Rectal IgE cells in inflammatory bowel disease

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SUMMARY Immunoglobulin-contained cells in the rectal mucosa of patients suffering from non-specific inflammatory bowel disease (IBD) were counted and compared with those in a control population. While the numbers of IgA, IgM, and IgG-containing cells in both ulcerative colitis and Crohn's disease did not differ from normal, both disease groups exhibited a marked increase in IgE-staining cells. This increase in IgE-cells did not correlate with severity, duration, or treatment of disease and it did not prove possible, using these immunological studies, to differentiate between Crohn's disease and ulcerative colitis.

The differentiation of ulcerative colitis from Crohn's disease confined to the large bowel is often difficult and in most surveys between 10-15% of patients defy classification (Lennard-Jones *et al.*, 1968; Schachter *et al.*, 1970). Immunological studies in both these disease states have, so far, tended to underline similarities rather than differences. However, a recent study (Heatley *et al.*, 1975a) has reported a vast increase in IgE-staining cells in the rectal mucosa of patients with 'proctitis'. This finding and the knowledge that many of these patients respond to disodium cromoglycate (Heatley *et al.*, 1975b) has led to renewed speculation on the role of a type-I hypersensitivity reaction in this condition. As approximately 20% of patients with 'proctitis' eventually develop ulcerative colitis (Powell-Tuck *et al.*, 1977) this study was undertaken to see if IgE-containing cells were also present in the rectal mucosa of patients with non-specific inflammatory bowel disease (IBD). Further, we wondered if the measurement of rectal immunoglobulin containing cells could help to differentiate ulcerative colitis from Crohn's disease.

Methods

PATIENTS

Details of the patients examined in this study are outlined in the Table. All 27 patients with IBD had active rectal disease at the time of biopsy. These biopsies were taken from non-ulcerated areas of rectal mucosa; two of the 15 patients with Crohn's disease had additional small bowel involvement and 10 of the 12 patients with ulcerative colitis had disease extending beyond the splenic flexure.

Table Details of patients studied

	No.	M	F	Age—years	
				Range	Mean
Crohn's disease	15	8	7	15-63	37.9
Ulcerative colitis	12	8	4	21-69	35.3
Controls	15	8	7	16-72	39.7

Eight patients with Crohn's disease were not receiving specific treatment; three were taking corticosteroids, two were on azathioprine alone, and two were receiving both corticosteroids and azathioprine. Seven patients with ulcerative colitis were untreated at the time of biopsy, three were taking low dose corticosteroids, and one was on corticosteroids plus azathioprine. Another patient was taking topical corticosteroids only.

The 15 control subjects all had a final diagnosis of the irritable bowel syndrome and had normal rectal histology.

Rectal biopsies were obtained as part of the routine evaluation of patients and divided into two pieces. The portion for immunological studies was processed according to the method of Savilahti (Savilahti, 1972). Sections 6 μ thick were prepared in a Bright's cryostat and stained with antisera to human IgA, IgM, IgG, and IgE (Behringwerke). These antisera were diluted with Coon's buffer in ratios of 1:30, 1:15, 1:90, and 1:15 respectively. Extra sections were prepared for use in the Nairn blocking technique (Nairn, 1964) and to test for autofluorescence. Sections were examined with transmitted fluorescent light and cells displaying fluorescent staining of the cytoplasm and eccentric nuclei were counted using an objective $\times 40$ and employing a grid to exclude glandular tissue. Areas of maximum fluorescence were counted and the

mean of the highest grid counts per biopsy for each antiserum was calculated. Fluorescent cells were expressed per mm² of lamina propria. Extracellular fluorescence was graded on a score of 0 to +3: 0 = none; +1 = minimal; +2 = moderate; and +3 = marked. A score of +2 or greater for IgA, IgM, or IgE was considered abnormal. Cell counts were independent of this extracellular staining.

Results

Figures 1 to 4 show the maximum number of immunoglobulin containing cells per mm² of rectal

Fig. 1 IgA-cells in rectal mucosa.

○: extracellular fluorescence 0 – +1.
●: extracellular fluorescence +2 – +3.

lamina propria for IgA, IgM, IgG, and IgE respectively. Closed symbols refer to those biopsies in which there was +2 or greater extracellular staining. Although four of the 15 patients with Crohn's disease, and six of the 12 with ulcerative

Fig. 2 IgM-cells in rectal mucosa.

colitis, had IgA cell counts below the normal range (Fig. 1) neither disease group differed significantly from the controls. Most of these patients with low IgA counts had excess extracellular IgA staining.

