Mast cells and immunoglobulin E in inflammatory bowel disease

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SUMMARY   The numbers of mast cells and of IgE-containing immunocytes in the bowel wall of patients suffering from Crohn's disease or ulcerative colitis have been estimated and the results compared with those found in normal control specimens. In ulcerative colitis there is a slight rise in the number of mast cells and it appears that these participate in the inflammatory process in a non-specific manner; the number of IgE-containing immunocytes is not significantly altered. In Crohn's disease there is an almost total absence of stainable mast cells in affected areas of the bowel, together with a marked decrease in IgE-containing immunocytes. It is suggested that these findings are due to degranulation of mast cells and consumption of IgE as a result of an immediate hypersensitivity reaction in the bowel wall, this being one component of the protean inflammatory and immunological response to the entry of a variety of antigenic material.

It is currently thought that both ulcerative colitis and Crohn's disease may be immunologically mediated, though the exact mechanisms responsible for both initiating and maintaining these two inflammatory bowel conditions are still far from clear. In recent years, the limited role of humoral antibodies in these diseases has become increasingly apparent and interest has tended to centre upon the possibility that they are the result of a cell mediated delayed hypersensitivity type of reaction in the bowel wall. In both these diseases, however, the inflammatory lesions are of a mixed type and features of an acute and a chronic inflammatory reaction are often both present. McGovern and Archer (1957) were the first to suggest that the acute inflammatory component of ulcerative colitis was mediated by mast cells, and subsequent workers have claimed that a marked increase in intestinal mast cells is a prominent feature of inflammatory bowel disease, some noting this increase only in ulcerative colitis (McAuley and Sommers, 1961; Bercovitz and Sommers, 1966; Sommers, 1966) and others finding it to be also a feature of Crohn's disease (Hiatt and Katz, 1962).

These studies were made at a time when it was not yet fully established that mast cells could react with antigen and IgE to produce an immediate hypersensitivity reaction (Stanworth, 1971). Thus the possibility exists that one of the immunological mechanisms involved in inflammatory bowel disease is an immediate hypersensitivity reaction and it was with this in mind that the mast cell and IgE-containing immunocyte populations of the normal and inflamed bowel were studied.

Methods

MAST CELLS
A preliminary study showed that bowel obtained at necropsy was unsuitable for the demonstration of mast cells and their distribution was therefore studied in surgically resected or biopsy specimens, all of which were obtained fresh. Thirty specimens of ileum were studied of which 12 were from patients with proven Crohn's disease; the remaining 18 were considered as normal controls and were attached to right hemicolectomy specimens resected for colonic carcinoma. Mast cell distribution was also studied in 44 surgically resected colons and in 60 specimens of rectum, some surgically resected and others obtained at biopsy. Of the colonic specimens, 15 were from patients with Crohn's disease, and 14 from cases of ulcerative colitis; multiple sections were taken from

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the macroscopically abnormal areas of each colon. Normal control sections of colon were taken from 15 specimens resected for neoplastic disease and were selected from macroscopically normal areas at some considerable distance away from the tumour. Of the rectal specimens studied, 16 were involved by Crohn's disease and 16 by ulcerative colitis; the 28 normal control specimens of rectum were either from surgical specimens resected for sigmoid carcinoma or were biopsies from patients with functional bowel symptoms.

A pilot study showed that mast cells were demonstrated best after fixation in alcohol-formalin and staining with uranyl nitrate Azure A (Hughesdon, 1949) and hence all the tissue for study was treated in this manner. When examining sections, counts were made separately in the lamina propria and in the submucosa. For quantitation of results a $1 cm^2$ grid subdivided into 100 squares was inserted into a $\times 10$ eyepiece and the section studied under a $\times 40$ objective. Using this magnification, the grid covered a field area of $0.09 mm^2$ and to obtain a mast cell count per mm^2 of tissue a total of 1100 small squares had to be counted; when making the count in the lamina propria only those squares which did not cover glandular or epithelial components were counted. At least 10 such counts were made on each specimen and a mean figure obtained; the results were analysed statistically using Student's t test.

