Clin. exp. Immunol. (1991) **85**, 504–509 ADONIS 0009910491002680

Changes in phenotypically distinct mucosal macrophage populations may be a prerequisite for the development of inflammatory bowel disease

M. C. ALLISON & L. W. POULTER* *Gastroenterology Unit, Royal Infirmary, Glasgow, and* **Department of Clinical Immunology, Royal Free Hospital School of Medicine, London, UK*

(Accepted for publication 19 April 1991)

SUMMARY

Previous studies have demonstrated the presence of much more marked macrophage heterogeneity in colonic mucosa affected by the idiopathic inflammatory bowel diseases (ulcerative colitis and Crohn's disease) than in normal mucosa. This study examines the morphology, distribution and phenotypic expression of mucosal macrophage-like cells in biopsies from patients with idiopathic inflammatory bowel disease in comparison with disease control samples from patients with colonic infection or ischaemia. Approximately 80% of macrophage-like cells in histologically normal mucosa co-express the antigens recognized by the monoclonal antibodies RFD1 (an interdigitating cell marker) and RFD7 (a marker for mature tissue macrophages). In idiopathic inflammatory bowel disease, the normal colonic macrophage population is partly replaced by cells staining positively with RFD7 alone, and, to a lesser extent, with RFD1$^+$ dendritic cells. Sections from patients with infections and ischaemia exhibited epithelial HLA-DR positivity and infiltration of the lamina propria by a more heterogeneous population of macrophages than that seen in histologically normal mucosa. However, the displacement of the normal colonic macrophage phenotype by RFD7$^+$ tissue macrophages occurred to a significantly greater extent in idiopathic inflammatory bowel disease than in disease control mucosa. A pathognomonic feature of the ulcerative colitis and Crohn's colitis sections was the clustering of RFD9$^+$ epithelioid cells at the bases of disrupted crypts and adjacent to areas of mucosal damage. It is concluded that a degree of macrophage heterogeneity and macrophage infiltration can occur as a non-specific response to colonic mucosal damage. The distinctive feature of idiopathic inflammatory bowel disease mucosa is the almost complete replacement of the normal colonic mucosal macrophage population by tissue macrophages and epithelioid cells, and this phenomenon may be important in promoting the development of a chronic inflammatory state.

Keywords inflammatory bowel disease ulcerative colitis Crohn's colitis ischaemic colitis macrophages

INTRODUCTION

Mononuclear cell infiltration is a characteristic feature of colonic mucosa affected by the idiopathic inflammatory bowel diseases. In Crohn's disease, there is an increased density of mucosal macrophages (Thyberg, Graf & Klingenstrom, 1981), and granuloma formation is commonly observed. In recent years it has become clear that a heterogeneous population of macrophage-like cells exists within these infiltrates in Crohn's colitis and in ulcerative colitis, and that this heterogeneity is more marked than in uninflamed colonic mucosa (Selby *et al.*, 1983; Wilders *et al.*, 1984; Mahida *et al.*, 1988).

We and others have recently employed panels of monoclonal antibodies (MoAbs) to examine the nature of normal colonic macrophage-like cells in comparison with those found in the mucosal infiltrates of the idiopathic inflammatory bowel diseases (Allison *et al.*, 1988; Seldenrijk *et al.*, 1989; Mahida *et al.*, 1989). In normal colonic mucosa, the superficial lamina propria is abundant in HLA-DR$^+$ macrophage-like cells, over 90% of which co-express the separate antigens recognized by the MoAbs RFD1 and RFD7 (Allison *et al.*, 1988). These two reagents are capable of distinguishing interdigitating cells from mature tissue macrophages respectively in other healthy tissues (Poulter *et al.*, 1986), and we have hypothesized that the dual positivity of the normal colonic 'macrophage' could be a sign of specialization appropriate to this antigen-rich mucosal environment (Allison *et al.*, 1988). In mucosa severely affected by ulcerative colitis or Crohn's colitis, the normal colonic macrophage population is replaced by at least three different subpopulations of HLA-DR$^+$ non-lymphoid cells, each of which exhibit a distinct morphology, distribution and surface antigen expression (Allison *et al.*, 1988; Mahida *et al.*, 1989).

Correspondence: Dr L. W. Poulter, Department of Immunology, Royal Free Hospital School of Medicine, Pond Street, London NW3 2QG, UK.

