Serum and intestinal secretory IgA in alcoholic cirrhosis of the liver

G PELLETIER, M J BRIANTAIS, C BUFFET,
J PILLOT, and J P ETIENNE

From the Liver and Gastrointestinal Diseases Service, Hôpital Bicêtre, Kremlin-Bicêtre, and the Department of Immunology and Bacteriology and Microbial Immunology Unit, Institut Pasteur, Paris, France

SUMMARY Serum and intestinal secretory IgA (sIgA) were investigated in control subjects and patients with alcoholic cirrhosis of the liver. Intestinal secretions were sampled by intraluminal perfusion with a balloon catheter. Monomeric IgA and sIgA were assayed by reversed radial immunodiffusion and nephelometry after separation by Ultrogel column filtration. High levels of serum sIgA were found only in patients with severe cirrhosis accompanied by jaundice. The intestinal rate of secretion of sIgA measured in these patients was significantly lower than that observed in either the controls or the patients with compensated cirrhosis. Such an intestinal sIgA deficiency, which could be explained either by a fall in small intestinal immunocyte synthesis or by a defect in the transenterocyte transport system, could be partially responsible for the high incidence of intestinal infection observed in severe cirrhosis.

The presence of secretory IgA (sIgA) in human serum is well documented.¹⁻³ Liver diseases, notably alcoholic cirrhosis of the liver, are associated with abnormally high amounts of sIgA in serum.⁴⁻⁶ In these cases, the observed structure of serum sIgA is identical with that of the sIgA found in external secretions.⁷ Apart from hypogammaglobulinaemia, the secretory component has never been detected free in serum; it has rather always been detected as covalently linked to dimeric IgA.^{1,3,7} Reports of small intestine involvement in cirrhosis⁸ decided us to undertake a parallel study of sIgA in serum and intestinal secretions in normal and cirrhotic subjects.

Methods

PATIENTS

These investigations were performed on three healthy subjects and six patients with alcoholic cirrhosis, three of whom presented serious signs of disease with jaundice (corresponding to Child's type C),⁹ while, in the other three, cirrhosis was compensated (Child's type A or B). The diagnosis of cirrhosis in each case was made on the basis of clinical and histological criteria and laboratory results. None of the subjects studied had received

antibiotics, steroids, or potassium chloride during the preceding month and none presented obvious intestinal infection.

PROCEDURE

Evaluation of serum sIgA

As it is well established that the secretory component is never found free in sera apart from in hypogammaglobulinaemia,⁷ it is possible to evaluate the amount of sIgA with anti-secretory component immune serum. Therefore serum sIgA was detected and measured by radial immunodiffusion in 0.6% Agarose, using an anti-secretory component antiserum.¹⁰ The results are semi-quantitative: normal sera contain: $12.1 \mu\text{g/ml} \pm 6.5$ sIgA; levels higher than mean value ± 2 SD ($30 \mu\text{g}$ sIgA/ml) are considered pathological. For certain serum specimens, in particular for those with a pathological sIgA level, a semi-quantitative assay was performed by haemagglutination inhibition.¹⁰

Sampling of intestinal secretions

Intestinal secretions were sampled by intraluminal perfusion with a balloon catheter.¹¹ The occlusive balloon was placed near the duodenojejunal junction and its position checked radiologically. Effective occlusion was confirmed both by inspection of the liquid collected at the sampling point and by injecting bromesulphonaphthalein proximal to the balloon and testing its appearance at

the sampling point. The segment explored extended from the balloon to point 40 cm distal to it. A SCOP PS 20 pump was used for perfusion at a constant rate of about 10 ml/mn (9.52–11.05 ml/mn). The tracer used was polyethyleneglycol (PEG) 4000, diluted at a concentration of 10 g/l in physiological saline solution and detected by Hyden's method.¹²

The flow rate (D) of a given substance (S) is given by the formula:

$$DS = V1 \left(S1 - S2 \times \frac{PEG1}{PEG2} \right),$$

in which V1 is the perfusion rate, S1 and S2 the concentrations of S at the perfusion and sampling points (here, S1=0), and PEG1 and PEG2 the tracer concentrations at these points.

In order to reach equilibrium, samples were taken only after one hour and secreted jejunal fluid was collected for one hour. The samples were stored at -20°C after the enzymatic inhibitor diisopropylfluorophosphate (DFP) had been added. Freezing at -20°C and the addition of DFP were confirmed to have no effect on the 7SIgA and sIgA levels measured.

