Macrophage subpopulations in lamina propria of normal and inflamed colon and terminal ileum

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SUMMARY The aim of this study was to characterise human intestinal macrophages in normal and inflamed ileum and colon. Immunoperoxidase staining with a panel of monoclonal antibodies and histochemical staining for acid phosphatase and non-specific esterase was performed. In the superficial lamina propria, normal colonic macrophages were larger and more strongly positive for acid phosphatase and non-specific esterase than those in normal terminal ileum. There were more macrophages staining with monoclonal antibody RFD1 in the superficial lamina propria of the latter. Studies in inflammatory bowel disease tissue showed the presence of macrophages staining with monoclonal antibodies RFD9 and 3G8 which were rarely present in normal tissue and represented a different pattern from that seen in infectious colitis. Studies on isolated intestinal macrophages confirmed the findings in tissue sections. Subpopulations of intestinal macrophages are likely to have different functional roles. Phenotypic changes during inflammation may be induced by mediators of inflammation or may represent a recently recruited population of cells.

Macrophages arise from the bone marrow where the monoblast differentiates into promonocytes and then into monocytes which are released into the blood.[1] From the blood, monocytes migrate to tissues where they become macrophages. During an inflammatory reaction, large numbers of monocytes are recruited into the lesion. The functionally diverse subpopulations of these cells as well as the resident population of macrophages are not easy to identify just on morphology and therefore new techniques are required to identify them.[2] Monoclonal antibodies have provided a powerful means to identify and study unique subsets of lymphocytes. By using similar antibodies it should be possible to study the heterogeneity of intestinal macrophages.

Human intestinal macrophages are found in the lamina propria, frequently close to the basal membrane of the epithelium.[3] They appear to be closely associated with lymphocytes, plasma cells, and epithelial cells and are likely to have a number of specialised functions. These include antigen presentation;[4] microbicidal and tumoricidal activity; secretion of mediators that regulate other cells and secretion of a variety of enzymes.[5]

In active inflammatory bowel disease, there is an increase in the mucosal macrophage population. Morphological and histochemical heterogeneity of intestinal macrophages has previously been demonstrated with a distinct population being prominent in active inflammatory bowel disease.[6] We have studied this heterogeneity further, in normal and inflamed small and large intestine, using a panel of monoclonal antibodies and histochemical staining. Tissue sections and cells isolated from normal and inflamed mucosa were studied.

Methods

PATIENTS

Normal colonic and ileal mucosa was obtained from 23 patients (16 women, median age 65 years (range 38–83)) undergoing intestinal resection for carcinoma. The mucosa was obtained at least 5 cm from tumour.

Mucosa involved with active inflammatory bowel disease was obtained from ileum and colon resected at operation or from rectal biopsy specimens from patients with active ulcerative colitis or Crohn's colitis (with rectal involvement). Thirty two patients were studied (20 women, median age 40 years (range 17–77)). Ten of the 32 patients were on intravenous

steroids, 13 on steroid enemas only an eighteen were on sulphasalazine. Rectal biopsy specimens from six patients with ulcerative colitis in remission (all on sulphasalazine only) and from three patients with *Clostridium difficile* colitis were also studied.

TISSUE SECTIONS

Tissue was placed on a small piece of cork and covered with OCT mounting medium (Ames) and frozen in isopentane in liquid nitrogen. Cryostat sections 4 μm thick were cut from the blocks at −25°C, fixed in acetone for 10 minutes, and stored at −20°C until used.

ISOLATION OF INTESTINAL MONONUCLEAR CELLS

Cells isolated from normal and inflamed colonic mucosa were studied. Macroscopically normal mucosa, greater than 5 cm from tumour and inflamed mucosa was obtained from fresh operation resection specimens.