Table 1. Monoclonal antibodies used in these studies

Antibody designation	CD	Major specificity in normal lymphoid tissues	Source
RFDR1 (IgM)	—	HLA-DR (monocytes, macrophages, dendritic cells, B cells)	RFHSM
RFD1 (IgM)	—	Interdigitating cells and subpopulation of B cells	RFHSM
RFD7 (IgG)	Gp11D*	Mature tissue macrophages	RFHSM
RFD9 (IgG)	—	Epithelioid cells, tingible body macrophages	RFHSM
UCHM1 (IgG)	CD14	Monocytes	N. Hogg, ICRF
EBM11 (IgG)	CD68	Monocytes and macrophages	J. O'D. McGhee, Oxford University

RFHSM, Royal Free Hospital School of Medicine, London; ICRF, Imperial Cancer Research Fund, London.
* Classification of unclustered macrophage MoAb at the 4th International Workshop (Gadd, 1989).

In the present investigation, a similar panel of MoAbs has been used to compare and contrast the mucosal macrophage subpopulations in ulcerative colitis and Crohn's colitis with those found in colonic disorders of known aetiology, such as infections and ischaemia. The aim of the study was to examine the possibility that macrophage heterogeneity is of primary pathogenetic importance in the idiopathic inflammatory bowel diseases and not simply a consequence of secondary events occurring in a damaged mucosa.

SUBJECTS AND METHODS

Patients with inflammatory bowel disease

Colonic mucosal biopsies or samples from surgical resection specimens were obtained from eight patients with Crohn's colitis and seven with ulcerative colitis. The diagnosis had been established independently by endoscopic, radiological and histological criteria in each case. All samples for this study were taken from areas of endoscopic or macroscopic involvement by the disease process. The median age of the patients with Crohn's colitis was 38 years (range 17–78), and in those with ulcerative colitis was 52 years (range 28–60). All were receiving standard medical treatment with sulphasalazine and/or topical or systemic corticosteroids at the time of biopsy or surgery.

Disease control patients

Biopsy or resection specimens were obtained from nine patients with colonic mucosal damage due to disorders other than ulcerative colitis or Crohn's colitis. There were three patients with antibiotic-associated colitis, each of whom had been symptomatic for at least 2 weeks at the time of biopsy. The diagnosis was based on three criteria: recent antibiotic therapy; presence of *Clostridium difficile* toxin in the faeces; and sigmoidoscopic and histological appearances in keeping with antibiotic-associated colitis. There were five patients with clinical and histological features of ischaemic colitis. One patient in this group had a solitary rectal ulcer, a disorder which is thought to result from mechanical damage from mucosal prolapse and straining at stool. The median age of the disease controls was 65 years (range 48–82). None of the disease controls had clinical or histological evidence of ulcerative colitis or Crohn's colitis.

Patients with normal mucosa

Histologically normal colonic mucosa was obtained from five patients. Four were undergoing segmental colectomy for colorectal carcinoma, and samples were taken at least 5 cm away from the tumour in each case. Another patient underwent colonoscopy for the investigation of altered bowel habit. Biopsies revealed histologically normal mucosa, and a clinical diagnosis of irritable bowel syndrome was made. The median age of the five patients from whom normal mucosa was obtained was 70 years (range 32–81).

Preparation of tissues

All specimens were processed within one hour of their removal. Tissue samples up to 1 cm^3 were orientated on cork, covered with OCT mounting medium (Ames) and snap frozen in isopentane in a liquid nitrogen bath. Sections of 6 μm were cut on a cryostat (Bright Instruments) maintained at $-35°C$, air dried, and fixed for 5 min in a 1:1 solution of chloroform and acetone. The slides were wrapped in plastic film and stored at $-20°C$ until used.

Monoclonal antibodies

The MoAbs used in this study, and their recognition patterns in normal lymphoid tissue, are shown in Table 1. RFDR1 binds to an HLA-DR framework epitope (Janossy *et al.*, 1986b). RFD1 recognizes a unique class II antigen which is associated with dendritic cells, and RFD7 detects a 77-kD antigen which is specific for acid phosphatase-positive tissue macrophages (Poulter *et al.*, 1986). At the Fourth International Workshop and Conference on Human Leucocyte Differentiation Antigens, RFD7 was placed in group 11D among the unclustered anti-macrophage antibodies examined (Gadd, 1989). RFD9 identifies epithelioid cells and giant cells in association with granulomas (Munro *et al.*, 1987), in addition to tingible body macrophages within germinal centres (Janossy *et al.*, 1986b). Thus, RFD1, RFD7 and RFD9 can be used for the distinction of different macrophage subpopulations in healthy lymphoid tissues. However, as stated earlier, >80% of macrophage like cells in histologically normal colonic mucosa are D1/D7 doubles—they co-express the antigens recognized by RFD1 and RFD7. UCHM1 is a CD14A MoAb that recognizes a 55-kD protein found chiefly on monocytes (Hogg *et al.*, 1984; Hogg & Horton, 1987). EBM11 is a CD68 reagent with high cellular

