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Characterization of antigen-presenting activity of intestinal mononuclear cells isolated from normal and inflammatory bowel disease colon and ileum

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SUMMARY

Antigen-presenting activity in mononuclear cells, isolated from normal and inflamed human ileum and colon, has been characterized using allogeneic mixed lymphocyte reaction with resting T cells as responders. Greatest proliferation was induced by fibronectin-adherent (macrophage-enriched) cells, and least by fibronectin non-adherent (macrophage-depleted) cells and by mononuclear cells depleted of macrophages by panning with monoclonal antibody 3C10. When intestinal mononuclear cells and allogeneic T cells were incubated in large numbers, clusters were observed. These clusters contained cells with a dendritic morphology that were strongly HLA-D-positive and which also stained with macrophage-specific monoclonal antibodies 3C10, EMB11 and Y1/82A. These cells were closely associated with proliferating T cells. Studies comparing mononuclear cells isolated from normal and inflamed colonic mucosa suggest that the latter may have enhanced antigen-presenting capacity.

INTRODUCTION

Induction of an immune response in T cells requires the presence of accessory cells. Most potent of these are dendritic cells, and features of these cells, isolated from mouse spleen and human peripheral blood, have been described elsewhere (Steinman & Nussenzweig, 1980; Steinman, Van Voorhis & Spalding, 1986; Austyn, 1987). They are irregularly shaped, non-phagocytic cells that express MHC class II molecules. They are potent stimulators of many lymphocyte responses including allogeneic mixed lymphocyte reactions (MLR), graft rejection, syngeneic MLR, cytolytic T cells, antibody-forming cells and proliferation to soluble antigens. It is believed that dendritic cells are essential to induce immune responses in previously resting T cells, whereas macrophages induce immune responses in primed or sensitized T cells (Austyn, 1987). Ia-positive dendritic cells have been described in rat Peyer's patches (Wilders *et al.*, 1983) and have been isolated from murine Peyer's patches (Spalding *et al.*, 1983). Cells with features of dendritic cells have also been demonstrated in lymphatics draining rat intestinal lymph (Pugh, MacPhersen & Steer, 1983).

Ulcerative colitis and Crohn's are chronic inflammatory disorders of unknown aetiology. They are characterized by an increase in the mucosal T-cell, B-cell and macrophage population. Increased turnover (Meuret, Bitzi & Hammer, 1978) and activation (Mee, Szawatakowski & Jewell, 1980; Doe & Forsman, 1982) of monocytes in inflammatory bowel disease has been demonstrated and it is likely that the increase in the mucosal macrophage population is derived from these cells. Wilders *et al.* (1984), using time-lapse photography, have demonstrated cells resembling veiled cells in suspensions of mononuclear cells isolated from inflammatory bowel disease mucosa. However, there is no information on the functional capacity of antigen-presenting cells isolated from human intestine. In this study, using allogeneic MLR with resting T cells as responders, we have characterized antigen-presenting cells isolated from mucosa of normal and inflammatory bowel disease ileum and colon.

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MATERIALS AND METHODS

Monoclonal antibodies

The monoclonal antibodies used in this study were obtained from various sources. Antibody EMB11, which is highly specific for tissue macrophages (Kelly *et al.*, 1988), was obtained from Professor J. O'D. McGhee, John Radcliffe Hospital, Oxford. 3C10 and Y1/82A are both specific for monocytes and macrophages (Van Voorhis *et al.*, 1983a; Hogg & Horton, 1987). 3C10 was from Dr Steinman, Rockefeller Institute, New York, and Y1/82A from Dr D. Y. Mason, John Radcliffe Hospital, Oxford. RFD1 (which labels dendritic cells) and RFB7 (anti-CD20) were obtained from Dr L. Poulter, Royal Free Hospital, London. Antibody 201521 (anti-HLA-D) was provided by Dr Fuggle, John Radcliffe Hospital, Oxford. Anti-Tac antibody was a gift from Dr T. Waldmann, National Institute of Health, Bethesda, MD. T910 (pan T cells), T310 (CD4-positive cells) and DK25 (CD8-positive cells) were all obtained from Dakopatts (High Wycombe, Bucks). B-cell monoclonal antibody TO15 (anti-CD22; gift from Dr D. Y. Mason) was also used.

