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IgA in alcoholic cirrhosis

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SUMMARY

Circulating IgA in patients with alcoholic cirrhosis was quantitated and characterized to determine the proportion of monomer and dimer present. Polymeric (p) IgA formed a significantly greater proportion of the total serum IgA in cirrhosis, but these individuals showed significant elevations of serum levels of monomeric (m) as well as pIgA. Thus a selective disturbance of pIgA metabolism could not entirely account for the abnormal serum IgA levels. Studies on spontaneous IgA synthesis *in vitro* by peripheral blood lymphocytes showed that greater amounts of both mIgA and pIgA were formed by cells from patients with cirrhosis compared with control individuals.

INTRODUCTION

High serum globulin levels, and particularly elevations of serum immunoglobulin, are common in chronic liver disease (Tomasi & Tisdale, 1964; Lee, 1965; Wilson *et al.*, 1967; Iturriaga *et al.*, 1977). This is true not only in patients in whom a primary immunological process may have been involved in the development of cirrhosis, for example, chronic active hepatitis—but in alcoholic cirrhosis. Although elevations of each class of immunoglobulin occur in most types of cirrhosis, elevated serum IgA levels are particularly marked in patients with alcoholic cirrhosis (Lee, 1965; Wilson *et al.*, 1967).

Recent studies in rodents have highlighted a central role for the liver in IgA metabolism (Lemaitre-Coelho, Jackson & Vaerman, 1977, 1978; Orlans *et al.*, 1978; Jackson *et al.*, 1978). Polymeric IgA in serum is rapidly transferred to bile, by an endocytic vesicular transport mechanism through the hepatocyte (Birbeck *et al.*, 1979; Mullock *et al.*, 1979; Renston *et al.*, 1980). Circulating dimeric IgA (dIgA) and higher polymers containing J-chain, link with secretory component (SC) on the liver cell surface to initiate this process (Fisher *et al.*, 1979; Socken *et al.*, 1979; Orlans *et al.*, 1979). Monomeric IgA (mIgA) cannot take this route. The pathway for polymeric IgA in rats is of major significance, and bile duct ligation leads to dramatic elevations in serum IgA (Lemaitre-Coelho *et al.*, 1978) and a fall in IgA concentrations in the gut lumen. Recent studies (Delacroix *et al.*, 1982c) using purified IgA polymers in man have also demonstrated selective transfer from serum to bile, although quantitatively the process appeared much less significant than in rodents. It has been frequently suggested in recent publications that interruption of this pathway might explain the elevated IgA levels observed in patients with liver disease (Sancho *et al.*, 1981; Kutteh *et al.*, 1982).

We have characterized circulating IgA in patients with alcoholic liver disease, and also measured the quantity and size of IgA synthesized *in vitro* by cultures of peripheral blood mononuclear cells. The results indicate that although the quantity of circulating dIgA is increased in patients with

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alcoholic cirrhosis, there is also a striking increase in mIgA in the circulation, and selective loss of clearance of dIgA by the liver cannot explain the high IgA levels seen in this disease.

MATERIALS AND METHODS

Patients and control individuals. Nine patients were studied, with biopsy proven alcoholic cirrhosis (three female, six male) aged 40–65 years; studies were also performed on nine healthy individuals (three female, six male) aged 32–50 years.

Cell cultures. Peripheral blood mononuclear cells (PBMC) were isolated from 25 ml venous blood by conventional Ficoll-Hypaque separation, washed five times and resuspended in 2 ml cultures in complete medium (RPMI 1640, GIBCO, containing 15% heat-inactivated fetal calf serum, 100 u/ml penicillin, 100 μg/ml streptomycin, GIBCO, and 4 mM glutamine) in round bottomed tissue culture tubes (Sterilin) at a concentration of 2×10^6/ml. Cultures were set up with or without pokeweed mitogen (40 μl of stock solution, Sigma Chemicals) and incubated in 5% CO_2 in air with continuous rotation for 7 days. After centrifugation supernatants were harvested and stored at −20°C until released Ig was measured.

Characterization of Ig. Serum samples (at a dilution of 1:6,000 or 1:8,000) or neat supernatants were fractionated on a 2·6 × 57cm column of Ultrogel AcA 22 (LKB) equilibrated in phosphate-buffered saline, pH 7·4. Two millilitre fractions were collected and IgA measured by immunoradiometric assay. The column was calibrated using pure standards of ^{125}I-dIgA and ^{131}I-mIgA (Delacroix, Meykens & Vaerman, 1982a) and purified IgG and IgM.