In addition to this absolute count of mast cells per unit area, an estimate was also made of the proportion of the total leucocyte count in the lamina propria of each specimen accounted for by mast cells. The number of mast cells and of total leucocytes was estimated per high power field and a mean count established by counting 10 such fields. The mast cells were then expressed as a percentage of total leucocytes.

IgE IMMUNOCYTES

The distribution of IgE-containing immunocytes was studied in 22 ileal specimens, of which 10 were from patients with ileal Crohn's diseases and 12 were normal controls. Thirty colons were studied (10 normal controls, 10 from cases of ulcerative colitis, and 10 from patients with Crohn's colitis) while 38 rectal specimens were examined (10 normal, 18 from patients with ulcerative colitis, and 10 from cases of Crohn's disease). Blocks were selected as described above and the tissue snap frozen in isopentane. Sections of 5 μ thickness were cut on a cyrostat at $-20°C$, dried at room temperature, and fixed for one minute in acetone. IgE was demonstrated by an indirect immunofluorescent technique using commercially prepared unconjugated rabbit monospecific anti-human IgE (Behringwerke 2049A) at a dilution

of 1 in 7 and fluorescein conjugated goat anti-rabbit globulin (Behringwerke F458E) at a dilution of 1 in 8. In the first stage of this procedure, the rabbit anti-IgE serum was placed on the section for 30 minutes in a moist chamber and the section then washed for 20 minutes in buffered saline. In the second stage, the conjugated anti-rabbit globulin was placed on the section for 30 minutes and this was followed by washing in buffer for 30 minutes. For each run, three negative controls were prepared using the following techniques:

1. The sections were, after the first stage, incubated with unconjugated second stage antiserum for 30 minutes before application of the conjugated antiserum.

2. The first stage was omitted and the section incubated only with the conjugated second stage antiserum.

3. Antibody-free serum was used in the first stage.

The treated sections were examined under UV light using a Leitz Orthoplan with a high vacuum burner, a 5 mm blue excitation filter, and a suppression filter. The distribution of immunocytes in the lamina propria was studied using a $\times 10$ eyepiece and a $\times 25$ objective with a $1 cm^2$ grid, divided into 100 squares, inserted into one of the eyepieces. The field under the grid at this magnification was estimated to be $0.748 mm^2$ and all the counts were expressed in terms of this area of lamina propria. A correction was made for the presence of epithelial and glandular elements by excluding those small squares covering such structures and then calculating the area of the grid covering true lamina propria.

Although not considered in detail in this study the total number of immunocytes—that is, producing IgA, IgM and IgG—was also estimated per unit area of the lamina propria (Lloyd, 1975); these were demonstrated by a direct immunofluorescent technique as previously described (Green and Fox, 1975).

Results

MAST CELLS

These were easily identified in the submucosa of all specimens by their size, cytoplasmic granularity, and metachromasia; they tended to be localized near to the muscularis mucosa. Mast cells were also easily recognizable in the lamina propria of normal bowel but their identification in this area in tissue from patients with inflammatory disease was more difficult largely because the non-specific chronic inflammatory cell infiltrate tended to distort and obscure the typical mast cell morphology; in these circumstances, the recognition of metachromasia was of supreme importance.

In the normal bowel the mean population density

	Ileum	Colon	Rectum
Normal	211·05 (± 8·4)	80·35 (± 2·95)	40·5 (± 2·6)
Crohn's disease	35·75 (± 6·3)	11·9 (± 2·1)	0·2 (± 0·1)
Ulcerative colitis	—	95·26 (±5·5)	81·2 (± 6·3)

Table 1 *Mean values of mast cells/mm² in the lamina propria (together with SE of mean)*

	Ileum	Colon	Rectum
Normal	84·38 (± 2·2)	86·78 (± 3·26)	98·2 (± 3·4)
Crohn's disease	64·83 (±4·23)	64·0 (± 4·10)	86·75 (± 2·9)
Ulverative colitis	—	97·0 (± 3·25)	154·9 (± 6·58)

Table 2 *Mean values of mast cells/mm² in the submucosa (together with SE of mean)*

of mast cells in the lamina propria of the ileum was much higher than that found in the lamina propria of the colon or rectum, while the mast cell content of the submucosa was relatively constant throughout the areas of the gut studied.