Staining

The peroxidase technique (Gatter, Falini & Mason, 1984) was used for staining with the antibodies EMB11, 3C10, Y1/82A, RFD1 and 201521.

For the rest of the antibodies, the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique (Cordell *et al.*, 1984) was used.

Tissue

Macroscopically and histologically normal colonic (seven) and terminal ileal (one) mucosa were obtained from patients undergoing intestinal resection for tumour (seven) or severe idiopathic constipation (one), respectively.

Inflamed, and in some cases non-inflamed, colonic and inflamed ileal mucosa were obtained from patients with inflammatory bowel disease (IBD) undergoing resection. Six patients had ulcerative colitis, one had colonic Crohn's disease and six had ileal Crohn's disease. All except two (one with colonic and one with ileal Crohn's disease) were on corticosteroids at the time of the operation.

No attempts were made to remove Peyer's patches from normal or inflamed ileum.

Isolation of intestinal mononuclear cells

Mononuclear cells (MNC) were isolated from normal and inflamed colonic and ileal mucosa by modification of the EDTA-collagenase technique described by Bull & Bookman (1977). In brief, small strips of mucosa were shaken with 5 mmol EDTA, in three half-hour steps, to remove the epithelial cells. After washing, the mucosa was cut into 2-mm pieces and digested with collagenase (from *Clostridium histolyticum*; Boehringer Mannheim, FRG) at a concentration of 1 mg/1 ml in culture medium (10% fetal calf serum in RPM1; Gibco, Paisley, Renfrewshire) for 3 hours. After filtration through a nylon mesh, the cells were washed. MNC were obtained by centrifugation on Ficoll–Paque (Pharmacia, Uppsala, Sweden).

Preliminary studies had shown that centrifugation on Ficoll–Paque does not alter the proportion of MNC staining with macrophage-specific monoclonal antibodies and antibody RFD1. In experiments using three colonic mucosal specimens (one normal, one ulcerative colitis and one colonic Crohn's disease), the proportion of MNC staining with the monoclonal antibodies, before and after centrifugation on Ficoll–Paque, were [mean($\pm$SD)] EBM11 10·3%($\pm$2·0) and 11·7($\pm$1·5); Y1/82A, 14·3%($\pm$2·5) and 15·7%($\pm$3·5); RFD1, 9%($\pm$3·6) and 11·7%($\pm$4·1), respectively.

Fibronectin adherence and panning

In some experiments, MNC adherent and non-adherent to fibronectin-coated petri-dishes (Lin & Gordon, 1979; Bevilacqua *et al.*, 1981) were obtained. A 0·1% solution of gelatin (Sigma, St Louis, MO) was prepared by boiling in distilled water. Small (50-mm) plastic petri-dishes were coated by adding 1 ml of cooled (to 37°) gelatin solution and incubated at 37° for 2 hr. After aspiration of excess gelatin solution, the petri-dishes were dried for 2 hr before incubating with 50% fresh human plasma (in RPMI) at 37° for 1 hr. The petri-dishes were then rinsed in phosphate-buffered saline (PBS; pH 7) before incubating intestinal mononuclear cells for 1 hr at 37°. Non-adherent cells were collected by gentle washing with warm (37°) 5% fetal calf serum (FCS) in RPM1. The removal of non-adherent cells was monitored by using an inverted microscope. The adherent cells were incubated at 37° in 10% FCS/RPM1 for about 12 hr. They were detached by incubation at 4° for 1 hr followed by vigorous pipetting.