The results for IgM cells (Fig. 2) were similar to IgA. Again there was no significant difference between either disease group and the control subjects. Five of the six patients with low IgM cell counts had increased extracellular staining.

IgG cell counts (Fig. 3) were the same for all three groups. However, all the patients with IBD and all the control subjects showed +2 or +3 extracellular staining despite the high dilution of the antisera and, for this reason, cell counting was more difficult than for any of the other immunoglobulin classes. Indeed, in three patients and one normal subject accurate counting was not possible.

Six of the 15 control subjects had IgE cells in the rectal mucosa and, although pale staining, they

Fig. 3 IgG-cells in rectal mucosa.

were more than could be explained by autofluorescence alone. However, nine of the 12 patients with ulcerative colitis had IgE cell counts above the absolute range of the normal subjects ($P < 0.01$), as had seven of the 15 patients with Crohn's disease ($P < 0.05$). There was no statistical difference between the disease groups. The rectal IgE-cells in patients with IBD were more brightly staining than the control subjects. Only one patient displayed extracellular fluorescence. The three patients with ulcerative colitis whose rectal IgE-cell counts were normal were receiving corticosteroids, as were two of the other patients with high IgE-counts. Likewise, neither treatment nor duration of disease could account for the differences in IgE-cell counts within the Crohn's disease group.

Discussion

The findings in this study differ in a number of

Fig. 4 IgE-cells in rectal mucosa.

ways from previous reports of mucosal immunoglobulin-containing cells in IBD. Despite employing different methods and counting areas of maximal fluorescence the number of IgA-containing cells in normal mucosa in our study is very similar to that recorded by Green and Fox (Green and Fox, 1975). However, we have failed to confirm the decreased numbers of mucosal IgA-cells in Crohn's disease that these authors have shown. Although a number of our patients with IBD had IgA-cell counts below the normal range all but one of them had marked extracellular staining with IgA. As the purpose of these lamina propria plasma cells is, presumably, to secrete immunoglobulin locally, this increase in extracellular staining (evidence that would be destroyed by an intensive washing technique) in the presence of lowered cell counts might represent utilisation rather than a true deficiency of the immunoglobulin. It is less easy to explain the complete contrast between our normal IgA results and

those of Skinner and Whitehead (Skinner and Whitehead, 1974) who found a very significant increase in IgA mucosal cells in both ulcerative colitis and Crohn's disease.

The increased extracellular staining in five of the six patients whose IgM cell counts were below the normal range likewise suggests that the immunoglobulin is more readily detached from its cell.

Because of the proportionally large amounts of extracellular IgG the tissue-processing technique used in this study is not ideal for the accurate counting of IgG-staining cells: A saline extraction technique such as that described by Balkien and Brandtzaeg (Balkien and Brandtzaeg, 1975) is most suited. Despite the limitation of our method, we found no evidence to support Balkien and Brandtzaeg's claim of a vast increase in IgG-containing cells in IBD.

Previous studies of mucosal IgE-containing cells in IBD have either failed to demonstrate these cells in numbers sufficient to measure (Skinner and Whitehead, 1974; Green and Fox, 1975) or have shown a decrease in cell counts compared with normal tissue (Lloyd *et al.*, 1975). An exception to these results is the work of Heatley and his colleagues who showed a vast increase in IgE-cells in the rectal mucosa of patients with proctitis (Heatley *et al.*, 1975a). Our results confirm this report and show that this increase in IgE-containing cells in rectal mucosa may be found both in ulcerative colitis and Crohn's disease. Brown and his colleagues reported a three-to-five fold increase in mucosal IgE-cells in two patients with Crohn's disease and one patient with ulcerative colitis (Brown *et al.*, 1975).

The absence of any correlation between the presence of IgE-cells and the extent or duration of disease or previous treatment is surprising. However, in the study of patients with proctitis reported by Heatley and his colleagues (Heatley *et al.*, 1975b) IgE-cells were present in the rectum, even in those patients whose disease was in remission.