specificity for human macrophages (Kelly *et al.*, 1988; Stockinger, 1989). EBM11 is considered to be a standard *pan*-macrophage reagent with a broader recognition pattern than RFD7, but a proportion of RFD1$^+$ interdigitating cells in tonsil and thymus may not be detectable using EBM11 (Bofill & Janossy, 1989).

Immunoperoxidase studies

This indirect immunoperoxidase method has been described in detail elsewhere (Mason *et al.*, 1983). Briefly, the slides were allowed to warm to room temperature and were incubated with normal rabbit serum (1/100 dilution) for 20 min. Tissue sections were then incubated with MoAb solutions at appropriate concentrations for 1 h.

One section from each patient was incubated in phosphate-buffered saline (PBS) alone and served as a negative control. Positive control sections (human palatine tonsil) were incubated in parallel with the study sections. After three rinses in PBS, all sections were incubated for 45 min with a rabbit anti-mouse peroxidase conjugate (P161, Dako Corporation). Further rinsing was followed by development with a solution containing diaminobenzidine and hydrogen peroxide. The slides were then counter-stained in Harris' haematoxylin, dehydrated, permanently mounted in DPX (BDH Chemicals) and examined by light microscopy.

Double immunofluorescence studies

The indirect two-colour method for the simultaneous demonstration of two antigens by immunofluorescence microscopy has been previously published in detail (Janossy, Bofill & Poulter, 1986a). Most of the dual labelling was done with a first layer combination of RFD1 (IgM class heavy chain isotype) and RFD7 (IgG class). After a 1-h incubation with the MoAb combination, the sections were thoroughly washed in PBS and incubated for 45 min with a second layer mixture of anti-IgG–FITC and anti-IgM–TRITC in PBS (fluorescent goat anti-mouse conjugates purchased from Southern Biotechnology Associates). Preparations were then rinsed in PBS, mounted in buffered glycerol and viewed with a Zeiss microscope equipped with epifluorescent illumination and selective filters for FITC and TRITC. Positive and negative control sections (as described above) were examined in parallel with the study sections.

Cell counting and statistical analysis

The slides were numerically coded at random prior to these assessments, which were all performed by M.C.A. Immunoperoxidase sections from each biopsy were analysed quantitatively by means of a computer-assisted morphometry device (Image Analysis System, Version 2, Video Victor Dynamics). This equipment enables the observer to draw a two-dimensional map of the mucosal field being studied under the microscope. Areas occupied by lymphoid follicles, luminal and glandular epithelium can be subtracted from the total mucosal area to give the area occupied by the lamina propria. Any cells within that area exhibiting identifiable reactions on their cell membranes distinct from background were scored as positive. No attempt was made to block endogenous peroxidase activity, which was clearly distinguishable from cell membrane positivity. The number of positive non-lymphoid mononuclear cells per mm^2 was calculated from the printout.

The method for quantifying proportions of mucosal macrophage-like cells recognized by RFD1, RFD7 or both MoAbs in indirect immunofluorescence preparations was as described previously (Allison *et al.*, 1988). At least 150 cells in successive high-power fields of each section were counted and classified as being positive under red light alone, green light alone, or both. These proportions were calculated as percentages of the total number of macrophage-like cells identified by the MoAb combination.

Initial pilot studies were done using serial immunoperoxidase and double immunofluorescence preparations to compare the staining properties of EBM11 with those of the RFD reagents. These confirmed that EBM11 has recognition patterns which approximate those of a specific *pan*-macrophage reagent. It detected the same populations of HLA-DR$^+$ macrophage-like cells as RFD1 and RFD7 combined, with few exceptions (see below). Therefore, the absolute numbers of macrophage-like cells recognized by EBM11 per mm^2 lamina propria were derived from immunoperoxidase sections of each biopsy, and the relative contributions of RFD1$^+$, RFD7$^+$ and D1/D7 doubles to this total were calculated from the immunofluorescence counts. This extrapolation, although only approximate, permitted an assessment of the proportions of RFD1$^+$, RFD7$^+$ and doubly positive cells in relation to the absolute numbers of mucosal macrophages per unit area of lamina propria in each section.