Measurement of IgA. Total serum IgA was measured by single radial immunodiffusion and by nephelometry, against a standard serum pool previously calibrated against WHO serum by nephelometry. Serum values determined by single radial immunodiffusion were corrected for the presence of dIgA as described by Delacroix *et al.* (1982a).

IgA in culture supernatants and in column eluates was measured by immunoradiometric assay. In brief, polyvinyl microtitre plates (Dynatech) were coated with affinity purified goat anti-human IgA (α-chain) ('Tago' Tissue Culture Services, Slough) at a concentration of 5 μg/ml in carbonate buffer, pH 9·6. Samples, and standards from pooled serum, were incubated for 4 h at 37°C, and after washing ^{125}I-anti-α (labelled by chloramine-T) was added. After extensive washing, wells were counted in an NE 1800 γ-counter (Nuclear Enterprises) and IgA concentrations measured from standard curves.

RESULTS

Serum IgA levels

The mean total IgA for nine patients with alcoholic cirrhosis was 8·9 mg/ml (±2·88 s.d.), compared with 1·69 (±0·97) for nine control individuals. The mean mIgA was 5·9 mg/ml (±2·78 s.d.) for the patients with cirrhosis, and 1·37 (±0·83) for control individuals; the mean dIgA was 3·0 mg/ml (±1·07 s.d.) for cirrhotic patients, compared with 0·32 (±0·18) for normal individuals (Table 1).

Thus (Fig. 1) amongst control individuals a mean of 85% of circulating IgA was monomeric, and 14·9% dimeric, compared with 68·8% mIgA and 31·2% dIgA amongst patients with cirrhosis. The higher proportion of dIgA amongst patients with cirrhosis is significant ($P < 0·01$, Wilcoxon Rank Sum test).

Synthesis of IgA (Fig. 2)

Supernatants from 7 day cultures of PBMC of normal individuals contained 195·8 ± 79 (s.d.) ng/ml of IgA in unstimulated cultures, and 537 ± 267 ng/ml in PWM stimulated cultures. In contrast supernatants from PBMC from cirrhotic patients contained 1,567 ± 1,089 ng/ml in unstimulated cultures, but less in cultures with pokeweed mitogen (717 ± 529 ng/ml). Comparing supernatants from patients and controls, unstimulated cultures from patients with alcoholic cirrhosis contained significantly more IgA ($P < 0·001$) but there was no significant difference between the cultures with pokeweed.

Table 1. Serum IgA in normal individuals and patients with cirrhosis

	mIgA	dIgA	Total serum IgA (mg/ml)
Normals			
1	0·28	0·05	0·33
2	1·26	0·30	1·56
3	2·92	0·48	3·40
4	1·40	0·09	1·49
5	0·37	0·25	0·62
6	2·24	0·54	2·78
7	1·22	0·50	1·72
8	1·61	0·44	2·05
9	1·00	0·23	1·23
Mean	1·37	0·32	1·69
s.d.	0·83	0·18	0·97
Cirrhotics			
1	4·75	3·33	8·08
2	2·60	2·64	5·24
3	4·00	5·27	9·27
4	5·50	3·57	9·07
5	8·20	2·00	10·20
6	6·52	2·18	8·70
7	8·01	2·19	10·20
8	11·02	3·68	14·70
9	2·82	2·18	5·00
Mean	5·94	3·00	8·94
s.d.	2·78	1·07	2·88

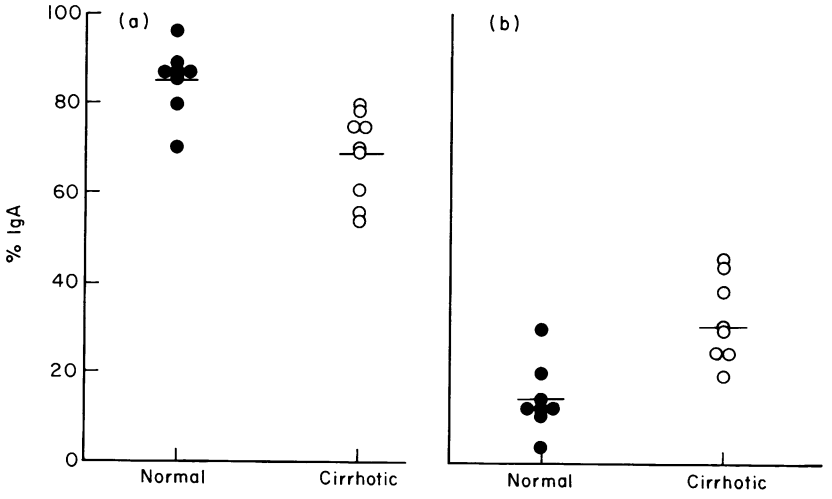

Fig. 1. (a) mIgA, and (b) dIgA, as proportion of total serum, in normal individuals and patients with cirrhosis.