In the ileum of patients with Crohn's disease there was, as compared with the normal, a marked, and statistically highly significant (P = < 0·001) decrease in the number of mast cells in both the lamina propria and the submucosa but particularly the former (Tables 1 and 2). In colons affected by Crohn's disease there was also a highly significant decrease in mast cell content as compared with the normal; similar findings were noted in the rectum. In patients with ulcerative colitis there was, by contrast, a moderately significant (P = < 0·05) increase in the mast cell population per mm² in the lamina propria and submucosa of both the colon and the rectum.

Considering the mast cells as a percentage of the total leucocyte population of the lamina propria (Table 3), it was clear that they formed a larger component of the total cell count in the normal ileum than in the normal large bowel. In areas of the bowel involved by Crohn's disease, the ratio of mast cells to total leucocytes declined markedly, while in lesions due to ulcerative colitis the proportion of the leucocyte population accounted for by mast cells was not significantly altered.

IgE-CONTAINING IMMUNOCYTES
The number of immunocytes containing IgE was higher in the normal large bowel than in the normal ileum (Table 4), this being in contrast with the total

immunocyte population, which tended to decline progressively from the ileum to the colon (Lloyd, 1975); thus the proportion of immunocytes which were producing IgE rose from approximately 1·5% in the normal ileum to 6·5-7% in the normal colon and rectum.

In the ileal specimens involved by Crohn's disease, there was a marked reduction, as compared with the normal, in the number of IgE-containing immunocytes (Table 4). There was a generalized decline in the total number of immunocytes in these specimens but the number of those containing IgE fell disproportionately to those in the other groups, their percentage incidence falling from 1·5% to 0·5%. Similarly, in specimens of colon and rectum affected by Crohn's disease, there was a very marked fall in the absolute number of IgE-containing immunocytes (Table 4); again, although there was an overall reduction in the total number of immunocytes in these specimens, the decrease in those containing IgE was out of proportion to the decrease in the other immunocyte populations, the proportion of such cells falling from 6·5-7% to 1-1·5%.

By contrast, in colons and rectums affected by ulcerative colitis, there was only a slight decline in the number of IgE-containing immunocytes and there was no significant alteration in the proportion they formed of the total immunocyte population.

Discussion

The results of this study indicate that there are significant differences between ulcerative colitis and

		Normal	Crohn's	Ulcerative colitis
	Ileum	7·75	1·25	—
	Colon	3·23	0·4	2·4
	Rectum	1·9	<0·1	1·9

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Table 3 *Mast cells as a percentage of the total leucocyte count in the lamina propria*

	Ileum		Colon			Rectum		
	Normal	*Crohn's*	*Normal*	*Ulcerative colitis*	*Crohn's*	*Normal*	*Ulcerative colitis*	*Crohn's*
Mean	4·5	0·8	11·3	6·7	1·2	14·6	5·8	0·7
± SE	0·6	0·1	1·8	1·9	0·3	2·2	0·9	0·2

Table 4   *Mean population of IgE-containing immunocytes per unit area of the lamina propria*

Crohn's disease in respect of the mucosal populations of mast cells and IgE-containing immunocytes. In Crohn's disease, the low number of stainable mast cells in the lamina propria of the ileum, colon, and rectum was, as compared with normal controls, highly significant. By contrast, in ulcerative colitis, there was an increased number of mast cells both in the lamina propria and the submucosa; this increase was, however, only marginally significant. Our results conflict with some previously published observations, for the increase in mast cells in ulcerative colitis was much less marked than that noted by other workers (Hiatt and Katz, 1962). Bercovitz and Sommers, 1966), while Rao (1973) was able to find considerable numbers of granulated mast cells in the lesions of Crohn's disease; we are not able to explain these discrepancies.