Significances of observed differences between groups in terms of percentage proportions of cells bearing one or both surface antigens, were assessed using unpaired Wilcoxon's tests.

RESULTS

Histology

There were no histological abnormalities in the five normal control sections. Those from patients with ischaemic colitis exhibited a mild-to-moderate infiltrate of acute and chronic inflammatory cells, crypt atrophy, mucosal oedema and areas of ulceration. All of the biopsies from patients with antibiotic-associated colitis showed varying degrees of mucosal infiltration with polymorphs, lymphocytes and plasma cells. A pseudomembrane was included in one biopsy. The solitary rectal ulcer specimen manifested focal accumulations of lymphoid cells and histiocytes. Sections from the ulcerative colitis and Crohn's colitis biopsies demonstrated the classical histological features of these two diseases. There were focal ulcers and deep fissures in three of the Crohn's colitis biopsies, and well-formed granulomas were included in two of these.

Pilot studies

Double-immunofluorescence preparations combining EBM11 with RFDR1 indicated that both reagents detected corresponding populations of colonic mucosal macrophage-like cells. RFDR1 bound to larger numbers of cells (some of which were lymphoid morphologically), but all EBM11$^+$ cells were HLA-DR$^+$. Combination immunofluorescence studies with EBM11 and RFD1 demonstrated almost identical recognition patterns in normal mucosa. In inflamed mucosa, however, there were large numbers of EBM11$^+$ cells that were negative for RFD1. Serial immunoperoxidase sections showed that nearly all of these RFD1$^-$ cells were recognized by RFD7 or RFD9. Occasional RFD1$^+$ dendritic cells were not detected by EBM11

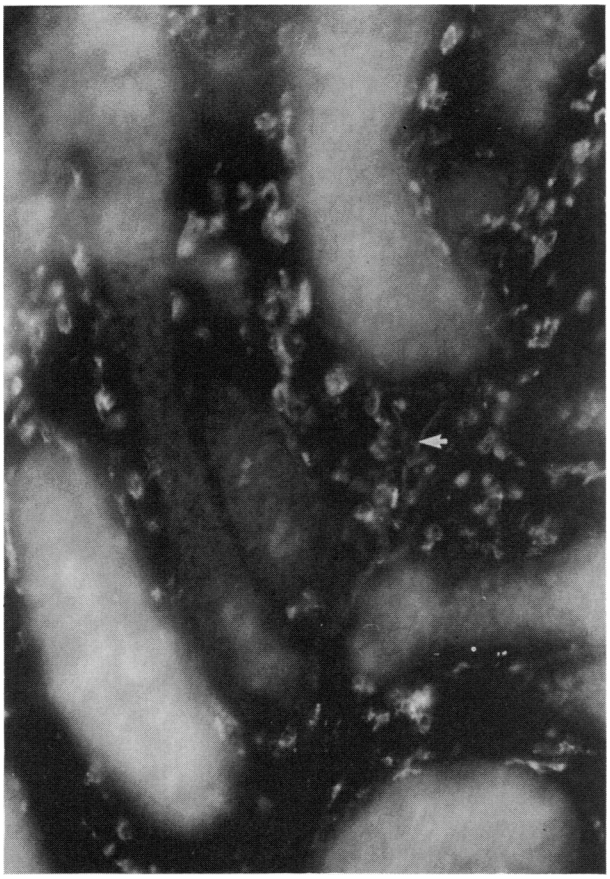

Fig. 1. RFD1/EBM11 double immunofluorescence preparation from a patient with infective colitis. All but one of the visible macrophage-like cells was positive for EBM11 alone. The exception is a dendritic cell, which was the only cell staining positively with RFD1 (arrow). Dendritic cells of this kind were not observed in normal mucosa (magnification × 180).

(an example of which is shown in Fig. 1). With this small population excepted, therefore, the pilot studies demonstrated that EBM11 is able to detect the combined populations recognized by RFD1 and RFD7. EBM11 also detected RFD9$^+$ cells and UCHM1$^+$ monocytes, but these populations never accounted for more than 10% of the total macrophage population in any section.

Immunoperoxidase studies

None of the histologically normal mucosal samples showed evidence of epithelial HLA-DR expression. Strong DR positivity was observed in the luminal and glandular epithelium of all the inflammatory bowel disease sections and seven out of the nine disease control sections.