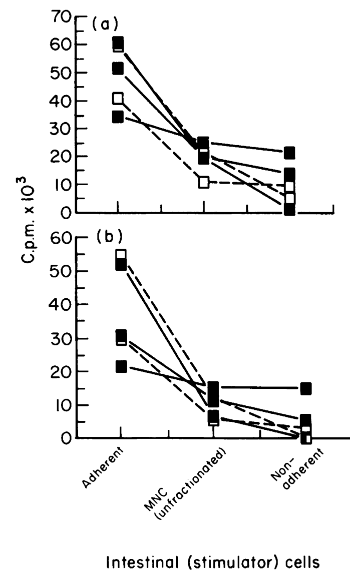

Figure 1. Proliferation of allogeneic T cells (10^5 per well) by unfractioned, fibronectin-adherent and fibronectin non-adherent intestinal MNC isolated from normal colonic mucosa (——■——) and from mucosa of ileum with active Crohn's disease (— □ —). (a) 10^5 intestinal MNC per well; (b) 5×10^4 intestinal MNC per well. For both (a) and (b) there was a significant difference between the different fractions of intestinal MNC: $P<0{\cdot}001$ (one-way analysis of variance).

In some experiments, the intestinal mononuclear cells were depleted of macrophages by a panning technique (Wysocki & Sato, 1978) using a macrophage-specific monoclonal antibody, 3C10 (IgG). Plastic petri-dises were coated with goat anti-mouse IgG (Zymed, San Francisco, CA) in 0·05 M Tris buffer pH 9·5. Intestinal mononuclear cells (2×10^7) were incubated with antibody 3C10 at 4° for 30 min and washed three times in 5% FCS/PBS. The coated petri-dishes were washed with cold PBS (pH 7) before adding the intestinal MNC. Following incubation at 4° for 2 hr, non-adherent cells were removed by gentle washing with cold (4°) 5% FCS/PBS.

Cytospin preparations of unfractionated MNC, fibronectin-adherent and non-adherent cells and cells after panning with 3C10 were made. Proportions of macrophages, CD4-positive and CD8-positive T cells and B cells were determined by staining with mononuclear antibodies as described above.

Preparation of allogeneic T cells

Purified, resting T cells were prepared from venous blood obtained from healthy volunteers (Smith *et al.*, 1986). Mononuclear cells were depleted of monocytes by adherence to plastic, tissue culture, petri-dishes for 1 hr at 37°. B cells and remaining monocytes were depleted further by passage through a nylon-wool column. Finally, activated T cells and any remaining B cells and monocytes were removed by complement lysis using monoclonal antibodies 201521 (anti-HLA-D), RFB7 (anti-CD20) and 3C10 (monocyte- and macrophage-specific antibody).

Table 1. Proliferation of allogeneic T cells (10^5 per well) by intestinal MNC, unfractionated and after depletion of fibronectin adherent cells (mitomycin C-treated) at concentrations of 10^5 and 5×10^4 per well

Experiment	Unfractionated MNC	Fibronectin non-adherent MNC
	10^5 intestinal (stimulator) MNC per well*	
1 (normal colon)	19,315	1161
2 (normal colon)	19,695	13,783
3 (normal colon)	25,145	21,260
4 (ulcerative colitis)	8215	2021
5 (ulcerative colitis)	21,931	16,950
6 (ulcerative colitis)	56,377	40,295
7 (normal ileum)	9812	6277
8 (ileal Crohn's)	19,203	11,809
9 (ileal Crohn's)	21,153	5223
10 (ileal Crohn's)	11,188	9565
	5×10^4 intestinal (stimulator) MNC per well*	
1 (normal colon)	6510	0
2 (normal colon)	11,534	5400
3 (normal colon)	15,244	14,930
4 (ulcerative colitis)	2045	237
5 (ulcerative colitis)	13,589	9900
6 (ulcerative colitis)	28,283	25,137
7 (normal ileum)	2628	2535
8 (ileal Crohn's)	13,443	7124
9 (ileal Crohn's)	12,183	2860
10 (ileal Crohn's)	5842	2882

Proliferation of allogeneic T cells (10^5 per well) by intestinal MNC, unfractioned and after depletion of fibronectin adherent cells (mitomycin C-treated) at concentrations of 10^5 and 5×10^4 per well.

* $P < 0{\cdot}01$ (paired *t*-test).

