p.f.) were found in all diagnostic groups. In Table 3 the material studied has been divided into four main diagnostic categories: cirrhosis (all types except primary biliary); primary biliary cirrhosis; miscellaneous hepato-biliary diseases and miscellaneous 'non-hepatic' diseases. In the diagnostic groups included in each category, 'Ig-cell' patterns were on the whole similar (Figs. 1, 3 and 4). In primary biliary cirrhosis and in cirrhoses of other types positive 'Ig-cell' counts were obtained in 86 and 70% of cases, respectively, and 'high' Ig/cell counts (more than 2/h.p.f.) in 71 and 44%, respectively.

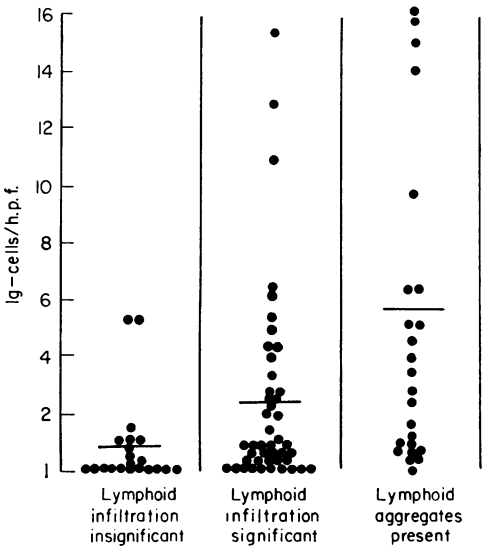

FIG. 5. Comparison of 'Ig-cells' (sum of IgG, IgA and IgM-cell counts) with lymphoid infiltration of the liver.

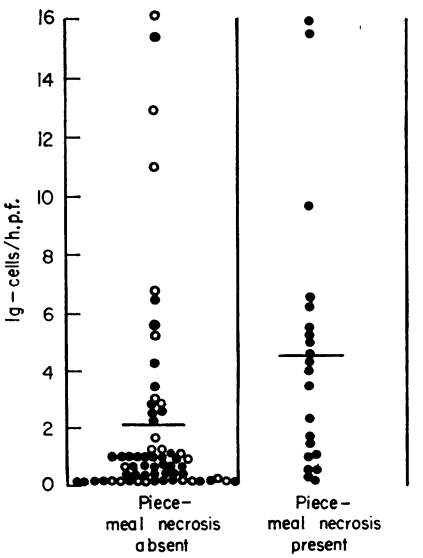

FIG. 6. Comparison of 'Ig-cells' (sum of IgG, IgA and IgM-cell counts) with the presence or absence of piecemeal necrosis. ●, Hepatobiliary disease; ○, non-hepatic disease.

These were in contrast to the lower figure in other hepato-biliary and miscellaneous 'non-hepatic' diseases. Nonetheless, 22% of patients without primary liver disease had high 'Ig-cell' counts.

In primary biliary cirrhosis the predominant cell type was IgM whereas in cirrhosis (other than primary biliary) and in other hepato-biliary diseases IgA cells were the predominant type. In contrast to the liver disease groups, IgG cells were the predominant cell type in miscellaneous 'non-hepatic' diseases.

Comparison of 'Ig-cells' with liver histology

The total 'Ig-cell' counts (sum of IgG-, IgA- and IgM-cell counts) were correlated with the presence or absence of significant lymphoid infiltration and with lymphoid aggregate formation (Fig. 5), and secondly with piecemeal necrosis (Fig. 6). The mean 'Ig-cell' counts of patients with lymphoid infiltration and with lymphoid aggregates were higher than those of patients without such infiltration and counts above 6 cells/h.p.f. were obtained only in patients with infiltration. But there was a wide range of cell counts in association with lymphoid infiltration (or aggregates) and in 50% of the samples with such infiltration 'Ig-cell' counts of less than 1/h.p.f. were obtained. The mean (total) 'Ig-cell' counts were higher in the presence than in the absence of piecemeal necrosis, but there was complete overlap in the range of cell counts in the two groups (Fig. 6).