Separation and detection of IgA intestinal juice

The jejunal juice was concentrated 20 to 50 times and then dialysed against phosphate buffered saline.

Monomeric IgA (7S IgA) was separated from polymeric IgA by gel filtration: a 0.6 ml sample of concentrate was applied to a 1.6×100 cm column (Pharmacia) of ACA 34 Ultrogel (IBF) at a flow rate of 4.4 ml/h. An example of the optical density of the eluted proteins is given in Fig. 1.

The IgA in the unconcentrated jejunal juice and its eluates was assayed without prior concentration by two techniques. The first employed nephelo-

metry (using an immuneph B device, Immuno) with an anti- α chain serum. The elution positions of 11S and 7S IgA were constant (with V_e/V_o ratios of 1.21 and 1.53 respectively). The fractions containing the 11S IgA were entirely distinct from those containing the 7S IgA. For a given jejunal juice sample, the relative proportions of 7S IgA and 11S IgA were calculated by comparing the surface areas under the peaks obtained by the nephelometric assay.

The second assay method that was used was reversed radial immunodiffusion, for which the jejunal juice was incorporated into an agarose support, with the anti- α chain immune serum (CDTS Bois Guillaume), used in dilutions of 1/1, 1/2, 1/4, and 1/8, placed in wells from which it was allowed to migrate by diffusion. The IgA level was measured in relation to that of a human serum containing a known amount of IgA. This procedure has been shown to underestimate the 11S IgA level by a factor close to 2.¹³

These two techniques were found to yield identical results (Fig. 2).

Serum albumin and jejunal liquid albumin levels

These were determined by radial immunodiffusion (Institut Pasteur production: Protéiplaques).

Statistical analysis

Analysis of the results was performed by the Mann-Whitney U test. All significance levels quoted are one-tailed.

Results

SERUM SIGA

Determined by radial immunodiffusion

The amounts measured in the three controls were

Fig. 1 Example of fractionation of jejunal juice on ACA 34 Ultrogel. — Optical density of eluted proteins. ---- IgA amounts determined by nephelometry.

Fig. 2 Example of fractionation of jejunal juice on ACA 34 Ultrogel. IgA assays in the eluted fractions, obtained by nephelometry (—) or inverse radial immunodiffusion (----).

normal (less than 30 $\mu\text{g/ml}$). In the compensated cirrhosis patients, the serum sIgA levels were also found to be normal. In the patients with severe cirrhosis, the serum sIgA levels were high (>30 $\mu\text{g/ml}$).

Determined by haemagglutination inhibition

The results are shown in Table 1. The patients with severe cirrhosis were found to have serum sIgA levels close to 85, 104, and 140 $\mu\text{g/ml}$.

PROPORTIONS OF 7S IGA AND 11S IGA IN JEJUNAL LIQUID (Table 2)

In the controls 11S IgA was found to constitute 89–92% of the total jejunal IgA. In compensated cirrhosis, this proportion was 84–92%, while in severe cirrhosis, the proportion observed was 59–69%.

JEJUNAL FLOW RATE OF SECRETION OF 7S IGA AND 11S IGA (Table 1)

The jejunal rate of secretion of 7S IgA was found to be comparable in all three groups: 78.3 $\mu\text{g/mn}$ (median) in the controls, 61.7 in compensated cirrhosis, and 102.7 in severe cirrhosis.

On the other hand, the rate of secretion of sIgA observed in the patients with severe cirrhosis (200.1 $\mu\text{g/mn}$) (median) was significantly lower ($p<0.05$) than that measured either in the controls (793.8) or in the patients with compensated cirrhosis (620.3).

Table 1 Serum sIgA levels

Medical state of subjects	Radial immunodiffusion* ($\mu\text{g/ml}$)	Haemagglutination inhibition ($\mu\text{g/ml}$)
Control	—	ND†
Control	—	28
Control	—	26
Compensated cirrhosis	—	ND†
Compensated cirrhosis	—	ND†
Compensated cirrhosis	—	24
Severe cirrhosis	+	85
Severe cirrhosis	+	104
Severe cirrhosis	+	140

* — = <30 $\mu\text{g/ml}$; + = >30 $\mu\text{g/ml}$.