Mononuclear cells were obtained by a modified EDTA-collagenase technique of Bull and Bookman.[7] Specimens were washed with calcium and magnesium free Hanks Balanced Solution (HBSS; Flow Laboratories). Mucosa was dissected from muscularis mucosa and fragments incubated with 1 mM dithiothreitol (Sigma) at 20°C for 15 minutes. After washing with HBSS, epithelial cells were removed by 3×30 minute incubations in 5 mM EDTA (BDH chemicals) at 37°C with shaking. Mucosa was subsequently cut into small pieces and digested with collagenase (from *Clostridium histolyticum*; Boehringer Mannheim) at a concentration of 100 mg/100 ml culture medium (containing 10% FCS/RPMI; Gibco) for three hours or 20 mg/100 ml culture medium for 12 hours. After washing, mononuclear cells were obtained by centrifugation over Ficoll-Paque (Pharmacia). All the media used contained 100 U/ml penicillin, 5 μg/ml gentamicin and 100 μg/ml streptomycin.

Cytospin preparations with about 60 000 mononuclear cells per slide were made, air dried and fixed with acetone. They were stored at −20°C until used.

MONOCLONAL ANTIBODIES

Table 1 lists the monoclonal antibodies used.

STAINING

Tissue sections and cytospin preparations were stained with the monoclonal antibodies using the peroxidase technique.[8] Endogenous peroxidase activity was blocked with methanol and hydrogen peroxide. The preparations were incubated with the monoclonal antibody for 60 minutes and washed in Tris buffered saline (TBS; pH 7). They were then

Table 1   *Monoclonal antibodies used*

Antibody	Cells labelled	Source
RFD1 (IgG)[15 16]	'Dendritic' cells	Dr Poulter Royal Free Hospital London
RFD7 (IgG)[11 15 16]	Mature macrophages	Dr Poulter Royal Free Hospital London
RFD9 (IgG)[16 21]	Epitheliod cells and tingible body macrophages	Dr Poulter Royal Free Hospital London
EBM11 (IgG)[12]	Tissue macrophages	Professor J O D McGee John Radcliffe Hosp. Oxford
3C10 (IgG)[13]	Monocytes and macrophages	Dr Ralph Steinman The Rockefeller Univ. New York
3G8 (IgG)[23]	Fcγ receptor on polymorphonuclear leucocytes natural killer cells and some macrophages	Dr H B Fleit State Univ of New York, New York
Y1/82A (IgG)[14]	Monocytes and macrophages	Dr D Y Mason John Radcliffe Hosp. Oxford
201521 (IgG)	Anti pan HLA-D	Dr S Fuggel John Radcliffe Hosp. Oxford
RDFR1 (IgM)[15]	Anti HLA-DR	Dr Poulter Royal Free Hosp. London

incubated with peroxidase-conjugated rabbit anti-mouse antibody (Dako; diluted in TBS/normal human serum) for 30 minutes, washed with TBS for five minutes and incubated with peroxidase conjugated swine antirabbit antibody (Dako; diluted in TBS/normal human serum) for 30 minutes. After further washing, peroxidase activity was developed with diaminobenzidine and hydrogen peroxide. The preparations were counterstained in Harris' haematoxylin, dehydrated, and mounted in DPX. Two controls were used, omitting the first antibody and secondly, using OX7 (mouse anti-rat Thy 1.1 monoclonal antibody kindly donated by Dr A Williams, MRC Cellular Immunology Unit, Oxford) as the primary antibody (at similar concentrations to those used for the macrophage monoclonal antibodies).

Tissue sections were also stained for acid phosphatase (ACP) using naphthol AS TR phosphoric acid sodium salt as substrate[9] and for non-specific esterase (NSE) using naphthyl acetate as substrate.[9] In some studies acid phosphatase activity was detected in combination with immunoperoxidase staining[10] using monoclonal antibodies as described.

For double immunofluorescence, monoclonal antibodies of different class (IgG and IgM) were used and labelled with fluorescein-conjugated goat anti-mouse IgG and TRITC-conjugated goat antimouse IgM

(Nordic).[10] Sections were examined under a standard 14 Zeiss microscope equipped with a ×40 phase oil objective and epifluorescence condenser containing selective filters for FITC and TRITC.

CELL COUNTS

For each monoclonal antibody and acid phosphatase staining, positive macrophages as a percentage of the total number of mononuclear cells in the superficial lamina propria was determined by using a graticule, using the same magnification objective, and counting in three different areas of each section.