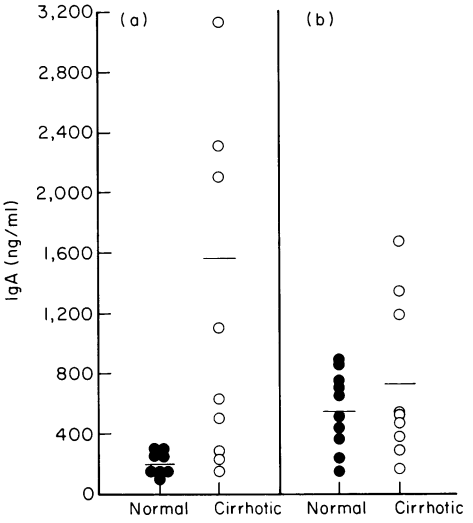

Fig. 2. IgA concentrations in supernatants from (a) unstimulated and (b) PWM stimulated *in vitro* cultures of PBMC from normal and cirrhotic individuals.

Characterization of synthesized IgA

The proportions of mIgA and dIgA in supernatants of *in vitro* cultures were compared in pokeweed mitogen stimulated cultures (the amount of IgA in unstimulated cultures from normal individuals was too low to allow accurate quantitation of IgA in fractions eluted from Ultrogel columns). In the stimulated cultures from normal individuals, 75·4% of IgA was dimeric and 24·6% monomeric; by comparison in the supernatants from cirrhotic patients, 63·6% was dIgA and 36·4% monomeric (Fig. 3). This proportion does not differ significantly between the two groups.

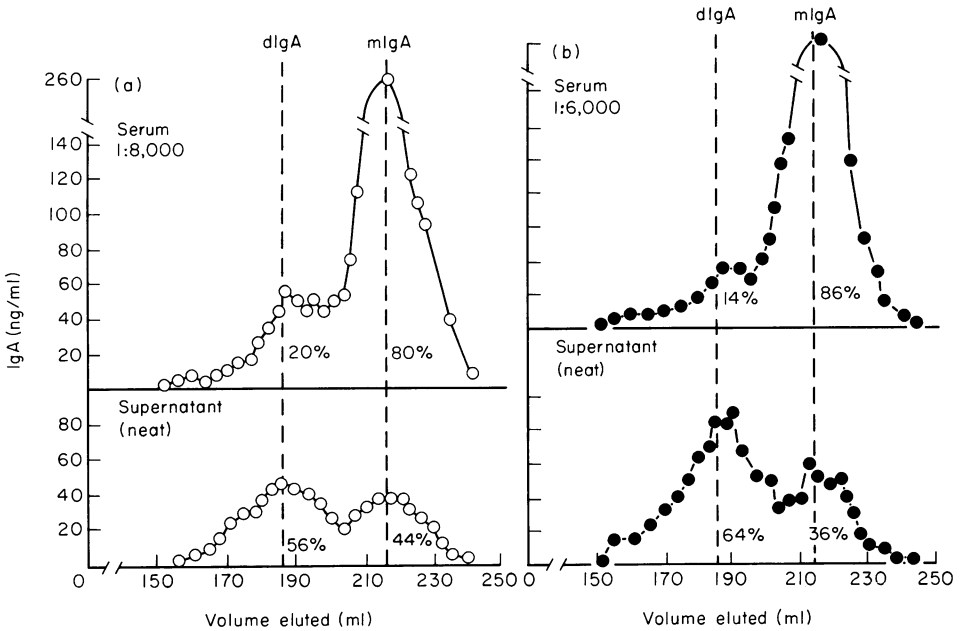

Fig. 3. (a) Elution profiles of (above) serum and (below) supernatant of *in vitro* pokeweed mitogen stimulated PBMC cultures, from a patient with alcoholic cirrhosis. Experimental details as in Methods. (b) Elution profiles of (above) serum and (below) supernatant of *in vitro* pokeweed mitogen stimulated PBMC cultures, from a normal individual.