EBM11 detected large numbers of mucosal macrophage-like cells in all sections studied. These were especially numerous in the superficial lamina propria beneath the luminal epithelial layer. Morphometric analysis of sections stained with this reagent revealed significantly greater numbers of positive macrophages per unit area of lamina propria in the disease control sections than in normal mucosa (medians 218/mm^2 and 126/mm^2, respectively, $P<0{\cdot}01$). Likewise, there were more EBM11$^+$ macrophages in the inflammatory bowel disease sections than in normal mucosa (medians 270/mm^2 in ulcerative colitis and 265/mm^2 in Crohn's colitis, $P<0{\cdot}05$ compared with normal mucosa). The degree of macrophage infiltration in the inflammatory bowel disease sections was similar to that seen in the disease control sections (Fig. 2).

The recognition patterns of RFD1 and RFD7 were simliar to those of EBM11 in normal mucosa and ischaemic colitis sections. Larger numbers of RFD7$^+$ cells and fewer RFD1$^+$ cells were observed in the inflammatory bowel disease and infective colitis sections in comparison with ischaemic colitis and normal mucosa. This qualitative observation was subsequently confirmed in the dual immunofluorescence counts. RFD9$^+$ cells were very rare in normal and disease control mucosa. They were confined exclusively to lymphoid follicles in sections from all but one of these biopsies. In this isolated example (ischaemic colitis with mucosal ulceration) there were three separate positive cells within an area of approximately 100 μm^2 adjacent to severe tissue damage. In contrast, two-thirds of the inflammatory bowel disease sections exhibited clusters of RFD9$^+$ cells that were generally located at the bases of disrupted crypts. In some cases there was complete replacement of crypts by RFD9$^+$ cells (Fig. 3a). Isolated RFD9$^+$ cells were commonly observed at sites of mucosal ulceration and fissuring, and there were some examples of organized clustering of RFD9$^+$ cells amounting to granulomas (Fig. 3b). Overall, however, the numbers of RFD9$^+$ cells were small in comparison with those identified by the other MoAbs.

Double immunofluorescence studies

Much more obvious macrophage heterogeneity was observed in infective colitis than in ischaemic colitis or normal colonic mucosa. Occasional RFD1$^+$ cells with long processes were observed in some of the infective colitis sections (Fig. 1). The proportions of cells bearing the normal D1/D7 macrophage phenotype were significantly lower in the disease control sections than in normal mucosa (medians 57% and 80%, respectively, $P<0{\cdot}05$). A much more pronounced reduction in this population was observed in the inflammatory bowel disease biopsies than in the disease control group (medians 6% and 57%, respectively, $P<0{\cdot}01$). The ulcerative colitis and Crohn's colitis sections exhibited significantly greater proportions of cells identified by RFD7 alone (medians 8% in normal mucosa, 22% in disease controls and 71% in the inflammatory bowel disease group; $P<0{\cdot}01$ between each of these three groups). These proportions are shown graphically in Fig. 2, where they have been extrapolated to take into account the total numbers of macrophage-like cells per unit area of lamina propria.

DISCUSSION

This study has demonstrated that ischaemic and infective insults to the colonic mucosa can lead to qualitative and quantitative changes in the lamina propria macrophage population. These alterations are associated with epithelial class II antigen expression, a feature which has been noted previously in infective colitis as well as idiopathic inflammatory bowel disease (McDonald & Jewell, 1987). The changes in the disease control biopsies were less marked than in ulcerative colitis or Crohn's colitis, in which the normal population of D1/D7 doubles was almost completely replaced by the other cell types.

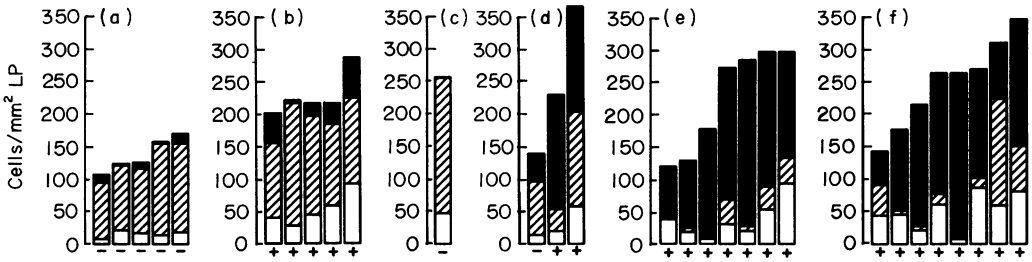

Fig. 2. Graphic representation of the relative proportions of macrophages recognized by RFD1 (□), RFD7 (■), and both MoAbs (■) in double-immunofluorescence preparations from biopsies studied: a, normal; b, ischaemic colitis; c, solitary ulcer; d, infective colitis; e, ulcerative colitis; and e, Crohn's colitis. These proportions have been extrapolated to illustrate the approximate numerical contributions of each subset towards the total population of macrophage-like cells detected by EBM11 per unit area of lamina propria (LP). +, HLA-DR$^+$ epithelium; −, negativity for HLA-DR.

