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THE IgA SUBCLASS DISTRIBUTION OF ANTIBODIES AGAINST LIPOPOLYSACCHARIDES FROM INTESTINAL BACTERIA

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SUMMARY

Using a novel technique for sampling of jejunal perfusion fluid, the IgA levels and subclass distribution of secreted antibodies against bacterial lipopolysaccharides were analysed in healthy controls and patients with various diseases. The highest level of jejunal fluid IgA was highest in patients with coeliac disease whereas the highest serum IgA levels were found among patients with ankylosing spondylitis.

Jejunal antibodies against lipopolysaccharide from *E. coli* were to a large extent of the IgA2 subclass, regardless of patient group whereas serum IgA antibodies from the corresponding patients were almost exclusively of the IgA1 subclass. IgA2 antibodies against lipopolysaccharide from *B. fragilis* were also found in jejunal secretions, again in contrast to the pattern found in serum.

These findings support the notion of a dichotomy between the secretory IgA system and the serum IgA system and suggests that these two systems are independently regulated.

INTRODUCTION

The mucosal immune system develops independently of the systemic immune system and maturity of the secretory IgA system is reached already at an early age whereas adult levels of serum IgA may not be seen until adolescence. During development there is an active interplay between the bacterial content of the gut and the differentiating immune system where the former may influence the subsequent immunoglobulin class pattern.

Human IgA may be subdivided into two biochemically distinct subclasses, each encoded by a separate gene (*Feinstein* and *Franklin*, 1966; *Vaerman* and *Heremans*, 1966). It has as yet not been fully elucidated whether these subclasses are also different with regard to biological and functional properties.

Previous studies have suggested that cells secreting IgA1 are far more abundant in the jejunum than cells producing IgA2 (*Brandtzaeg* et al., 1986), a finding which also reflected in the subclass distribution of the secreted antibodies (*Delacroix* et al., 1982).

The subclass distribution of specific

Table 1: IgA concentrations in serum and jejunal fluid[a]

Patient category	n	Serum IgA (g/l)	n	Jejunal IgA (mg/l)
Healthy controls	7	2.2 (0.9-4.6)	9	14.8 (5.2-56.5)
Coeliac disease[b]	24	2.1 (0.5-3.9)	30	24.4 (4.8-136.3)
Ankylosing spondylitis	22	3.5 (0.9-10.3)	21	18.9 (3.3-60.0)
Rheumatoid arthritis	17	2.2 (0.5-4.7)	19	11.9 (3.2-42.5)

a. Results are given as mean levels of IgA (range).
b. Patients with IgA deficiency excluded from the calculation.

serum IgA antibodies against a number of antigens have been described to date. In most instances, a marked dominance of IgA1 is seen. Far less is however known about the subclass distribution of secreted antibodies although limited studies in saliva and breast milk (for review see *Mestecky* and *Russel*, 1986) suggests that some IgA2 antibodies may be found against selected bacterial antigens.

The purpose of this study was to analyse the IgA subclass distribution of antibodies against bacterial lipopolysaccharides in serum and jejunal perfusion fluid in patients with various disorders.

MATERIALS AND METHODS

Patient samples

Jejunal perfusion was performed as described previously in detail (*Knutson* et al., 1989). The samples were collected and stored at -70°C until used. Sera were obtained simultaneously and similarly stored until assayed. Samples were obtained from 9 normal control subjects, 30 patients with coeliac disease, 19 patients with rheumatoid arthritis and 22 patients with ankylosing spondylitis. Total IgA in jejunal fluid was measured using alpha-specific rabbit antibodies in a sandwich ELISA (Dakopatts, Copenhagen, Denmark). Two mM of a protease inhibitor (phenylmethylsulfonylfluoride, Sigma chemical Co., MO, USA) was added to the jejunal fluid in all experiments in order to avoid degradation of IgA. Serum levels of IgA were measured by nephelometry.