Cells in and around Peyer's patches were not considered, they are the subject of a separate investigation. For all sections, the same length of superficial lamina propria was used. In the cytospin preparations, the percentage of positive macrophages was determined by counting at least 200 mononuclear cells per preparation. All the counts were performed by one investigator (YRM) but many were confirmed independently by other investigators.

STATISTICAL ANALYSIS

Analysis between median percentages of positive macrophages (staining with monoclonal antibodies or for ACP or NSE in tissue sections or cytospin preparations) was performed using Wilcoxon's rank-sum tests for unpaired samples. Results are expressed as a median and range.

Results

In control tissue sections or cytospin preparations (after either omitting the primary antibody or using an irrelevant monoclonal antibody, OX7), no peroxidase staining was seen.

Table 2 summarises the different staining patterns of macrophages in tissue sections stained for ACP and NSE and with monoclonal antibodies RFD1, RFD9, and 3G8.

NORMAL COLON

In tissue sections, macrophages were most frequent in the superficial lamina propria just below the epithelium. Here they were large, round and strongly ACP and NSE positive (Fig. 1). In the deeper lamina propria they became smaller, more irregular and were only weakly ACP and NSE positive or frequently ACP and NSE negative.

Monoclonal antibodies RFD1 and EBM11 stained macrophages predominantly in the superficial lamina propria whereas the other antibodies (RFD7, 3C10, Y1/82A, and 201521) stained macrophages both in the superficial and deep lamina propria. Combined staining with monoclonal antibodies and for acid phosphatase activity showed that 30–45% of RFD1

Table 2 *Macrophages in tissue sections stained for ACP and NSE and with monoclonal antibodies RFD1, RFD9, and 3G8*

	ACP and NSE	RFD1	RFD9 and 3G8
Normal colon	Large, strongly positive cells in superficial lamina propria	Positive cells predominantly in superficial lamina propria	Rarely any positive cell in lamina propria
Normal terminal ileum	Smaller, less strongly positive cells in superficial lamina propria	Positive cells throughout lamina propria superficial> deep	Rarely any positive cell in lamina propria
UC and colonic Crohn's	Strongly positive cells throughout mucosa	Positive cells throughout lamina propria	Positive cells in lamina propria in all sections
Ileal Crohn's	Strongly positive cells throughout mucosa and submucosa	Positive cells throughout lamina propria but fewer in superficial lamina propria compared to normal ileum	Positive cells in lamina propria of most sections

positive macrophages were also ACP positive. For antibodies RFD7 and 3C10, 70–95% of cells in the superficial lamina propria were also ACP positive whereas in the deep lamina propria, only 5–20% of cells were ACP positive. More than 90% of the ACP positive cells were HLA-D (201521) positive. There were no differences between proximal and distal colon in the distribution of macrophages.

NORMAL TERMINAL ILEUM

Macroscopically normal terminal ileum and ascending colon (at least 5 cm from tumour) from six right hemicolectomy specimens resected for caecal carcinoma were studied.

Compared with normal colonic mucosa, macrophages in the superficial lamina propria of the terminal ileum were smaller and less strongly ACP and NSE positive (Fig. 2). In the deeper lamina propria, the macrophages became smaller and weakly ACP and NSE positive or ACP and NSE negative. In most sections, all the monoclonal antibodies stained macrophages in the superficial as well as deep lamina propria.

Cell counts performed using a graticule (Table 3) showed that there was a significantly higher proportion of RFD1 positive cells in the villi of the terminal ileum compared with the superficial lamina propria of the colon.