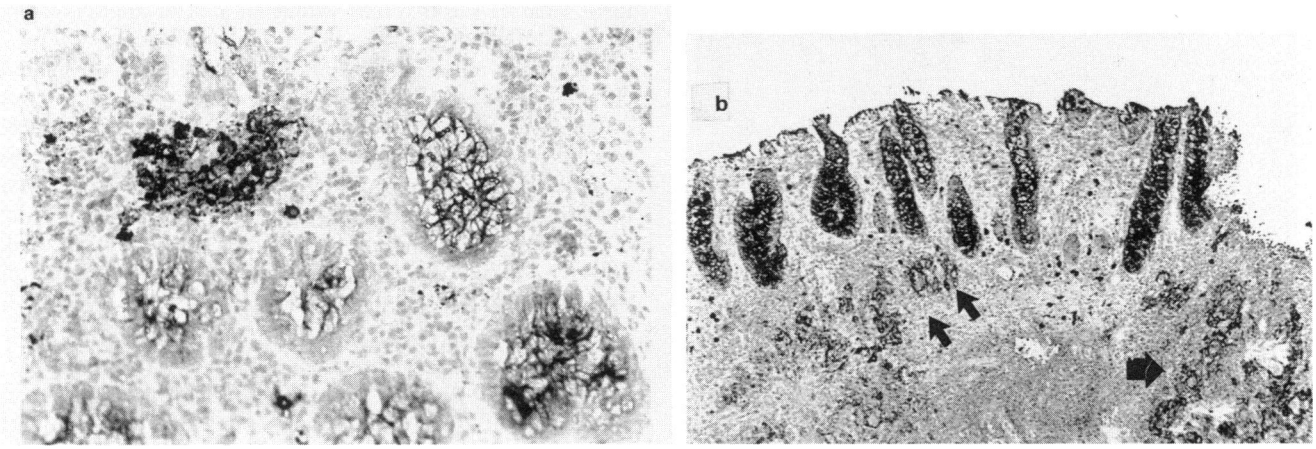

Fig. 3. Immunoperoxidase preparation of a section of rectal mucosa from a patient with active Crohn's colitis. (a) RFD9 has identified a cluster of epithelioid cells replacing a crypt (magnification × 130); (b) the distribution of RFD9$^+$ cells at lower power. Well-formed granulomas are seen at the bases of disrupted crypts (thin arrows) and within a lymphoid aggregate situated at the base of an ulcer (thick arrow) (magnification × 57).

In considering the possible importance of these results, it is of interest to compare findings in normal and inflamed colonic mucosa with those obtained in similar studies of other tissues in health and disease. As stated earlier, the normal colonic mucosal macrophage is phenotypically distinct from its counterparts elsewhere in the body in that it stains positively with both RFD1 and RFD7. Infiltration by D1/D7 doubles, however, is a feature of chronic inflammatory disorders affecting other tissues (Salisbury, Duke & Poulter, 1987; Noble, du Bois & Poulter, 1989; Spiteri, Clarke & Poulter, 1988). The small subset of D1/D7 doubles isolated from normal human lung exhibits suppressor properties in functional studies (Spiteri & Poulter, 1991). It is therefore tempting to speculate that D1/D7 doubles in normal colon are suppressor macrophages also; their function might be to inhibit inappropriate cell-mediated immune responses to antigenic material in faeces.