† Not done.

JEJUNAL ALBUMIN RATE OF SECRETION

This was always found to be within the normal range, thus excluding the possibility of associated exudative enteropathy (Table 3).

Discussion

The quantitative study of intestinal sIgA levels involves two methodological problems: sampling of the intestinal juice and measurement of sIgA in a mixture containing monomeric and polymeric IgA.

Samples obtained by ordinary intestinal suction, with levels expressed in terms of concentration, are difficult to interpret,¹⁴ as the intestinal juice is mixed with salivary, gastric, biliary, and pancreatic secretions including proteolytic enzymes, and its degree of dilution depends on the state of absorption or secretion of the intestine. The technique of intestinal perfusion with an occlusive balloon¹¹ avoids these causes of error by blocking proximal secretions and yielding results in terms of

Table 2 Relative proportions of 7S and 11S IgA in jejunal juice and jejunal IgA rates of secretion

Medical state of subjects	Percentage of 11S IgA in jejunal juice	Jejunal rate of secretion 7S IgA	11S IgA ($\mu\text{g/min/40 cm jejunum}$)
1 Control	84	160.7	845.2
2 Control	92	54.5	634.5
3 Control	91	78.3	793.8
4 Compensated cirrhosis	92	54.2	620.3
5 Compensated cirrhosis	89	61.7	502.4
6 Compensated cirrhosis	78	189.6	674
7 Severe cirrhosis	59	145.5	209.7
8 Severe cirrhosis	66	102.7	200.1
9 Severe cirrhosis	69	72.4	160

Table 3 Serum albumin and jejunal albumin rates of secretion

Medical state of subjects	Serum albumin (g/l)	Jejunal albumin rate of secretion ($\mu\text{g}/\text{min}/40\text{ cm jejunum}$)
1 Control	35	316.5
2 Control	44.5	79.5
3 Control	36.8	35
4 Compensated cirrhosis	39.5	21
5 Compensated cirrhosis	47	157
6 Compensated cirrhosis	30	338
7 Severe cirrhosis	28.6	428
8 Severe cirrhosis	24	193.1
9 Severe cirrhosis	31.4	84

flow rates. Rambaud *et al*¹⁵ have demonstrated the validity of this method, the results of which are not influenced by the presence of the balloon, by the flow rate, or by transintestinal absorption/secretion.

As far as the assay of IgA in a mixture of monomeric and polymeric IgA is concerned, the usual immunological techniques, using radial immunodiffusion in agarose and 7S or 11S standard samples, are unsatisfactory, as the 7S scale underestimates the IgA level,¹⁶ while the 11S one overestimates it.¹⁷ Duprey *et al*¹³ have shown that reversed radial diffusion yields a valid estimate of 7S and 11S IgA if the latter is corrected by a factor of about 2. We obtained identical results by nephelometry. The relative proportions of the two types of IgA were ascertained by column gel filtration. Intestinal 11S IgA is virtually entirely sIgA.¹⁸

Serum sIgA is known to be raised in cirrhosis.⁴⁻⁶ We found this rise in three cases of severe cirrhosis with jaundice. On the other hand, the serum sIgA level was observed to be normal in the three cases of compensated cirrhosis, none of which was associated with jaundice. The presence or absence of cholestasis in these two groups of patients may in part explain this difference. In this connection, it was shown recently that experimentally produced bile duct obstruction in rats produced a rapid and considerable increase in the serum sIgA level.¹⁹ A similar process can be observed in human liver disease: the highest serum sIgA levels are seen when cholestasis is present (unpublished data). Another study²⁰ demonstrated the role of the secretory component in the active and selective transport of dimeric serum IgA into the bile; Nagura *et al* suggest that the cellular pathway for entry of polymeric IgA into the bile is an endocytic transport across biliary epithelium mediated by secretory component.²¹

The proportion of sIgA relative to total IgA in the jejunal juice as measured in our control subjects

(about 90%) is in agreement with Tomasi's results.¹⁸ Other authors have found figures of 70%¹⁶ or from 64 to 80%.¹³

The possibility of associated exudative enteropathy, a possible finding in cirrhosis, and one which can modify the IgA rate of secretion, was eliminated by measuring the rate of secretion of jejunal albumin; this was shown to be within the normal range, as defined by Nouel,²² in both the controls and the cirrhotic patients.

