Colonic bacterial proteases to IgA₁ and sIgA in patients with ulcerative colitis

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SUMMARY The colonic faecal and mucosal associated bacterial populations of five patients with ulcerative colitis and four control patients were studied in detail to assess their ability to produce IgA₁-proteases. A total of 330 bacterial strains were isolated from the patients with ulcerative colitis and IgA₁-protease activity was unable to be reliably shown in any. It is therefore unlikely that such enzyme production by colonic bacteria plays a significant role in the pathogenesis of ulcerative colitis.

Secretory IgA (sIgA) is an important immunological defence for the intestinal mucosa. It is present as two subtypes (sIgA₁, sIgA₂) which are present in roughly equal proportions.¹

Certain bacteria produce a specific protease that cleaves IgA₁ and sIgA₁ into intact Fab and Fc fragments. IgA protease activity was originally recognised in the faeces of volunteers.² *Streptococcus sanguis*, isolated from dental plaque, was the first organism shown to be capable of producing such an enzyme.³ Similar proteases have subsequently been isolated from cultures of variety of other pathogenic bacteria.¹⁸ One example is *Neisseria gonorrhoeae*, an organism which attacks mucosal surfaces.⁵ When this bacterium causes a proctitis the histological appearances can be similar to those of acute ulcerative colitis.

The pathogenesis of ulcerative colitis is obscure. Immunological studies suggest that there is increased antigenic absorption through the local intestinal immune system and that immune responses to such antigens may play a role in the pathogenesis of the condition.⁶ Any factor which diminishes sIgA antibody activity could promote such antigenic absorption. Although there is no significant change in the faecal flora of patients with ulcerative colitis,⁷⁻⁹ the colonic flora can produce potentially destructive enzymes such as haemolysins and necrotoxins.¹⁰ The hypothesis that certain bacterial strains in patients with ulcerative colitis might produce IgA₁ proteases has been tested in this study.

Methods

PURIFICATIONS OF IgA AND sIgA

IgA was prepared from the serum of patients with IgA₁ multiple myeloma. Identification of patients with the IgA₁ subtype was achieved using the chromic chloride red cell labelling technique.¹¹ Anti-IgA₁ antibody was kindly supplied by Professor I C M McLennan (Birmingham, UK). IgA₁ was isolated by ammonium sulphate precipitation of serum, followed by elution from a DEAE cellulose column using phosphate buffer.¹² sIgA was prepared from pooled human colostrum.¹³ After removal of other milk proteins by centrifugation and filtration, the sIgA was purified using Sephadex G-200.

The purity of the sIgA and IgA₁ isolated was confirmed by polyacrylamide gel electrophoresis, immunoelectrophoresis and double diffusion in agarose using Dako antisera to IgA and sIgA.

SAMPLES FOR BACTERIOLOGY

Rectal biopsy specimens and a sample of rectal faeces were taken at sigmoidoscopy. The samples were immediately placed in a weighed 'bijou' bottle containing 4.5 ml cryoprotective glycerol broth, frozen, and stored at below -40°C. All samples were analysed within four months of collection and such a procedure has previously been shown to result in a minimal loss of bacteria.¹⁴

ISOLATION OF BACTERIA

The methods of Hudson *et al*¹⁵ were used with the following exceptions: the atmosphere of the anaerobic chamber was 10% CO₂, 10% H₂, balance nitrogen and supplemented brain heart infusion blood

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agar was modified to contain neutralised liver digest (Oxoid Ltd) 5 g/l and a volatile fatty acid mixture 3·1 ml/l added as a filter sterilised solution of the acids as their potassium salts.¹⁶

Briefly, quantitative bacterial analysis was carried out in a flexible film anaerobe chamber. Biopsy tissue was carefully washed, weighed, and macerated. Decimal dilutions of macerate and of faeces were subjected to a quantitative bacterial analysis using a range of non-selective, selective and differential agar media. Isolates were identified at least to genus level.

Isolates were then individually subcultured in reduced Todd-Hewitt broth and when in a logarithmic phase of growth were frozen at -40°C until ready for detection of IgA-protease activity.

DETECTION OF IgA-PROTEASE ACTIVITY

Samples of the individual bacteria in Todd-Hewitt broth were thawed and then centrifuged at 1500 g. Fifty microlitre aliquots of broth were incubated at 37°C for 20 hours with 50 µl IgA₁ which had a protein concentration of 5 mg/ml. The same method was used for the incubation with purified sIgA.

Fifty microlitres of the digests were boiled for two minutes with 12·5 µl sample buffer. After addition of 3 grains sucrose, 20 µl samples were used for polyacrylamide gel electrophoresis on 12% gels using the discontinuous buffer system of Laemmli,¹⁷ but without a stacking gel.

Immunoelectrophoresis was carried out using the Corning Universal agarose film system, and Dako anti-IgA and anti-sIgA antibodies.