In contrast to the other members of the macrophage family studied, RFD9$^+$ epithelioid cells were almost completely confined to the ulcerative colitis and Crohn's colitis biopsies. The clustering of RFD9$^+$ cells adjacent to and within disrupted crypts and mucosal fissures can be likened to their distribution within remnants of alveolar air spaces in cryptogenic fibrosing alveolitis (Noble *et al.*, 1989), and their participation within granulomas parallels that observed in sarcoidosis (Munro *et al.*, 1987). Foci of free-lying RFD9$^+$ cells can be found within tiny rectal mucosal lesions detected by magnifying colonoscopy in patients with ulcerative colitis (Gionchetti *et al.*, 1988). It is likely that they would also be found in 'microgranulomas' which have been identified in macroscopically normal rectal mucosa from patients with Crohn's disease (Rotterdam, Korelitz & Sommers, 1977). Taken together with the results of the present study, these observations demonstrate that the presence of extrafollicular RFD9$^+$ cells in colonic mucosa from patients with idiopathic inflammatory bowel disease cannot be attributed to non-specific mucosal damage. Although their detection may be helpful in the differential diagnosis of colonic inflammatory disorders, RFD9$^+$ cells can also occur in granulomas due to infections, such as schistosomiasis (Mahida *et al.*, 1987).

The precise role of RFD9$^+$ cells in these disorders remains unknown, but their concentration within sites of tissue damage and cryptolysis suggests that they may have an important role in perpetuating the chronic inflammatory response.

It might have been expected that the biopsies from patients with Crohn's colitis would show a greater degree of macrophage infiltration than the disease control samples. The failure to demonstrate a significant difference in the present study is considered to be a consequence of the morphometric method employed. Mucosal involvement with Crohn's disease is asso-

ciated with oedema, crypt atrophy and expansion of the basal lymphoid aggregates. Each of these architectural changes serves to increase the area of lamina propria in relation to the length of mucosa studied. It is probable that significant differences between Crohn's colitis and the other disorders would have emerged if the macrophages had been quantified according to unit length of mucosa or muscularis propria.

The results of this study indicate that various colonic inflammatory disorders are associated with macrophage infiltration and greater macrophage heterogeneity than that seen in normal colonic mucosa. Replacement of the normal colonic macrophage population by dendritic cells, mature phagocytes and epithelioid cells is much more obvious in idiopathic inflammatory bowel disease than in ischaemic or infective colitis. Functional studies of isolated mucosal macrophage subsets using density separation techniques will be required in order to examine the pathogenetic relevance of these findings in more detail.

ACKNOWLEDGMENTS

This study was funded by the Research Support Group of the Greater Glasgow Health Board. Assistance with purchase of equipment and consumables was provided by the West of Scotland divisions of the Ileostomy Association and the National Association for Colitis and Crohn's Disease. L.W.P. was in receipt of a grant from the Wellcome Trust.

REFERENCES

ALLISON, M.C., CORNWALL, S., POULTER, L.W., DHILLON, A.P. & POUNDER, R.E. (1988) Macrophage heterogeneity in normal colonic mucosa and in inflammatory bowel disease. *Gut*, **29,** 1531.

BOFILL, M. & JANOSSY, G. (1989) The reactivity of macrophages and interdigitating cells with the Fourth workshop mAb. In *Leucocyte Typing IV. White Cell Differentiation Antigens* (ed. by W. Knapp) p. 916. Oxford University Press, Oxford.

GADD, S. (1989) Report on unclustered mAb. In *Leucocyte Typing IV. White Cell Differentiation Antigens* (ed. by W. Knapp) p. 846. Oxford University Press, Oxford.

GIONCHETTI, P., MAHIDA, Y.R., PATEL, S. & JEWELL, D.P. (1988) Macrophage and lymphocyte subpopulations in magnifying endoscopic lesions of Crohn's disease. *Clin. exp. Immunol.* **72,** 373.

HOGG, N. & HORTON, M.A. (1987) Myeloid antigens: new and previuosly defined clusters. In *Leucocyte Typing III. White Cell Differentiation Antigens* (ed. by A. J. McMichael) p. 576. Oxford University Press, Oxford.

HOGG, N., MACDONALD, S., SLUSARENKO, M. & BEVERLEY, P.C. (1984) Monoclonal antibodies specific for human monocytes, granulocytes and endothelium. *Immunology*, **53,** 753.

JANOSSY, G., BOFILL, M. & POULTER, L.W. (1986a) Two-colour immunofluorescence: analysis of the lymphoid system with monoclonal antibodies. In *Immunocytochemistry: Practical Applications in Pathology and Biology* (ed. by J. Polak & S. van Noorden) p. 438. J. Wright, Bristol.

JANOSSY, G., BOFILL, M., POULTER, L.W., RAWLINGS, E., BURFORD, G.D., NAVARETTE, C., ZIEGLER, A. & KELEMAN, E. (1986b) Separate ontogeny of two macrophage-like accessory cell populations in the human fetus. *J. Immunol.* **136,** 4354.