Combined immunoperoxidase and acid phosphatase staining gave similar results to those obtained for normal colon. Thus, in the superficial
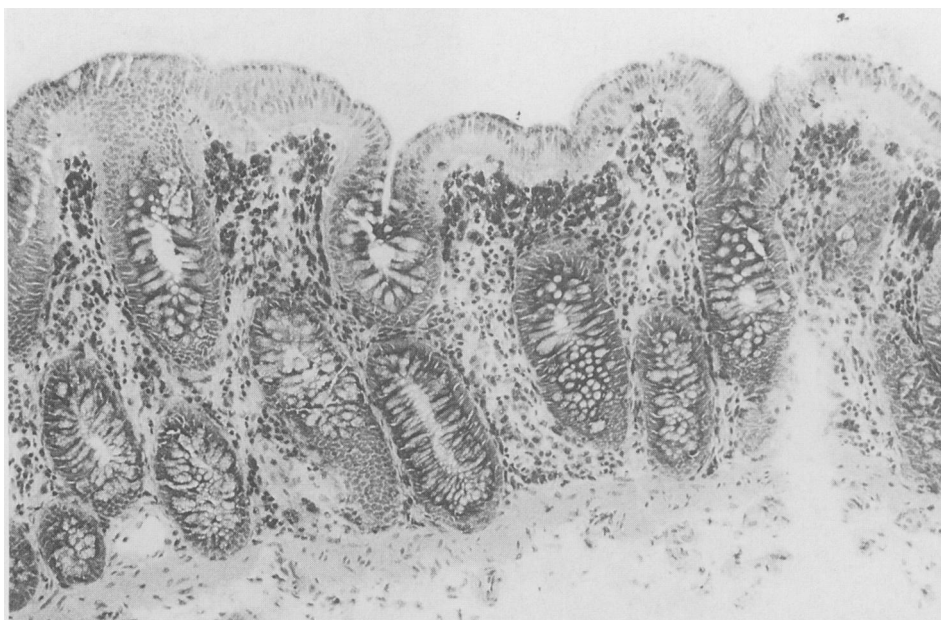
Fig. 1   *Section of normal colonic mucosa stained for acid phosphatase showing strongly positive macrophages in superficial lamina propria.*

lamina propia 20–40% of RFD1 positive macrophages were also ACP positive whereas 80–90% of cells that stained with RFD7 and 3C10 also showed ACP activity. In the deeper lamina propia, only about 5–25% of cells that stained with RFD1, RFD7, and 3C10 demonstrated ACP activity. More than 90% of the ACP positive cells were HLA-D (201521) positive.

INFLAMED COLONIC MUCOSA

In active inflammatory bowel disease mucosa, although the overall pattern of large round macrophages in the superficial lamina propria and smaller more irregular cells in the deeper regions was maintained, there was a general increase in the number of macrophages which were irregular in shape. Acid phosphatase and NSE positive macrophages were seen throughout the lamina propria and submucosa (the latter, especially with Crohn's colitis) (Fig. 3). Cells staining with the antibodies RFD1 and EBM11 were also similarly distributed. Combined staining showed that 70–90% of RFD1, RFD7, and 3C10 positive macrophages throughout the lamina propia (as well as the submucosa) showed ACP activity. Most (more than 90%) of ACP positive cells were HLA-D (201521) positive.

Cell counts using a graticule showed that there was a significant increase in RFD9 positive and 3G8 positive mononuclear cells in ulcerative colitis and Crohn's colitis (Table 4). These findings were confirmed by counts on cytospin preparations of isolated mononuclear cells stained with the same monoclonal antibodies (Table 5). Of the 13 normal colons, there were no RFD9 positive cells in the lamina propria in nine and no 3G8 positive mononuclear cells in 10. In contrast, all the ulcerative colitis and Crohn's colitis sections showed the presence of RFD9 and 3G8 (Fig. 4) positive mononuclear cells. Staining of sequential sections suggested that RFD9 and 3G8 positive mononuclear cells were largely distinct populations of cells. Combined staining demonstrated that 80–90% of both RFD9 and 3G8 positive macrophages demonstrated ACP activity.

Many of the RFD9 positive cells were largely aggregated in seven of 14 ulcerative colitis and all the Crohn's colitis sections (Fig. 5). The unaggregated cells tended to be less intensely stained than the aggregated cells.

Examination of rectal biopsy specimens from six patients with ulcerative colitis in remission (confirmed by routine histology) showed occasional RFD9 positive cells in two but no 3G8 positive cells in any section.