Table 2. Proliferation of allogeneic T cells (10^5 per well) by intestinal MNC (mitomycin C-treated), unfractionated and after panning with monoclonal antibody 3C10 at concentration of 10^5 and 5×10^4 per well

Experiment	Unfractionated MNC	3C10-depleted MNC
	10^5 intestinal (stimulatory) MNC per well*	
1 (normal colon)	20,038	14,754
2 (normal colon)	27,789	15,191
3 (normal ileum)	9812	5590
4 (ileal Crohn's)	21,153	6606
5 (ileal Crohn's)	21,412	11,900
	5×10^4 intestinal (stimulator) MNC per well	
1 (normal colon)	6161	4002
2 (normal colon)	13,833	10,711
3 (normal ileum)	2628	2175
4 (ileal Crohn's)	12,183	499
5 (ileal Crohn's)	13,527	2555

* $P < 0{\cdot}02$ (paired *t*-test).

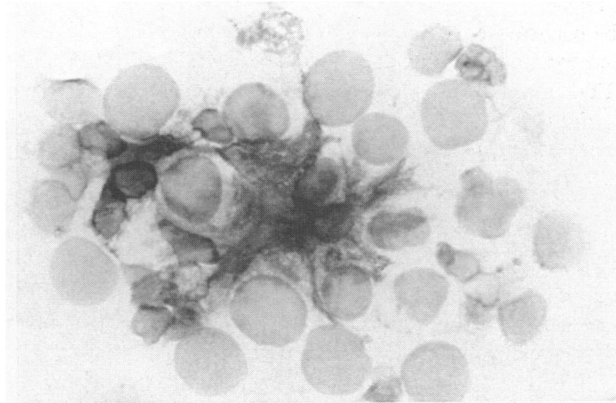

Figure 2. Cluster of proliferating lymphocytes and cells with dendritic morphology stained with anti-HLA-D monoclonal antibody 201521 (magnification × 442).

Mixed lymphocyte reaction

Peripheral blood allogeneic T cells (resting, unprimed) were used as responders. 10^5 T cells, in 20% responder serum (heat-inactivated) in RPM1, were used per well. Intestinal MNC (unfractionated, fibronectin-adherent, fibronectin non-adherent and 3C10-depleted) were used as stimulators and were treated with mitomycin C 25 μg/ml for 30 min at 37°. After three washes the intestinal cells were resuspended in 20% responder serum (heat-inactivated) in RPM1. 10^5, 5×10^4 and, in some cases, $2{\cdot}5 \times 10^4$ intestinal cells per well were used. Assays were performed in triplicate (in flat-bottomed microtitre wells; Celcult, Feltham, Middlesex) and incubated for 6 days. Eighteen hours before harvesting, 1 μCi of [^{3}H]thymidine was added to each well and proliferation measured. Results were expressed as maximal counts per minute (c.p.m. in stimulated cultures minus c.p.m. in non-stimulated cultures of purified T cells). C.p.m. in cultures of purified, allogeneic T cells only (non-stimulated cultures) was usually < 400.

Clusters

In some experiments, 10^6 intestinal (mitomycin C-treated) and allogeneic T cells were incubated (in 2 ml, 20% responder serum). Over the following days, clusters were observed. After 6 days' incubation, cytospin preparations were made, fixed in acetone and stored at $-20°$. Using the macrophage-specific and some of the T-cell antibodies described earlier, the preparations were stained using the peroxidase or APAAP techniques.

Statistics

Statistical analysis was performed using one-way analysis of variance and Student's *t*-test (paired or unpaired, as indicated).

RESULTS

Viability of responder (allogeneic T cells) and all fractions of stimulator (intestinal) cells was always $>90\%$, as assessed by exclusion of trypan blue.

Purity of allogeneic T cells was assessed by staining cytospin preparations with monoclonal antibodies. Staining with antibodies 201521, 3C10, Y1/82A and RFB7 showed that the proportion of cells labelled with these antibodies (HLA-D-positive, monocytes and B cells) was $<0{\cdot}5\%$.

