Local immunity in ulcerative colitis: evidence for defective secretory IgA production

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SUMMARY   To investigate local humoral immunity in ulcerative colitis (UC), immunoglobulin (Ig) contents and net Ig production *in vitro* was assessed using organ cultures of colonic biopsies from 21 patients with quiescent disease and 11 controls. Ig was estimated by enzyme linked immunoassay (ELISA) for IgA, secretory IgA (sIgA), IgM, and IgG. In parallel, numbers of IgA plasma cells were estimated by indirect immunoperoxidase staining of tissue sections for IgA. IgA was the dominant Ig isotype found pre-existing in colonic mucosae, and secreted *in vitro*. In UC patients, preformed tissue IgA and IgA produced *in vitro* were significantly increased compared with controls. There was no concomitant increase in amounts of sIgA synthesised in culture, however, although numbers of IgA plasma cells were increased in UC patients by an amount comparable with the increased *in vitro* IgA production. These results directly show a dysfunction of transepithelial IgA secretion in quiescent ulcerative colitis. Despite a significantly raised concentration of tissue IgG in UC patients, little was produced *in vitro* in patient and control groups alike, suggesting that mucosal IgG was serum derived, and not linked to local IgA production.

Studies of systemic humoral immunity in patients with ulcerative colitis (UC) have not shown major perturbations. At the level of the colonic mucosa, however, there are clearer indications that a humoral immune response is involved in UC, seen mainly as an increase in the numbers of plasma cells, particularly of the IgA isotype.[1 2] There is also evidence of increased local Ig production in patients with UC, as shown by *in vitro* culture of biopsies[3 4] and isolated intestinal cells.[5] Nevertheless, *in vitro* production of the IgA isotype by isolated intestinal mononuclear cells is decreased rather than raised in UC, when calculated on a 'per cell' basis.[6–8]

Immunohistological studies have shown a marked decrease, or even absence of IgA from colonic epithelium in UC, despite the presence of secretory component[9–11] and the normal production of secretory component *in vitro*.[3 12] These data are suggestive of a defect in production of secretory component-bound secretory IgA (sIgA) in patients

with UC, possibly as a result of decreased J-chain production by mucosal plasma cells,[11] or because of an increased proportion of the IgA being produced in monomeric form as part of the normal local immune response.[8] No direct evidence for this hypothesis has been forthcoming, however. Clearly, the production of non-secretory and secretory forms of IgA in the colonic mucosa of patients with UC needs further investigation. To these ends, we have studied *in vitro* synthesis of immunoglobulins, with special emphasis on IgA and secretory IgA as a correlate of plasma cell density.

Methods

PATIENTS AND TISSUE SAMPLES

Twenty one patients with UC (10 men, age range 24–66 years, mean=42), in whom the diagnosis had been substantiated by conventional means of endoscopy, biopsy histology and often radiology, were studied at follow up clinics. The duration since first diagnosis ranged from three to 40 years (mean=9), although none of the patients had active disease at the time of study. Nine patients were on cortico-

steroid treatment during the month preceding the study, 12 were on sulphazaline, four were receiving both treatments and four were not on any specific therapy. The control group included 11 patients (five men; age range 26–49 years, mean=39) with non-inflammatory conditions (five with diverticular disease, four with irritable bowel syndrome, one with benign adenomatous polyps, and one with angio-dysplasia of the caecum); none were receiving any specific therapy at the time of study. Multiple biop-sies were obtained from adjacent sites of the descend-ing colon from each patient during routine colono-scopy. Two biopsies were placed immediately into tissue culture medium and cultured within five minutes of collection, two were weighed and homogenised for estimation of tissue Ig contents, and a fifth biopsy (when available) was fixed in neutral buffered formalin for histological sectioning and subsequent plasma cell enumeration.

ORGAN CULTURE

A grid organ culture method was used[13] as previously described in detail.[14 15] Briefly, biopsies were orien-tated luminal side uppermost and placed on stainless steel grids in organ culture chambers (Falcon) filled with 1·3 ml RPMI 1640 culture medium, supple-mented with 10% fetal bovine serum and penicillin/ streptomycin (Flow Laboratories), just sufficient to ensure that a thin layer of liquid was drawn over the mucosal surface by capillary action. Duplicate cul-tures were maintained at 37°C in a humidified atmos-phere of 5%CO_2, 95%O_2. After three days culture, the supernatants were carefully collected, and the biopsies were weighed, homogenised in 2 ml saline, and clarified by centrifugation. Samples were stored aliquoted at −20°C before assay for Ig contents.