ELISA for IgA subclass distribution of specific antibodies

Specific serum and jejunal fluid antibodies of the IgA class were measured at a concentration of 10 µg/ml of IgA. The levels were measured in single wells in ELISA as has been described in detail previously (*Engström* et al., 1990). The antigens (lipopolysaccharide from *E. coli* and *B. fragilis* which were both gifts from Dr. A. Weintraub, Dept. of Microbiology, Huddinge Hospital, Huddinge, Sweden) were coated with bicarbonate buffer at a concentration of 2 µg/ml. An alkaline phosphatase conjugated rabbit anti human IgA antiserum (Dakopatts, Copenhagen, Denmark) was used for assessment of total IgA levels whereas monoclonal reagents were used for subclass determinations (anti-IgA1 and anti-IgA2 both from Nordic Laboratories, Tilburg, The Netherlands) followed by rabbit anti-mouse antibodies (Dakopatts) and alkaline phophatase conjugated sheep $F(ab)_2$ anti-rabbit antiserum.

A monoclonal chimeric IgA2 antibody directed against the hapten NP

Table 2: IgA antibodies against bacterial lipopolysaccharides[a]

	n	Antigen	Serum	Jejunal fluid
Controls	5	*E. coli* LPS	0.24 (0.13-0.36)	1.80 (0.78-2.62)
Patients	28	*E. coli* LPS	0.25 (0.06-0.53)	1.31 (0.25-2.86)
Controls	5	*B. fragilis* LPS	0.12 (0.02-0.19)	1.89 (0.32-2.65)
Patients	28	*B. fragilis* LPS	0.18 (0.03-0.41)	0.97 (0.05-2.77)

a. Results are given as mean net absorbance (range) after 40 minutes.

(*Brüggeman* et al., 1987) served as standard for semiquantitative measurements of specific antibodies of the IgA class and IgA2 subclass (tested on separate plates coated with NP-BSA, a gift from Prof. O. Mäkelä, Dept. of Bacteriology and Immunology, University of Helsinki, Helsinki, Finland) which were run in parallel as has been described previously (*Engström* et al., 1990). Serum and saliva from donors with IgA class or subclass deficiency served as specificity controls as described previously (*Engström* et al., 1988, 1990).

RESULTS

Concentrations of IgA in serum and jejunal fluid

The levels of both serum IgA and jejunal fluid IgA differed markedly between patient groups, serum levels being highest in the patients with ankylosing spondylitis (Table 1). Four coeliac patients were deficient in serum IgA; these patients also lacked IgA in jejunal fluid (<0.01 µg/ml).

Subclass distribution of anti-lipopolysaccharide antibodies

Selection of samples to be tested for IgA subclass distribution was based on the presence of concentrations of IgA in the jejunal fluid of more than 10 µg/ml. Samples from 5 normal control subjects, 10 patients with coeliac disease, 5 patients with rheumatoid arthritis and 12 patients with pelvospondylitis were assayed in two separate experiments where the samples were diluted to give a final concentration of 10 µg/ml. The corresponding serum samples were similarly diluted.

In a first set of experiments, the subclass pattern of serum IgA antibodies against lipopolysaccharide from *E. coli* and *B. fragilis* was determined. At the level tested, only IgA1 antibodies were detected (data not shown). The pattern in jejunal fluid samples was markedly different. First, levels of specific anti-lipopolysaccharide antibodies, as determined by absorbance values, were around tenfold higher in jejunal fluid as compared to serum (Table 2). Second, the subclass pattern was quite distinct, with major contributions from IgA2, again as determined by absorbance levels, in all patient categories without any major deviance in any particular disease (Table 3).