KELLY, P.M.A., BLISS, E., MORTON, J.A., BURNS, J. & MCGHEE, J.O'D. (1988) Monoclonal antibody EBM/11: high cellular specificity for human macrophages. *J. clin. Pathol.* **41,** 510.

MCDONALD, G.B. & JEWELL, D.P. (1987) Class II antigen (HLA-DR) expression by intestinal epithelial cells in inflammatory diseases of colon. *J. clin. Pathol.* **40,** 321.

MAHIDA, Y.R., PATEL, S., GIONCHETTI, P., VAUX, D. & JEWELL, D.P. (1989) Macrophage subpopulations in lamina propria of normal and inflamed colon and terminal ileum. *Gut*, **30,** 826.

MAHIDA, Y.R., PATEL, S., WU, K. & JEWELL, D.P. (1987) Macrophage and lymphoid subpopulations in the granulomata of Crohn's disease. *Gut*, **28,** A1388.

MAHIDA, Y.R., PATEL, S., WU, K. & JEWELL, D.P. (1988) Interleukin 2 receptor expression by macrophages in inflammatory bowel disease. *Clin. exp. Immunol.* **74,** 382.

MASON, D.Y., ABDULAZIZ, Z., FALINI, B. & STEIN, H. (1983) Double immunoenzymatic labelling. In *Immunocytochemistry: Practical Applications in Pathology and Biology* (ed. by J. Polak & S. van Noorden) p. 113. J. Wright, Bristol.

MUNRO, C.S., CAMPBELL, D.A., COLLINGS, L.A. & POULTER, L.W. (1987) Monoclonal antibodies distinguish macrophages and epithelioid cells in sarcoidosis and leprosy. *Clin. exp. Immunol.* **68,** 282.

NOBLE, B., DU BOIS, R.M. & POULTER, L.W. (1989) The distribution of phenotypically distinct macrophage subsets in the lungs of patients with cryptogenic fibrosing alveolitis. *Clin. exp. Immunol.* **76,** 41.

POULTER, L.W., CAMPBELL, D.A., MUNRO, C. & JANOSSY, G. (1986) Discrimination of human macrophages and dendritic cells by means of monoclonal antibodies. *Scand. J. Immunol.* **24,** 351.

ROTTERDAM, H., KORELITZ, B. & SOMMERS, S.C. (1977) Microgranulomas in grossly normal rectal mucosa in Crohn's disease. *Am. J. clin. Pathol.* **67,** 550.

SALISBURY, A.K., DUKE, O. & POULTER, L.W. (1987) Macrophage-like cells of the pannus area in rheumatoid arthritis joints. *Scand. J. Rheumatol.* **16,** 263.

SELBY, W.S., POULTER, L.W., HOBBS, S., JEWELL, D.P. & JANOSSY, G. (1983) Heterogeneity of HLA-DR-positive histiocytes in human intestinal lamina propria: a combined histochemical and immunohistological analysis. *J. clin. Pathol.* **36,** 379.

SELDENRIJK, C.A., DREXHAGE, H.A., MEUWISSEN, S.G.M., PALS, S.T. & MEIJER, C.J.L.M. (1989) Dendritic cells and scavenger macrophages in chronic inflammatory bowel disease. *Gut*, **30,** 484.

SPITERI, M.A. & POULTER, L.W. (1991) Characterisation of immune inducer and suppressor macrophages from the normal human lung. *Clin. exp. Immunol.* **83,** 155.

SPITERI, M.A., CLARKE, S.W. & POULTER, L.W. (1988) Phenotypic and functional changes in alveolar macrophages contribute to the pathogenesis of pulmonary sarcoidosis. *Clin. exp. Immunol.* **74,** 359.

STOCKINGER, H. (1989) Cluster report. In *Leucocyte Typing IV. White Cell Differentiation Antigens* (ed. by W. Knapp) p. 841. Oxford University Press, Oxford.

THYBERG, T., GRAF, W. & KLINGENSTROM, P. (1981) Intestinal fine structure in Crohn's disease. Lysosomal inclusions in epithelial cells and macrophages. *Virchows. Archiv. [A]*, **39,** 141.

WILDERS, M.M., DREXHAGE, H.A., KOKJE, M., VERSPAGET, H.W. & MEUWISSEN, S.G.M. (1984) Veiled cells in chronic idiopathic inflammatory bowel disease. *Clin. exp. Immunol.* **55,** 377.