PATIENTS

Five patients with well documented ulcerative colitis were studied. One was suffering from clinically and sigmoidoscopically active distal disease and had

relapsed despite sulphasalazine therapy. Two had active pancolitis and were on no therapy, and two patients had left sided disease in remission whilst taking sulphasalazine. Four control patients were also studied – two with constipation, one with symptomatic *Pneumatosis coli* and the other with Crohn's colitis who was in remission whilst taking sulphasalazine. No patients were taking any other medication at the time the samples were taken.

A rectal biopsy was not taken from one of the patients with constipation and a faecal sample was not available from the patient with pneumatosis coli nor from one of the patients with active untreated ulcerative colitis. Otherwise, all had both a rectal biopsy specimen and a faecal sample collected.

Results

The results of the bacteriological assessment of the samples obtained are listed in Tables 1 and 2. A total of 330 separate bacterial strains were isolated from the faecal and mucosal associated flora of the five patients with ulcerative colitis. These were all tested for IgA₁-protease production and compared with the 182 bacterial strains similarly isolated from the four controls.

An unclassified bacillus, isolated from the mucosal flora of one patient with untreated ulcerative colitis demonstrated weak IgA₁ protease activity on polyacrylamide gel electrophoresis which was not confirmed on immunoelectrophoresis. It showed no demonstrable sIgA-protease activity. Otherwise, there was no evidence of IgA₁ or sIgA protease activity in any of the patients with ulcerative colitis.

Streptococcus mitis was identified from the mucosal flora of the patient with *Pneumatosis coli* and demonstrated IgA₁-protease activity. This

Table 1 Number of bacterial isolates from mucosal (M) and faecal (F) flora of patients with ulcerative colitis tested for IgA-protease activity

Bacterial groups	Patient 1 Active UC untreated		Patient 2 Active UC untreated		Patient 3 Inactive UC on SASP		Patient 4 Inactive UC on SASP		Patient 5 Active UC on SASP	
	M	F	M	F	M	F	M	F	M	F
Anaerobic bacteroides	9	7	14	—	4	7	6	16	15	20
Anaerobic bifidobacteria	3	2	3	—	0	0	4	4	2	8
Other anaerobic gram positive rods	0	8	0	—	1	6	4	3	4	7
Anaerobic cocci	1	1	0	—	0	0	5	3	3	1
Anaerobic veillonellae	0	1	0	—	1	1	0	0	0	2
Other anaerobes	0	2	8	—	0	0	0	4	0	1
Microaerophilic lactobacilli	11	14	0	—	4	1	0	5	2	1
Microaerophilic streptococci	0	0	0	—	0	0	0	1	1	3
Aerobic coliforms	7	8	0	—	5	4	0	0	2	3
Aerobic streptococci	0	1	0	—	20	25	0	6	5	2
Other aerobes	3	3	0	—	0	0	0	2	0	0

Table 2 Number of bacterial isolates from mucosal (M) and faecal (F) flora of control patients tested for IgA-protease activity

Bacterial groups	Patient 1 Constipation		Patient 2 Constipation		Patient 3 Pneumatosis coli		Patient 4 Crohn's disease	
	M	F	M	F	M	F	M	F
Anaerobic bacteroides	—	5	8	13	8	—	13	15
Anaerobic bifidobacteria	—	0	5	1	0	—	1	3
Other anaerobic gram positive rods	—	2	3	4	0	—	9	5
Anaerobic cocci	—	4	2	3	0	—	1	4
Anaerobic veillonellae	—	0	0	0	0	—	0	1
Other anaerobes	—	0	0	6	0	—	0	7
Microaerophilic lactobacilli	—	0	0	2	0	—	1	0
Microaerophilic streptococci	—	0	0	2	0	—	2	1
Aerobic coliforms	—	0	8	7	3	—	5	4
Aerobic streptococci	—	1	0	2	3	—	9	8
Other aerobes	—	0	0	0	1	—	0	0

organism has been previously reported to produce such an enzyme when isolated from the oral cavity.⁴ None of the mucosal associated or faecal flora isolated from the other control patients showed any IgA₁ or sIgA protease activity.

Discussion

IgA₁-proteases have now been shown to be produced by a variety of bacterial species.¹⁸ All such bacteria are pathogenic to man and affect mucous membranes. Their pathogenicity is probably related to the production of such enzymes because it has been shown that, although IgA₁-proteases uniquely release intact Fc and Fab fragments, the cleavage products lose over 90% of the antigen binding capacity of the original IgA₁ molecule.¹⁹

The precise immunological role of sIgA is not fully known but it is generally believed that it blocks foreign antigen uptake by mucosal epithelial cells. A local deficiency of sIgA has been reported in patients with ulcerative colitis^{20,21} and may contribute to the perpetuation of the disease by allowing increased antigenic absorption and a subsequent immune response directed toward the colonic mucosa.⁶ Although it is conceivable that such a breakdown in local immune defence could be the result of chronic bacterial IgA₁-protease production, this study does not support such a hypothesis. Although only five patients with ulcerative colitis were studied, over 300 bacterial species from both their faecal and mucosal associated flora were tested for such enzyme activity and none could be reliably demonstrated. It would therefore seem reasonable to conclude that colonic bacterial IgA₁-protease production does not contribute to the pathogenesis of ulcerative colitis.

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