Relationship between serum IgA and IgA synthesized in vitro

The relationship between circulating IgA levels and *in vitro* synthesis rate was investigated both for mIgA and dIgA. For neither molecular form, in either group of subjects, was there a correlation between serum IgA levels and the amount of IgA in supernatants from *in vitro* peripheral blood lymphocyte cultures.

DISCUSSION

The serum level of IgA, as any other protein, is determined by the synthetic and catabolic rates, the plasma volume, and distribution between intra- and extravascular compartments. In patients with alcoholic cirrhosis there are theoretical reasons for assuming that all of these may be altered. Hypoalbuminaemia and a lowered colloid osmotic pressure tend to reduce intravascular volume, whilst the development of ascites and oedema indicate a gross expansion of extravascular volume. Reticuloendothelial cell dysfunction, and portal–systemic shunting, probably result in persistence of antigen in the circulation, forming a continuous stimulus to antibody production (Triger, Cynamon & Wright, 1973; Pomier-Layrargnes *et al.*, 1980). The liver has a well defined role in removing effete plasma proteins, so that defective liver function might be expected to decrease protein catabolism (Ashwell & Morell, 1974). We have mentioned above the particular role of the rodent liver in removing polymeric IgA (pIgA) from the circulation to the bile, so that interruption of this pathway dramatically elevates serum IgA polymer levels.

The results presented here confirm again the striking elevation in serum IgA seen in alcoholic cirrhosis, with a significant increase in the proportion of IgA that is polymeric. Our results are similar to those of Kutteh *et al.* (1982), who in a smaller group of patients (four controls and four with cirrhosis) found pIgA to form 8% of total IgA in the serum of normal individuals, and 25–45% of IgA in patients with cirrhosis. Compared with this study, our results show a higher proportion of pIgA in normal individuals (10–30% in normals) but our results in cirrhotic patients are similar (20–46%). Such differences probably reflect methodological differences in the estimation of small amounts of pIgA, and in particular the effects of size heterogeneity on the assay for pIgA (Kutteh *et al.*, 1982, not using a correction factor for this in their assays on serum, although they used such corrections in bile). Different techniques of estimating pIgA have recently been compared (Delacroix *et al.*, 1982a; Delacroix, Deherinin & Vaerman, 1982b; Delacroix & Vaerman, 1982) and this explains the discrepancies in pIgA concentrations reported in various studies (Nagura *et al.*, 1981; Delacroix *et al.*, 1982c). Despite this, there is no basic disagreement between Kutteh and ourselves on the finding that this paper highlights: in alcoholic cirrhosis, there is a striking increase in circulating mIgA as well as in pIgA: in our patients, mIgA was increased nearly five-fold.

Thus interruption of the heavily emphasized transfer of pIgA from serum to bile forms an inadequate explanation for the abnormal levels of IgA in patients with alcoholic cirrhosis. Recent estimates (Delacroix *et al.*, 1982c) of the amount of pIgA transferred in man support this conclusion—e.g., only 2% of injected pIgA being transferred to bile over 24 h in man, compared with over 50% in rodents after 4 h (Dooley *et al.*, 1982). If this figure is generally applicable, interruption of the transfer of 2% of circulating pIgA from serum could only make a minor contribution to the nearly 10-fold increase in pIgA in these cirrhotic patients, bearing in mind the half life of pIgA of 3–5 days.

The explanation for the elevation in circulating mIgA is unclear and requires catabolic studies using labelled proteins *in vivo* in patients in a steady state. As an indirect approach to the problem we measured IgA synthesized *in vitro* by peripheral blood lymphocytes both spontaneously and in response to the polyclonal B cell stimulator pokeweed. Clearly the peripheral blood B cell population is not representative of the total IgA synthetic capacity of the body, a comment emphasised by the lack of correlation between serum Ig levels and the amount of IgA synthesized, and illustrated in recent comparative studies (Kutteh, Prince & Mestecky, 1982). There was, however, in unstimulated cultures, a striking increase in the amount of IgA synthesized compared with controls, both m- and pIgA being greater. This suggests that enhanced synthesis rather than, or in addition to, decreased catabolism contributes to the high IgA levels in alcoholic cirrhosis.

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