ESTIMATION OF IMMUNOGLOBULINS

A sensitive enzyme linked immunoassay (ELISA) technique was used to estimate Ig content of biopsies and culture supernatants for total IgG, IgM, non-secretory IgA, and secretory IgA (sIgA), as des-cribed previously.[16] For estimation of total immuno-globulins, a polyvalent antihuman Ig purified anti-body was used as the solid-phase 'capture' reagent, whereas for estimation of sIgA, a specific anti-secretory component purified antibody (Seward Laboratories) was used. Alkaline phosphatase-conjugated isotype-specific antihuman IgG, IgM, and IgA affinity purified second layers were used for the quantitation of each isotype. All antibodies and conjugates were obtained from Sigma, except where noted. Immunoglobulin standards were derived from pooled sera (Seward), colostral IgA (Sigma) was used as the sIgA standard.[16] To obtain values of non-secretory IgA, the sIgA concentration was sub-tracted from the total IgA value. In this assay, fetal bovine serum in culture media does not interfere.[15] The concentration of preformed material in duplicate uncultured biopsies was calculated, and subtracted from the gross Ig production in culture (derived from the total amount found in culture supernatant plus the contents of the biopsy after culture) to arrive at a figure for net synthesis *in vitro*, expressed as 'ng Ig/mg weight of biopsy'.

IgA PLASMA CELL ENUMERATION

An indirect immunoperoxidase technique was used to label trypsinised 5 μm paraffin sections, as pre-viously described.[15] Briefly, after quenching of endo-genous peroxidase and pre-incubation with normal swine serum, unconjugated antibodies to IgA (Dakopatts), IgA_1 and IgA_2 (Nordic) were incubated on serial sections. The reaction was detected with swine antirabbit peroxidase conjugated second antibody (Dakopatts), and visualised with di-aminobenzidine. Controls included omission of primary and/or second layer antibodies, and absorp-tion of primary antibodies with purified IgA and/or lyophilised normal human serum.[15] Microscope slides were projected onto a large sheet of graph paper using a Leitz projecting microscope at a standard distance. The number of peroxidase labelled cells identifiable morphologically as plasma cells were counted per unit area of the lamina propria to obtain a figure on a 'per mm^2' basis. A stage micrometer slide (Graticules Ltd) was used to obtain the exact magnification factor. At least 200 cells were counted per specimen.

STATISTICAL ANALYSIS

The two-tailed Mann-Whitney U-test was used to evaluate comparative statistical significance; means and standard deviations were used as descriptive statistics.

Results

IMMUNOGLOBULIN CONTENTS OF COLONIC BIOPSIES

IgA was the predominant Ig isotype found pre-existing in the tissues (Fig. 1). In control patients, the isotype ratios IgA:sIgA:IgM:IgG were 4:1:1:1:5 respectively, whereas in the UC patients, the isotype ratios were 6:1:1:1:5 respectively, reflecting an over-all increase in IgA tissue content (Fig. 1). The amount of Ig present overall in the colonic mucosa of patients with UC was higher than that found in the controls; the amount of IgA was more than doubled (p<0·01) and IgG levels were significantly raised (p<0·01), although to a lesser extent. Although tissue levels of sIgA and IgM were also slightly
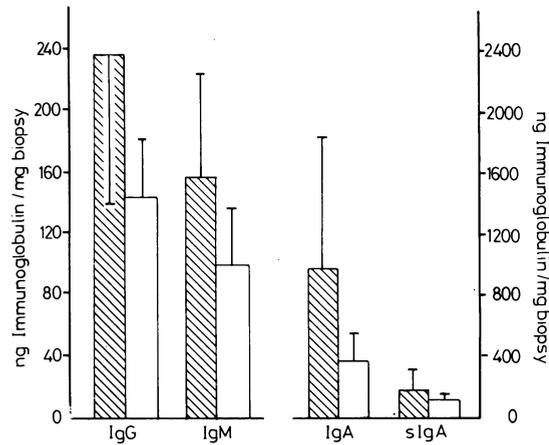
Fig. 1   *Immunoglobulin contents of colonic mucosal biopsies estimated by ELISA for IgG, IgM, IgA and secretory IgA (sIgA). Means and standard deviations plotted. Shaded bars – ulcerative colitis patients; open bars – control patients. NB 10-fold scaling factor for IgA and sIgA.*