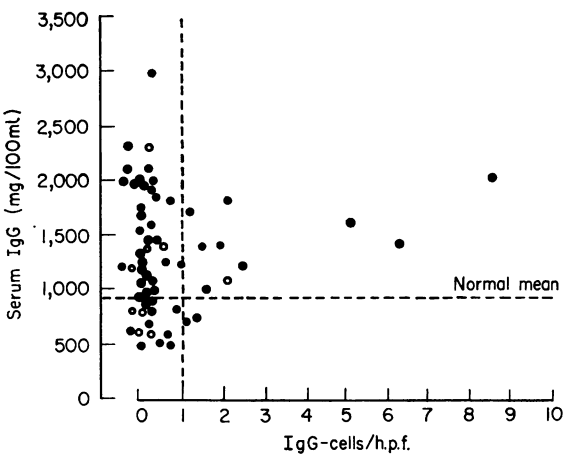

FIG. 7. Correlation of serum IgG levels with IgG-cells in the liver. Key as Fig. 6.

Correlation with serum immunoglobulin levels

Comparison in sixty-one cases of the number of cells containing immunoglobulins with the respective serum immunoglobulin levels failed to show an overall correlation. Separate comparisons in each of the diagnostic groups also showed a lack of correlation. However, when more than 1 cell/h.p.f. containing a certain immunoglobulin was seen in a liver specimen, the respective serum immunoglobulin level was, with few exceptions, above the normal mean (Figs. 7, 8 and 9). This suggests that in some patients immunoglobulin production in mononuclear cells of the liver could be a contributory factor in serum immunoglobulin levels.

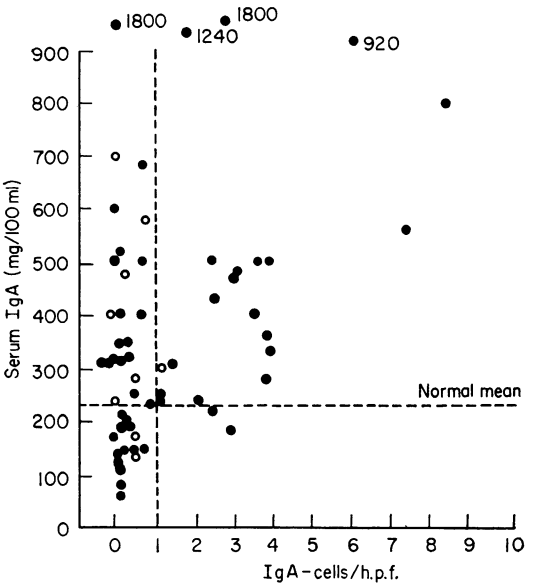

FIG. 8. Correlation of serum IgA levels with IgA-cells in the liver.

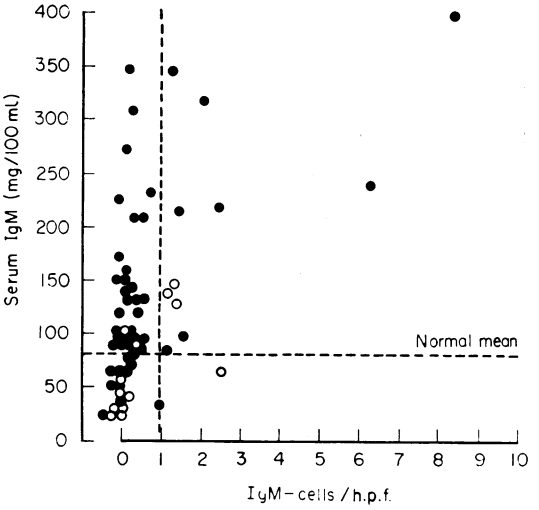

FIG. 9. Correlation of serum IgM levels with IgM-cells in the liver.