Also studied were rectal biopsies from three patients with *Clostridium difficile* colitis (diagnosed by the presence of the toxin and compatible

Fig. 2   *Section of normal terminal ileal mucosa stained for acid phosphatase showing weak activity in the macrophages in the superficial lamina propria.*
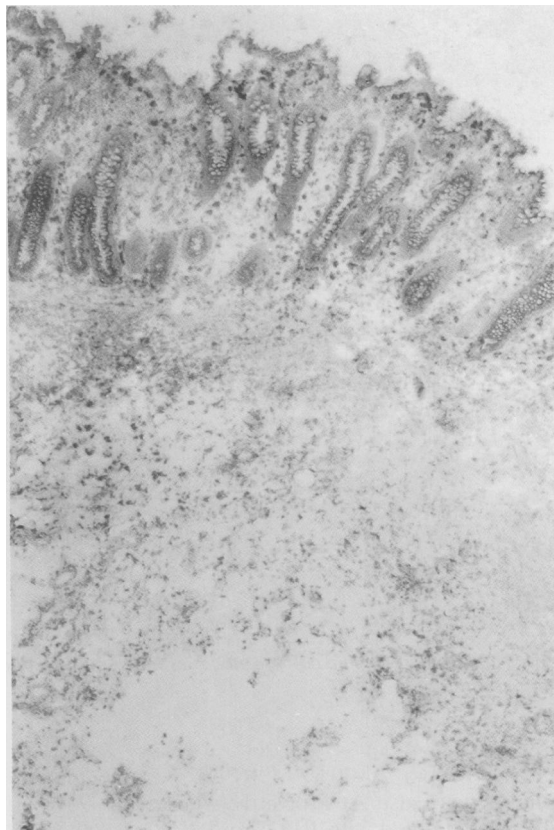

Fig. 3   *Section of colonic Crohn's disease stained for acid phosphatase. Strongly positive cells are seen in the mucosa as well as the submucosa.*

Table 3   *Median (range) % of positive macrophages in superficial lamina propria in tissue sections from normal terminal ileum and ascending colon (of six intestinal resections for carcinoma of caecum)*

Monoclonal antibody	Normal terminal ileum (n=6)	Normal colon (n=6)
RFD1	32 (28–35)	20·5 (13–24)*
RFD7	22 (14–26)	19·5 (14–24)
RFD9	0 (0–2)	0 (0–3)
EB11	26 (16–34)	22·5 (18–34)
3C10	18·5 (15–24)	22 (13–43)
3G8	0 (0–1)	0 (0–1)
Y1/82A	31·5 (30–36)	31 (23–34)
ACP	19 (16–29)	23 (20–29)

*p<0·01. Macrophages staining with each monoclonal antibody or ACP as a percentage of the total mononuclear cell population in the superficial lamina propria was determined by using a graticule. Three different areas of superficial lamina propria of each section were examined.

Table 4   *Median (range) % positive macrophages in superficial lamina propria in tissue sections of normal and inflammatory bowel disease colon*

Monoclonal antibody	Normal colon (n=13)	UC (n=14)	Crohn's colitis (n=8)
RFD1	23 (13–25)	19·5 (10–27)	20·5 (9–28)
RFD7	20 (10–28)	19·5 (12–29)	23 (13–37)
RFD9	0 (0–5)	12·5 (5–27)*	7·5 (2–15)*
EBM11	22 (18–34)	22·5 (9–31)	21·5 (13–29)
3C10	29 (13–43)	24 (15–31)	21·5 (14–33)
3G8	0 (0–5)	9·5 (1–15)*	11·5 (2–20)*
Y1/82A	31 (23–32)	29 (17–36)	29 (15–43)
ACP	20 (16–29)	21 (12–26)	22·5 (18–28)

*p<0·01 (normal *v* inflamed). Macrophages staining with each monoclonal antibody or for ACP as a percentage of the total mononuclear cell population in the superficial lamina propria was determined using a graticule. Three different areas of superficial lamina propria of each section were examined.

Table 5 *Median (range) % positive macrophages in cytospin preparations of isolated colonic mucosal mononuclear cells*

Monoclonal antibody	Normal colon (n=10)	IBD colon (n=16)
RFD1	9·5 (5–19)	9·0 (4–16)
RFD7	5 (1–12)	7·5 (2–9)
RFD9	0·5 (0–2)	4 (1–7)*
EBM11	11 (9–24)	12 (8–23)
3C10	14 (6–20)	12 (5–20)
3G8	1 (0–2)	4 (2–11)*
Y1/82A	17·5 (8–24)	12 (7–21)

*$p<0·01$. Macrophages staining with each monoclonal antibody or for ACP as a percentage of the isolated mononuclear cell population was determined by counting at least 200 cells per preparation.

histology). There were no RFD9 positive cells in any, but 3G8 positive mononuclear cells were present in all three.