Our findings do not support the view that a considerable increase in bowel mast cells not only occurs in ulcerative colitis but is an important factor in the pathogenesis of the disease. Sommers (1966) suggested that the four sigmoidoscopic stages recognized by Buie (1926) in the evolution of ulcerative colitis could be correlated with an increasing number of mast cells in the bowel, but in this study no relationship was found in cases of ulcerative colitis between the severity of the disease, as judged by histological and sigmoidoscopic criteria, and the number of mast cells in the bowel. The fact that the proportion of the total leucocytes accounted for by mast cells was the same in colons affected by ulcerative colitis as in normal colons suggests that, in this disease, the mast cells participate in the inflammatory process in a non-specific manner.

The almost total absence of mast cells in areas of the bowel affected by Crohn's disease is a phenomenon that is open to two interpretations; there may be a real reduction in mast cells or, alternatively, mast cells may be present in normal or even increased numbers but are degranulated and therefore not recognizable on light microscopy when using conventional staining methods. This latter interpretation would appear the more likely to be correct, for electron microscopic studies have shown that degranulated mast cells can be seen with some

frequency in the inflammatory lesions of Crohn's disease (Ranlov *et al.*, 1972; Cook and Turnbull, 1975). The presence of this abundance of degranulated mast cells would indicate that they play a role in the inflammatory process and, indeed, some of the histological features characteristic of Crohn's disease are most readily explained on this basis; thus, the marked oedema could well be due to release of vasoactive amines from mast cells, while focal mucosal ulceration could result from the release of proteolytic enzymes.

Although several mechanisms of mast cell degranulation are possible, it seems likely that in Crohn's disease this is most probably due to an immediate hypersensitivity reaction. In reactions of this type, antibody (usually IgE) attaches itself to the mast cell *via* membrane receptors in such a manner that the Fab part of the antibody is left free for union with antigen, a union which results in mast cell degranulation (Stanworth, 1971). That a reaction of this type is occurring in Crohn's disease is suggested by the decrease in IgE-containing immunocytes in the bowel of patients with this disease; this decrease was not simply a dilution effect due to the local oedema, for the overall number of immunocytes was only moderately reduced and not in any way comparable with the marked decline in the IgE-immunocyte population. There is evidence that IgE is actually consumed during an immediate hypersensitivity reaction, insofar as the plasma membrane of the immunocytes appear to be disrupted during a mast cell-IgE-antigen reaction (Miller, 1970), and hence the combination of a depletion of IgE-containing immunocytes and extensive degranulation of mast cells suggests strongly that an immediate type hypersensitivity reaction is occurring in the bowel in Crohn's disease.

It has been proposed that an important factor in the pathogenesis of Crohn's disease is an increased mucosal permeability to antigenic material (Aluwihare, 1972; Green and Fox, 1975). If this be the case, then the inflow of antigenic material into the bowel wall would be expected, because of the protean nature of the antigens, to provoke a variety of immunological responses and hence a variable and pleomorphic type of inflammatory lesion; it

seems possible from our results that, among the immune mechanisms invoked by antigenic entry, is an immediate hypersensitivity reaction. A reaction of this type would not only contribute to the complex histological picture but may also have important functional consequences. It has been shown that, in the intestinal mucosa of the rat, mast cell discharge and release of vasoactive amines opens up the intercellular spaces between the epithelial cells and creates a mucosal hyperpermeability to macromolecules (Murray *et al.*, 1971). If this mechanism be also operative in human Crohn's disease, it could have a double-edged effect, for while the hyperpermeability would facilitate the rapid transfer of protective antibody across the mucosal epithelium it could also allow for a still greater entry of alien antigens into the bowel wall which will, in turn, provoke a further immune response.

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