Proportion of anti-lipopolysaccharide antibodies

The ELISA is not a quantitative method and the above levels of absorbance may be misleading in terms of true antibody levels. We have previously developed a semiquantitative

Table 3: IgA subclass distribution of anti-lipopolysaccharide antibodies in jejunal perfusion fluid[a]

Patient category	n	*E. coli* LPS IgA1	*E. coli* LPS IgA2	*B. fragilis* LPS IgA1	*B. fragilis* LPS IgA2
Healthy controls	5	1.91	0.95	1.07	0.89
Coeliac disease	12	1.35	0.74	1.02	0.64
Ankylosing spondylitis	11	1.48	0.76	1.14	0.52
Rheumatoid arthritis	5	1.50	0.78	1.02	0.45

a. Results are given as mean net absorbance after 30 minutes.

method for IgA and IgA2 antibodies in serum and secretions based on comparison from a standard curve established with the aid of a human monoclonal antibody directed against NP. Thus, in one experiment, an attempt was made to quantify the antibodies found in jejunal fluid. As evident in Table 4, a fair proportion of antibodies against bacterial lipopolysaccharides were of the IgA2 subclass. Antibodies against the two antigens tested constituted a few percent of the total amount of IgA antibodies present in jejunal fluid (Table 4). Since the mean total level of IgA was in jejunal fluid was around 15 µg/ml, approximately 30 ng/ml of specific anti-*E. coli* lipopolysaccharide antibodies are normally found in jejunal fluid.

DISCUSSION

Concentrations of specific IgA antibodies in jejunal secretions are difficult to determine due to technical reasons. The abundance of proteolytic enzymes may degrade the antibodies during processing and the presence of enzyme inhibitors both during the jejunal lavage and the subsequent assays is a necessary precaution. Second, hitherto available systems for sampling (open or semi-open techniques) frequently suffer from proximal or distal contamination thus introducing errors in the evaluation of the samples. Furthermore, such techniques often require high perfusion rates and the low and variable recovery of perfusion fluid results in a low precision in the analysis of IgA. The recent development of an adequate technique for sampling has allowed the reliable measurement of complement factors (*Ahrenstedt* et al., 1990) and antibodies (unpublished results) in jejunal lavage fluid.

Specific antibodies in jejunal fluid against bacterial lipopolysaccharides constituted a few percent of total IgA, markedly higher than in serum. This suggests that the local bacterial load influences the level of specific antibodies. The observed levels of antibodies also indicate that gastrointestinal bacteria induce local immunity rather than tolerance.

The subclass distribution of the specific anti-lipopolysaccharide antibodies shows a dominance of IgA2. This is not a reflection of the total amount of IgA1 and IgA2 synthesised since there is normally a predominance of IgA1 producing cells in the jejunum (*Brandtzaeg* et al., 1986). Our findings thus confirm and extend previous findings (*Moldoveanu* et al., 1987) on the subclass distribution of anti- lipopolysaccharide antibodies in secretions. Since the former study was performed using saliva as a source of antibodies,

Table 4: Quantitative estimate of specific IgA and IgA2 antibodies in jejunal perfusion fluid[a]

E. coli LPS		*B. fragilis* LPS	
IgA (ng/ml)	IgA2 (%[b])	IgA (ng/ml)	IgA2 (%)
20.9	62	9.6	82

a. Results are given as mean levels in the 15 randomly chosen subjects tested (3 healthy controls, 7 patients with coeliac disease, 2 patients with ankylosing spondylitis and 3 patients with rheumatoid arthritis; all tested at a fixed concentration of 10 µg/ml of IgA).
b. Results are given as mean % of specific IgA2 antibodies.

the finding of a similar subclass pattern in jejunal fluid supports the notion of a common secretory immune system with homing of IgA producing cells in distal mucosal tissues after initial priming in the gut (for review see *Mestecky* and *McGhee*, 1987).

Since protein antigens mainly induce IgA1 in secretions (for review see *Mestecky* and *Russel*, 1986),the IgA2 response to polysaccharide antigens appears to reflect a distinct regulatory pathway. This mechanism, although still elusive with regard to its molecular basis, is reminiscent of the subclass restriction of IgG antibodies where polysaccharide antigens mainly induce IgG2 whereas protein antigens preferentially elicit IgG1 antibodies (for review see *Hammarström* and *Smith*, 1986).

It remains to be determined whether the mucosal response to different antigens (or epitopes on these antigens) is identical to that of the systemic immune response. Our results suggest that responses may be different at least in terms of subclass composition. The continued utilisation of the sampling technique employed in this paper may allow a resolution of this question.

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