ILEAL CROHN'S DISEASE

Acid phosphatase and NSE positive cells were present throughout the mucosa and also the submucosa. In many sections there was a general increase in the intensity of staining for ACP and NSE in the lamina propria compared with normal ileal tissue. There was also an increase in the number of irregular macrophages especially in the superficial lamina propria.

Cell counts using a graticule revealed an increase in the proportion of RFD9 and 3G8 positive mononuclear cells (Table 6). The RFD9 positive cells were largely aggregated in seven of 10 ileal Crohn's sections. As in the active inflammatory bowel disease colon, the unaggregated cells tended to be less intensely stained than the aggregated cells.

There was a significant decrease in the proportion of RFD1 positive cells in the superficial lamina propria of the inflamed ileum compared with normal ileal mucosa. Combined staining showed that 70–100% of macrophages staining with the antibodies RFD1, RFG7, 3C10, RFD9, and 3G8 were also positive for ACP. As for the colon, more than 90% of ACP positive cells were stained with antibody 201521 (HLA-D).

Cytospin preparations of mononuclear cells isolated from nine specimens of ileal Crohn's showed RFD9 positive cells in all (median 3%; range 1–7%) and 3G8 positive macrophages in preparations of eight out of nine specimens (median 2%; range 1–4%). In contrast no RFD9 positive or 3G8 positive cells were seen in mononuclear cells isolated from thee specimens of normal terminal ileum.

DOUBLE IMMUNOFLUORESCENT STAINING

Double immunofluorescent staining of normal and inflamed colonic and ileal tissue showed that more than 90% of macrophages staining with the monoclonal antibodies listed in Table 1 were HLA-DR positive.
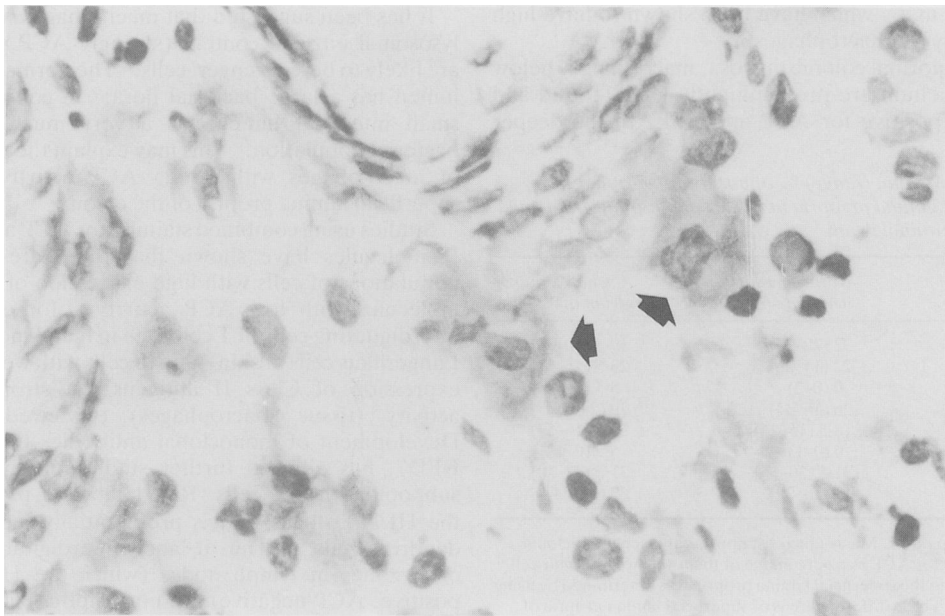