DISCUSSION

All types of liver specimen studied (operative, needle and autopsy) were found to be suitable for immunocytochemical observations of lymphoid cells. Operative biopsies are the best because they provide adequate samples of fresh tissue. Autopsy specimens though adequate in size have a number of drawbacks. Such factors as superimposed infections, shock or a prolonged agonal period, influence the liver histology and autolysis prevents the assessment

of piecemeal necrosis. Needle biopsies provide only small amounts of tissue and raise the problem of sampling error. Needle samples were used only in three instances of primary biliary cirrhosis and one of alcoholic cirrhosis.

For the quantitation of 'Ig-cells' in the liver the mean population density rather than the maximal count was used. These results cannot be compared on a numerical basis with those of previous studies in which maximal counts of cells were presented (Cohen *et al.*, 1960; Paronetto *et al.*, 1962, 1964, Bonomo *et al.*, 1966). If a comparison is to be attempted the equivalents given in the 'Materials and methods' section should be used.

In the present study mononuclear cells containing immunoglobulins in counts above 0·5/h.p.f. (equal to + of other studies) were seen not only in liver diseases but also in miscellaneous 'non-hepatic' disorders. As many as 22% of the 'non-hepatic' cases had high 'Ig-cell' counts (i.e. more than 2 cells/h.p.f., equivalent to + + or more.) This is in contrast to the observations of Cohen *et al.* (1960) and Paronetto *et al.* (1962), who reported the absence of or the occurrence of only a few (+) 'Ig-cells' in the liver of patients with miscellaneous diseases and non-hepatic hypergammaglobulinaemias. Bonomo *et al.* (1966), however, in studies directed at the demonstration of rheumatoid factor-containing cells did find γ-globulin containing cells in the livers of patients with miscellaneous non-hepatic diseases.

It is not certain whether 'Ig-cells' are present in the livers of healthy persons and in what numbers. Cases of accidental death and a variety of 'non-hepatic' diseases were included in an attempt to examine histologically normal livers. Only three of fourteen (21%) of the 'non-hepatic' cases without histological evidence of mononuclear infiltration had 'Ig-cell' counts above 0·5/h.p.f. whereas in those with a significant mononuclear infiltration, the incidence of positive Ig-cell counts was seven out of nine (77%).

In liver diseases IgA-containing cells were the most abundant except in primary biliary cirrhosis where, as previously described by Paronetto *et al.* (1964), IgM-cells were the predominant cell type. They were present, however, as minor components in other liver diseases and in 'non-hepatic' conditions. IgG-cells were seen amongst most diagnostic groups, they were the predominant cell type in the 'non-hepatic' group in two patients with cryptogenic and in one with alcoholic cirrhosis. In contrast to the observations of Paronetto *et al.* (1962), the heavy lymphoid infiltrate in patients with active chronic hepatitis was not necessarily accompanied by high IgG-cell counts; furthermore, IgG was not the predominant cell type. Whether these differences are related to the clinically inactive stage of the cases in this study or to previous steroid therapy is not possible to say.

From these immunohistological observations it may be concluded that the IgA system of cells may be represented in the liver in proximity to the bile ducts much as in other mucosal surfaces, that various local and systemic infections may result in increased numbers of various Ig-containing cells in the liver and that in chronic active hepatitis, treatment with prednisolone may result in a substantial decrease of Ig-cells in the liver.

The 'Ig-cells' in the liver are probably producing immunoglobulins, rather than phagocytosing them (Vazquez, 1961; Paronetto *et al.*, 1962; Schaffner & Popper, 1962). The lack of albumin-containing mononuclear cells supports the former assumption. The lack of overall correlation between 'Ig-cell' counts and the respective serum immunoglobulin levels indicates that 'Ig-cells' in the liver are not the main source of the raised serum immunoglobulin levels in liver disease, but when present in large numbers in the liver, it is possible that they contribute to the serum levels (Figs. 7, 8 and 9).