Table   *IgA containing plasma cells and IgA subtypes in the colonic mucosa*

	Absolute no of IgA cells*	IgA₁ cells (%)	IgA₂ cells (%)
Ulcerative colitis	788	68	32
	810	57	43
	625	60	40
	588	62	38
	627	63	37
	808	63	37
	420	64	36
	1167	71	29
	729	58	42
Mean	729·11	62·89	37·11
SD	207·58	4·48	4·48
Controls	337	45	55
	220	57	43
	369	69	31
	223	52	48
	453	56	44
	320	56	44
Mean	320·33	55·83	44·17
SD	89·20	7·83	7·83
Significance	p<0·01	NS	NS

*Absolute numbers expressed as cells/mm² lamina propria; SD=standard deviation; NS=not statistically significant.

increased in UC, the results were not statistically significant.

IMMUNOGLOBULIN PRODUCTION *IN VITRO*
In biopsies from control patients, increased production of IgA and IgM (Fig. 2) altered the isotype ratio to 20:6:2:1 for IgA:sIgA:IgM:IgG, respectively. Net IgG production was very low, and in four cases was effectively zero. In cultured biopsies from UC patients, IgA production predominated, and resulted in similar isotype ratios compared with controls (20:2:1:1), although the absolute net amount of IgA produced was more than double (p<0·05). There was no commensurate rise of sIgA production *in vitro*, however, the amounts produced being no higher than those of control biopsies (Fig. 2). Thus, whereas in the control group, sIgA accounted for nearly one third of all IgA produced, in the patients with UC, net sIgA production was only about one tenth of total IgA secretion. In UC patients, production of IgM was higher than in controls, although the difference was not statistically significant. Net IgG production was negligible in three individuals with UC, whereas several other patients showed quite high production. Because of large individual scatter, however, the difference between control and UC patients was not statistically significant.

IgA PLASMA CELLS
Tissue sections from nine UC patients and six controls were available for the study (Table). There was a considerable increase in absolute numbers (per mm² of lamina propria) of IgA plasma cells in the

colonic mucosa of patients with UC, compared with controls (p<0·01). The magnitude of increase (over two-fold) was comparable with the increased IgA contents and IgA secretion rates noted above. There was no correlation, however, between IgA plasma cells and either tissue content or production of sIgA.

The increase in numbers of IgA plasma cells was observed for both IgA subtypes: the ratio of IgA₁ to IgA₂ plasma cells in UC patients (62·9% to 37·1%,
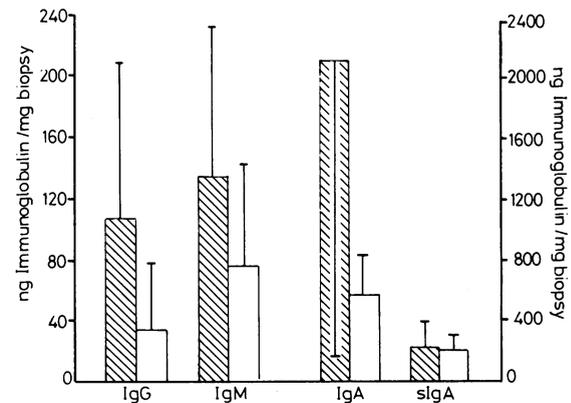

Fig. 2   *Net immunoglobulin secretion by colonic mucosal biopsies in vitro (means and standard deviations). Shaded bars – ulcerative colitis patients; open bars – control patients. NB 10-fold scaling factor for IgA and sIgA. Although overall IgA secretion is considerably increased in ulcerative colitis patients, there is no concomitant increase of sIgA.*

respectively), although slightly increased, was not significantly altered from the proportions observed in control patients (55·8% to 44·2%, respectively) (Table).

Discussion

IgA-secreting cells are the dominant plasma cell type in the colonic mucosa, and our data indicate that an important feature of local mucosal immunity associated with ulcerative colitis involves the IgA response. Previous studies of colonic mucosal plasma cells have reported increases in numbers in UC,[12 17-20] although individual reports show some discrepancies. In recent reviews,[12] such discrepancies were attributed to methodological problems and the fact that selection of tissue samples poses considerable problems due to histopathological variability within specimens, especially in cases of active inflammatory bowel disease. We chose to study patients with quiescent disease as the variability is less marked in the mucosa of such patients, and our data are in good overall agreement with most previous reports.