Fig. 4 *Macrophages (arrowed) stained with monoclonal antibody 3G8 in a section of colonic Crohn's disease.*
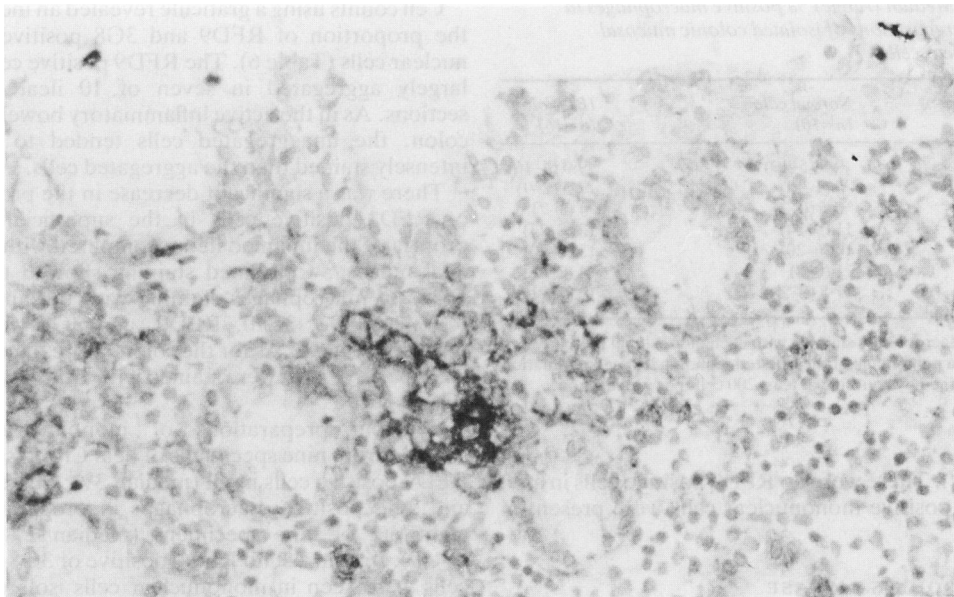
Fig. 5   Aggregate of RFD9 positive cells in section of colonic Crohn's disease.

Discussion

We have studied heterogeneity of macrophages of normal and inflamed terminal ileal and colonic mucosa using a panel of monoclonal antibodies and ACP and NSE staining. Monoclonal antibodies used included many which have been shown to have high specificity for macrophages.[27-30]

In the normal colonic mucosa, macrophages below the epithelium are predominantly large, round and strongly positive for ACP and NSE. In the deeper

Table 6   Median (range) % of positive macrophages in superficial lamina preopria in tissue sections of normal and Crohn's terminal ileum

Monoclonal antibody	Normal terminal ileum (n=6)	Crohn's terminal ileum (n=10)
RFD1	32 (28–35)	19·5 (10–29)*
RFD7	22 (14–26)	23·5 (15–32)
RFD9	0 (0–2)	5·5 (1–14)*
EMB11	26 (16–34)	29 (24–35)
3C10	18·5 (15–24)	23·5 (16–37)
3G8	0 (0–1)	5·5 (0–18)†
Y1/82A	31·5 (30–36)	31·5 (21–40)
ACP	19 (16–29)	23 (11–34)

*p<0·01; †p<0·05. Macrophages staining with each monoclonal antibody or for ACP as a percentage of the total mononuclear cell population in the superficial lamina propria was determined by using a graticule. Three different areas of superficial lamina propria of each section were examined.

lamina propria they became smaller, more irregular and showed weak activity for ACP and NSE. Macrophages in normal terminal ileum of the same patients showed a similar distribution but generally the cells tended to be smaller and less strongly positive for ACP and NSE.

It has been suggested that macrophages with high lysosomal enzyme content (strongly ACP positive) are likely to be 'scavenger' cells.[16] The normal colonic lumen has a large bacterial flora;[17] in contrast, the small intestinal lumen has a very much smaller bacterial population.[18] This may explain the presence of macrophages with strong ACP activity in the superficial lamina propria of the colon.