ACKNOWLEDGMENTS

This work was supported by grants from the Prudential Life Insurance Company (for S. Hadziyannis); the Royal Free Hospital and the Medical Research Council (for Ten Feizi).

REFERENCES

BONOMO, L., TURSI, A. & MINERVA, V. (1966) Immunofluorescence study of rheumatoid factor in liver tissue of patients with rheumatoid arthritis and hepatic diseases. *J. Path. Bact.* **92,** 423.

COHEN, S., OHTA, G., SINGER, E.J. & POPPER, H. (1960) Immunocytochemical study of gamma globulin in liver in hepatitis and post-necrotic cirrhosis. *J. exp. Med.* **111,** 285.

DONIACH, D., ROITT, I.M., WALKER, J.G. & SHERLOCK, S. (1966) Tissue antibodies in primary biliary cirrhosis, active chronic hepatitis (lupoid) hepatitis, cryptogenic cirrhosis and other liver diseases and their clinical implications. *Clin. exp. Immunol.* **1,** 237.

FAHEY, J.L. & MCKELVEY, E.M. (1965) Quantitative determination of serum immunoglobulins in antibody-agar plates. *J. Immunol.* **94,** 84.

FEIZI, T. (1968) Immunoglobulins in chronic liver disease. *Gut,* **9,** 193.

HOBBS, J.R. (1966) Primary biliary cirrhosis: positive antibody tests associated with increased immunoglobulin IgM. *Proc. roy. Soc. Med.* **59,** 568.

LEE, F.I. (1965) Immunoglobulins in viral hepatitis and active alcoholic liver disease. *Lancet,* **ii,** 1043.

MANCINI, G., CARBONARA, A.O. & HEREMANS, J.F. (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. *Int. J. Immunochem.* **2,** 235.

MCKELVEY, E.M. & FAHEY, J.L. (1965) Immunoglobulin changes in disease: quantitation on the basis of heavy polypeptide chains, IgG (γG), IgA (γA) and IgM (γM) and of light polypeptide chains, type K (I) and type L (II). *J. clin. Invest.* **44,** 1778.

MILLER, L.L. & BALE, W.F. (1954) Synthesis of all plasma protein fractions except gamma globulins by the liver. The use of zone electrophoresis and lysine-E-C14 to define plasma proteins synthesized by the isolated perfused liver. *J. exp. Med.* **99,** 125.

PARONETTO, F., RUBIN, E. & POPPER, H. (1962) Local formation of γ-globulin in the diseased liver, and its relation to hepatic necrosis. *Lab. Invest.* **11,** 150.

PARONETTO, F., SCHAFFNER, F. & POPPER, H. (1964) Immunocytochemical and serologic observations in primary biliary cirrhosis. *New Engl. J. Med.* **271,** 1123.

RAIA, S. & SCHEUER, P.J. (1968) Method for obtaining both frozen and paraffin sections from the same liver biopsy. *J. clin. Path.* **21,** 412.

SCHAFFNER, F. & POPPER, H. (1962) A phagocytic and protein-forming mesenchymal cell in human cirrhosis. *Nature* (*Lond.*), **196,** 684.

SCHEUER, P.J. (1967) Primary biliary cirrhosis. *Proc. roy. Soc. Med.* **60,** 1257.

SHERLOCK, S. (1968) *Diseases of the Liver and Biliary System,* 4th edn. Blackwell Scientific Publications, Oxford and Edinburgh.

STIEHM, E.R. & FUDENBERG, H.H. (1966) Serum levels of immune globulins in health and disease: a survey. *Pediatrics,* **37,** 715.

VASQUEZ, J.J. (1961) Antibody- or gamma globulin- forming cells as observed by the fluorescent antibody technique. *Lab. Invest.* **10,** 1110.