As far as we know, no studies of the intestinal rate of secretion of sIgA and 7S IgA in normal or cirrhotic subjects have been published. Our results demonstrate a decrease in the jejunal sIgA rate of secretion in severe cirrhosis. Of course, only a small number of cases could be explored, but the results are homogeneous and the difference between this rate of secretion and that observed in the controls and the cases of compensated cirrhosis is significant ($p < 0.05$). On the other hand, the intestinal secretion of 7S IgA was found to be identical in all nine subjects. Although for such small groups the statistical risk β of incorrectly concluding that there is no difference among them is high, it can at least be said that the mean values observed in the three groups are extremely close. It must be noted that the mechanisms responsible for the secretion of 7S IgA and sIgA are not the same: the former occurs by a passive and non-specific process^{23, 24} whereas, in the latter, a specific system exists for the transport of the dimeric immunoglobulins across the crypt enterocytes by the secretory component.^{23, 25-27}

Several hypotheses can be proposed to explain the deficit in intestinal sIgA in severe cirrhosis with jaundice:

1 A cholestasis-induced deficiency in sIgA of biliary origin cannot be maintained, as the occlusive balloon technique of intestinal perfusion prevents inclusion of bile secretions in the samples.

2 Decreased sIgA synthesis by digestive system immunocytes could be implied, but this possibility was not explored in our study as coagulation disorders in our cases of severe cirrhosis made intestinal biopsy impossible. In this connection, it can be mentioned that a decrease in intestinal submucosal immunocytes, particularly IgA immunocytes, has been reported in cirrhosis.⁸

3 Impaired transport of dimeric IgA across the enterocytes by the secretory component is a possibility, as 7S IgA secretion, which occurs by simple diffusion, remains normal. The dimeric IgA secreted by the small intestine immunocytes would then enter the general circulation rather than the intestinal lumen. This hypothesis is supported by Brandtzaeg and Baklien's report²⁸ of an adolescent with very pronounced polyclonal IgA hyper-

secretion in which an excessive population of small intestine IgA immunocytes was observed. This hypersecretion was seen to produce a considerable increase in serum IgA, while the sIgA in the intestinal secretions (sampled by ordinary intestinal suction) remained normal. Such a finding implies a defect in the secretory component transport system or its saturation.

Furthermore, F and C André⁵ have shown that the considerable increase in serum IgA seen in cirrhosis is due in large part to a rise in dimeric IgA, the concentration of which was found to be about seven times higher than normal. This implies that some of the dimeric IgA which is accumulated in the serum may arise from that secreted by the intestinal immunocytes which is neither secreted as sIgA into the intestinal liquid nor secreted into the bile.

4 The sIgA secreted into the intestine could be reabsorbed by the altered mucosa, but this possibility is unlikely, as it was shown that the uptake of dimeric IgA from the intestine into the serum is inhibited by the presence of secretory component on IgA.²⁹

The lowered intestinal sIgA level observed in our cases of severe cirrhosis may lead to a defective small intestinal defence system and hence be partially responsible for the increased incidence of infection-diarrhoea, septicaemia of digestive origin, or of infected ascites that is observed in such patients.

We wish to thank Professor J C Rambaud and Dr F Duprey for their helpful advice.