The relevance of this increase in IgE-cells is unknown. Similar cells are found in the bronchial mucosa of patients with asthma (Gerber *et al.*, 1971) and in the jejunal mucosa of children with milk protein intolerance (Kilby *et al.*, 1975), both syndromes associated with type 1 hypersensitivity reactions. This raises the question of an allergic mechanism in the pathogenesis of IBD and, indeed disodium cromoglycate has been found to be of use in patients with 'proctitis' (Heatley *et al.*, 1975b). Benefit has also been claimed for this drug in chronic ulcerative colitis when high dosage is used (Dronfield and Langman, 1977).

The inference that the presence of IgE-cells in rectal mucosa would differentiate ulcerative colitis

from Crohn's disease (Goodman *et al.*, 1977) has not been supported by this study. Our inability to distinguish these two diseases by these immunological methods lends some support to the theory that both conditions represent the ends of a spectrum of a single disease entity, the expression of which depends on the genetic/immunological make-up of the host (Cave *et al.*, 1976).

References

- Balkien, K., and Brandtzaeg, P. (1975). Comparative mapping of the local distribution of immunoglobulin-containing cells in ulcerative colitis and Crohn's disease of the colon. *Clinical and Experimental Immunology*, **22**, 197-209.
- Brown, W. R., Borthistle, B. K., and Chen, S-T. (1975). Immunoglobulin E (IgE) and IgE-containing cells in human gastrointestinal fluids and tissues. *Clinical and Experimental Immunology*, **20**, 227-230.
- Cave, D. R., Mitchell, D. N., and Brooke, B. N. (1976). Evidence of an agent transmissible from ulcerative colitis tissue. *Lancet*, **1**, 1311-1314.
- Dronfield, M. W., and Langman, M. J. S. (1977). Controlled comparison of sodium cromoglycate and sulphasalazine in the maintenance of remission in ulcerative colitis (Abstract). *Gut*, **18**, A973.
- Gerber, M. A., Paronetto, F., and Kochwa, S. (1971). Immunohistochemical localisation of IgE in asthmatic lungs. *American Journal of Pathology*, **62**, 339-349.
- Goodman, M. J., Kirsner, J. B., and Riddell, R. H. (1977). Usefulness of rectal biopsy in inflammatory bowel disease. *Gastroenterology*, **72**, 952-956.
- Green, F. H. Y., and Fox, H. (1975). The distribution of mucosal antibodies in the bowel of patients with Crohn's disease. *Gut*, **16**, 125-131.
- Heatley, R. V., Calcraft, B. J., Rhodes, J., Owen, E., and Evans, B. K. (1975a). Disodium cromoglycate in the treatment of chronic proctitis. *Gut*, **16**, 559-563.
- Heatley, R. V., Rhodes, J., Calcraft, B. J., Whitehead, R. H., Fifield, R., and Newcombe, R. G. (1975b). Immunoglobulin E in rectal mucosa of patients with proctitis. *Lancet*, **2**, 1010-1012.
- Kilby, A., Walker-Smith, J. A., and Wood, C. B. S. (1975). Small intestinal mucosa in cow's milk allergy (Letter). *Lancet*, **1**, 531.
- Lennard-Jones, J. E., Lockhart-Mummery, H. E., and Morson, B. C. (1968). Clinical and pathological differentiation of Crohn's disease and proctocolitis. *Gastroenterology*, **54**, 1162-1170.
- Lloyd, G., Green, F. H. Y., Fox, H., Mani, V., and Turnberg, L. A. (1975). Mast cells and immunoglobulin E in inflammatory bowel disease. *Gut*, **16**, 861-866.
- Nairn, R. C. (1964). *Fluorescent Protein Tracing*, p. 120. Livingstone: Edinburgh.
- Powell-Tuck, J., Ritchie, J. K., and Lennard-Jones, J. E. (1977). The prognosis of idiopathic proctitis. *Scandinavian Journal of Gastroenterology*, **12**, 727-732.
- Savilahti, E. (1972). Immunoglobulin-containing cells in the intestinal mucosa and immunoglobulins in the intestinal juice in children. *Clinical and Experimental Immunology*, **11**, 415-425.
- Schachter, H., Goldstein, M. J., Rappaport, H., Fennessy, J. J., and Kirsner, J. B. (1970). Ulcerative and granulomatous colitis—validity of differential diagnostic criteria.

Annals of Internal Medicine, 72, 841-851.
Skinner, J. M., and Whitehead, R. (1974). The plasma cells

in inflammatory disease of the colon: a quantitative study. *Journal of Clinical Pathology*, 27, 643-646.

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