Alterations in IgA subtype have not been studied extensively in UC. It has been reported that there is a preferential increase in IgA_1 subtype secretion by isolated colonic cells in UC,[8] and very recently, a substantial increase in numbers of IgA_1 cells has been reported.[21] Our data suggested that the increase in IgA_1 plasma cells was modest, but not statistically significant. As all our patients were in remission, these observations suggest that a marked increase in IgA_1 occurs only in patients with active disease.

Several groups have studied Ig production in the colonic mucosa of UC patients. The increased production reported previously using organ cultures[3 4] was not specifically compared with numbers of plasma cells, nor to the pre-existing tissue contents. Careful studies using cultures of isolated cells indicated a reduction, however, rather than a rise in IgA secretion on a 'per cell' basis.[7 8] Our data demonstrate that the increase of IgA production by organ cultures is a function of numbers of plasma cells, and does not reflect any increase in rate of synthesis on a 'per cell' basis. These observations therefore agree with functional studies of isolated cell populations[8] and with immunohistological investigations.[21]

Whereas the production of IgA in the colonic mucosa of patients with UC was substantially raised, there was no concomitant increase of secretory IgA production, either preformed in the tissues, or secreted during organ culture. Thus a 'leakage' effect of *de novo* synthesised IgA through the stroma during culture is unlikely to wholly account for our data, and would equally apply to the control tissues.

Although secretory IgA production has not been previously directly estimated in the colonic mucosa, several reports have suggested that secretory IgA production may be deficient in patients with UC. Immunohistological studies have shown that amounts of IgA found within colonic epithelial cells are low,[9-11] implying that despite the raised numbers of plasma cells and increased overall rate of production, there is a defect of secretory component binding and transepithelial transport.

Damage to enterocytes in UC may impair synthesis of secretory component, or perturb IgA binding to secretory component expressed on the basolateral surface of enterocytes, its subsequent internalisation and resecretion into the gut lumen. Immunohistological studies, however, suggest that amounts of epithelial secretory component are not markedly perturbed[9-11] and normal production of secretory component was reported *in vitro*,[9 12] suggesting that the defect lies elsewhere. Indeed, functional assays have shown that less dimeric IgA is secreted by isolated mucosal cells in UC,[8] and immunohistological evidence has been presented for a defect in dimeric IgA production by the plasma cells, manifest as reduced expression of the J-chain component,[11] thereby reducing the ability of IgA to bind secretory component.

Defective secretory IgA production may play a crucial role in the pathogenesis of UC. Impaired defences against influx of potentially antigenic material would increase local immunostimulation, resulting in raised local production of IgA, which, however, would remain ineffective. A second consequence would be a local inflammatory reaction, leading to increased tissue permeability, thereby promoting ingress of gut derived antigen(s) into the systemic circulation and development of a subsequent systemic IgG response. Relatively little IgG is normally synthesised within the large intestine,[22] as we have shown above. In UC, however, the amounts of preformed tissue IgG are increased, as shown immunohistologically,[11] and by ourselves by direct measurement. Our data thus confirm the previous suggestion that most mucosal IgG is extravascular, and not synthesised locally.[4] Only in active UC is there any evidence for substantially raised local IgG production,[3 7] whereas the patients we studied were all in remission and showed only a modest increase.

IgG is the predominant immunoglobulin isotype associated with immune complex formation and deposition. It is therefore likely that IgG type antigen-antibody complexes against disease related antigens are formed in the colonic mucosa in UC.[23] Immune complexes are known to contribute substantially to inflammatory responses, and the occurrence of immune complexes in UC[24-26] may play a major

role in the maintenance of the chronic inflammatory state.[19 27] Our observations of a modest increase of IgM production agrees with the above hypothesis, as IgM antibodies are principally elicited to primary antigenic exposure, and hence should not be markedly increased during chronic immunostimulation. It is, however, worth remembering that J-chain linked IgM can also be secreted in secretory component bound form; although we have not yet measured secretory IgM production, a defect of J-chain production in UC[11] would suggest impaired sIgM secretion in addition to impaired sIgA production, thereby exacerbating the problem of defective local secretory immunity.

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