Studies using combined staining for ACP and Class II molecules have shown that there are distinct populations of cells with high expression of Class II molecules with low ACP activity – for example, interdigitating cells of T cell zone in lymph nodes and Langerhans cells of skin – and of cells with weak or no expression of Class II antigens but strong ACP activity (tissue macrophages) (reviewed in[16 19]). Development of monoclonal antibodies RFD1 and RFD7, has allowed further studies on these two subpopulations of cells. RFD1 labels a product of the HLA-D locus that is preferentially located on dendritic cells.[11 20] Thus it labels interdigitating cells of T zones in lymph nodes (which are HLA-DR positive, ACP negative) but not Kupffer cells of the liver.[11] RFD7 labels tissue macrophages (ACP

positive) – for example, macrophages in the marginal sinus of lymph nodes, but not interdigitating cells of T zones and thymic medulla.[11 16]

In the villi of the normal terminal ileum, a higher proportion of macrophages staining with the antibody RFD1 were present compared with the superficial lamina propria of the colon. There was no significant difference in the proportion of macrophages staining with the other monoclonal antibodies.

In the superficial lamina propria of both the normal terminal ileum and colon, the majority of RFD7 and 3C10 positive macrophages were ACP positive compared with about 30% of RFD1 positive cells.

The normal small intestinal mucosa comes in contact with a large variety of soluble food antigens and this may account for the higher proportion of RFD1 positive ('antigen presenting') cells in the villi of the terminal ileum.

In inflammatory bowel disease, there was an increase in the intensity of ACP staining as well as in the number of ACP positive cells in deep lamina propria and submucosa. This may be caused by increased penetration of bacteria and its products after damage to the epithelial barrier.

Monoclonal antibody staining also showed the presence of two new subpopulations of macrophages in the lamina propria of mucosa with active inflammatory bowel disease. These subpopulations stained with the antibodies RFD9 and 3G8. These cells were absent or only occasionally present in the normal tissue or in ulcerative colitis in remission.

The significance of the appearance of these two subpopulations of macrophages in the inflamed tissue is unknown. RFD9 is a marker for epithelioid cells and 'tingible body' macrophages.[16 21] Epithelioid cells in sarcoid granulomas are strongly positive with this antibody.[21] We have recently shown strong staining of epitheliod cells in Crohn's granulomas by this antibody.[22] Aggregates of these cells occur in active ulcerative colitis or Crohn's disease, especially in the latter. It is possible that, under certain conditions, some of these aggregates may go on to form granulomas.

Monoclonal antibody 3G8 recognises the low affinity FcγR on polymorphs, natural killer cells and some macrophages.[23 24] There are two types of receptors with low affinity for monomeric IgG; one with broad electrophorectic mobility (51-73kd) is found on neurtophils,[23] natural killer cells[24] and macrophages (identified by 3G8), and one on platelets[25] and human monocyte lines.[26] The FcγR recognised by 3G8 is absent on monocytes but is present on 60% of alveolar macrophages. The 3G8 determinant appears on monocytes cultured *in vitro* for seven days suggesting that it is an inducible protein.[27] Sections of human spleen and liver show

staining of red pulp macrophages and Kupffer cells by this antibody; white pulp of spleen and lymph nodes were negative.[28] Our study shows that normal intestinal macrophages are virtually negative in their expression of the 3G8 antigen but in inflamed ileal and colonic tissue, some macrophages showed reactivity with this antibody. Macrophage expression of this antigen occurred in inflammation as a result of active inflammatory bowel disease or infection (in contrast with reactivity with the RFD9 antibody).

The significance of this is uncertain. It has been suggested, however, that low-affinity FcγRs may be important in the clearance of immune complexes. This has been supported by some recent animal studies.[28] Thus it is conceivable that macrophage expression of this low-affinity FcγR in inflamed intestine may have the role of clearing immune complexes.

In conclusion, subpopulations of macrophages in normal and inflamed human colon and terminal ileum in tissue and in isolated cells have been shown using a panel of monoclonal antibodies and histochemical staining. The phenotypes of these cells are likely to be influenced by their environment which in the intestine is determined to a large extent by the luminal contents and the integrity of the epithelial barrier. The latter is deranged in inflamed mucosa and the increased penetration of luminal antigens as well as the mediators released during inflammation could influence the phenotype of macrophages. In addition, recently recruited monocytes from the circulation are also likely to be phenotypically different from the resident population of macrophages.

Finally, this study also shows that antigenic determinants on macrophages that are recognised by monoclonal antibodies used in this study do not appear to be affected by the isolation procedure.

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