References

- Thompson RA, Asquith P, Cooke WT. Secretory IgA in the serum. *Lancet* 1969; **2**: 517-9.
- Waldman RH, Mach JP, Stella MM, Rowe DS. Secretory IgA in human serum. *J Immunol* 1970; **105**: 43-7.
- Brandtzaeg P. Occurrence of secretory piece in human serum. *J Immunol* 1971; **106**: 318-23.
- Thompson RA, Carter R, Stokes RP, Geddes AM, Goodall JAD. Serum immunoglobulins, complement component levels and auto-antibodies in liver diseases. *Clin Exp Immunol* 1973; **14**: 335-46.
- André F, André C. Participation de l'Immunoglobuline A monomérique et dimérique à l'hypergammaglobulinémie de la cirrhose éthylique. *Biol Gastroenterol* 1976; **9**: 147-50.
- Iscaki S, Buffet C. Secretory IgA increase in sera of patients with liver diseases. *La Ricerca Clin Lab* 1978; **8**: 287-91.
- Iscaki S, Geneste C, Pillot J. Molecular state of secretory component in human sera. *Immunol Letters* 1980; **1**: 217-21.
- Dubrisay J, Demelier JF, Barbier JPH, Renault P, Carnot F, Debetz J, Dadoume JP. Cirrhose du foie et intestin grêle. Confrontations cliniques, biologiques, histologiques, immunologiques et bactériologiques. *Sem Hôp Paris* 1973; **49**: 1639-48.
- Child G. *The liver and portal hypertension*. Philadelphia: Saunders, 1964: 50.
- Iscaki S, Geneste C, D'Asambuja S, Pillot J. Human secretory component in human sera. *J Immunol Methods* 1979; **28**: 331-9.
- Modigliani R, Rambaud JC, Bernier JJ. The method of intraluminal perfusion of the human small intestine. *Digestion* 1973; **9**: 176-92.
- Hyden S. A turbidimetric method for the determination of the higher polyethylene glycols in biological materials. *Ann R Agr Coll* 1955; **22**: 139-45.
- Duprey F, Delpech B, Rambaud JC. Méthode de dosage de l'IgA 7S et de l'IgA 11S dans le perfusé de la lumière jéjunale de l'homme. *Gastroenterol Clin Biol* 1979; **3**: 79-80.
- Brandtzaeg P, Fjellanger I, Gjeruldsen ST. Human secretory immunoglobulins. I. Salivary secretions from individuals with normal or low levels of serum immunoglobulins. *Scand J Haematol* 1970; suppl 12: 54-6.
- Rambaud JC, Duprey F, Nouel O. Étude méthodologique de la détermination par perfusion segmentaire des débits sécrétoires d'IgA dans la lumière jéjunale de l'homme. *Gastroenterol Clin Biol* 1979; **3**: 89.
- Bull DM, Bienenstock J, Tomasi TB. Studies on human intestinal immunoglobulin A. *Gastroenterology* 1971; **60**: 370-80.
- Samson RR, Clelland PBL, Shearmann DJC. Studies on the quantitation of immunoglobulin in human intestinal section. *Gut* 1973; **14**: 616-26.
- Tomasi TB. Secretory immunoglobulins. *N Engl J Med* 1972; **287**: 500-6.
- Lemaitre-Coelho I, Jackson GDF, Vaerman JP. High levels of secretory IgA and free secretory component in the serum of rats with bile duct obstruction. *J Exp Med* 1978; **147**: 934-9.
- Fisher M, Nagy B, Bazin H, Underdown BJ. Biliary transport of IgA: role of secretory component. *Proc Natl Acad Sci USA* 1979; **76**: 2008-12.
- Nagura H, Smith PD, Nakane PK, Brown WR. IgA in human bile and liver. *J Immunol* 1981; **126**: 587-95.
- Nouel O. Étude méthodologique de la détermination par perfusion segmentaire des débits de sérum, d'albumine et d'immunoglobuline A sécrétés dans la lumière jéjunale. Université Paris VI: Thesis, 1976.
- Brandtzaeg P, Baklien K. Intestinal secretion of IgA and IgM: a hypothetical model. *Immunology of the gut. Ciba Foundation Symposium* 1977; **46**: 77-113.
- Walker WA, Isselbacher KJ. Intestinal antibodies. *N Engl J Med* 1977; **297**: 767-73.
- Brown WR, Isobe K, Nakane P, Pacini B. Studies on translocation of immunoglobulins across intestinal epithelium: evidence for binding of IgA and IgM to secretory component in intestinal epithelium. *Gastroenterology* 1977; **73**: 1333-9.

- 26 Brandtzaeg P. Mucosal and glandular distribution of immunoglobulin components: differential localization of free and bound SC in secretory epitheliums cells. *J Immunol* 1974; **112**: 1553–9.
- 27 Brandtzaeg P. Polymeric IgA is complexed with secretory component (SC) on the surface of human intestinal epithelial cells. *Scand J Immunol* 1978; **8**: 39–52.
- 28 Brandtzaeg P, Baklien K. Characterization of the IgA immunocyte population and its product in a patient with excessive intestinal formation of IgA. *Clin Exp Immunol* 1977; **30**: 77–88.
- 29 David PJ, Porter P. A mechanism for secretory IgA mediated inhibition of the cell penetration and intracellular development of *Eimeria terella*. *Immunology* 1979; **36**: 471–7.