Table 3. Mean percentage (±SD) of macrophages, B cells and T cells and CD4/CD8 ratio, identified using monoclonal antibodies, in cytospin preparations of different fractions of isolated intestinal mononuclear cells

	Unfractionated MNC ($n=10$)	Fibronectin-adherent MNC ($n=5$)	Fibronectin non-adherent MNC ($n=10$)	3C10-depleted MNC ($n=5$)
Macrophages	18·2(±2·9)	43·8(±9·0)*	10·0(±4·3)*	6·8(±2·0)†
B cells	16·8(±3·8)	4·4(±1·7)	18·7(±5·2)	17·2(±5·1)
T cells	52·6(±8·6)	21·8(±2·5)	59·0(±14·9)	49·4(±20·3)
CD4/CD8 ratio	3·5(±2·0)	2·5(±2·1)*	3·7(±1·0)	2·7(±1·7)

* vs. unfractionated MNC: $P<0{\cdot}001$.
† vs. unfractionated MNC: $P<0{\cdot}01$ (paired t-test).

Table 4. Proliferation of allogeneic T cells (10^5 per well) by MNC isolated from normal and inflamed (one to six ulcerative colitis; seven Crohn's disease) colonic mucosa

	Intestinal (stimulator) MNC per well		
	10^5	5×10^4	$2{\cdot}5\times10^4$
Normal colon			
1	19,315	6510	
2	19,695	11,534	
3	27,278		
4	20,693	10,607	2547
5	25,145	15,244	4806
6	20,038	6161	3852
7	27,789	13,833	5755
Mean	22,851	10,648	4240
SEM	1415	1520	685
IBD			
1	8044	2021	
2	21,931	13,589	7782
3	56,377	28,283	19,203
4	40,080	14,904	5084
5	49,200	25,800	16,800
6	42,674	27,207	19,258
7	25,713	8708	2238
Mean	34,860	17,216	11,727
SEM	6402	3829	3098

At each concentration (of intestinal MNC), normal vs. inflamed (IBD) mucosa: $P>0{\cdot}05$.

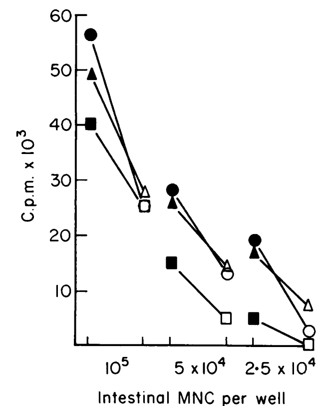

Figure 3. Proliferation of allogeneic T cells by MNC isolated from inflamed (closed symbols) and non-inflamed (open symbols) mucosa of three colons with distal active ulcerative colitis. 10^5 intestinal MNC per well: $P<0{\cdot}05$; 5×10^4 intestinal MNC per well: $P<0{\cdot}02$; $2{\cdot}5\times10^4$ intestinal MNC per well: $P=0{\cdot}05$ (paired t-test).

Lack of functional antigen-presenting cells in the allogeneic, resting (responder) T cells was confirmed by an experiment in which MLR (as described in the Materials and Methods) was performed using the purified T cells isolated from two healthy (allogeneic) individuals. Using 10^5 T cells (from each individual) per well, there was no significant proliferation (c.p.m. of T cells incubated separately: 158 and 237; c.p.m. of T cells incubated together: 357; c.p.m. of T cells of one individual incubated with adherent cells of second individual: 15,105).

Characterization of antigen-presenting cells

To study characteristics of cells with antigen-presenting activity, proliferation of allogeneic (resting) T cells in response to unfractionated MNC, MNC adherent and non-adherent to fibronectin-coated petri-dishes and MNC after depletion of macrophages (with antibody 3C10) was determined.

In cells isolated from five specimens (three normal large bowel and two with ileal Crohn's disease), unfractionated MNC, fibronectin-adherent and non-adherent cells were studied. Studies using three different concentrations of intestinal (stimulator) cells showed that proliferation of responder cells was greater with adherent (macrophage-enriched) cells and least with non-adherent (macrophage-depleted) cells (Fig. 1).

In a further five specimens (three ulcerative colitis, one normal terminal ileum and one ileal Crohn's disease), it was possible to study unfractionated MNC and non-adherent cells. Table 1 shows the results of all experiments using unfractionated MNC and fibronectin non-adherent cells (from the same specimens). Significantly less proliferation was induced by fibronectin non-adherent (macrophage-depleted) cells than by unfractioned MNC.

In five specimens (two normal large bowel, one normal terminal ileum and two with ileal Crohn's disease), unfractionated MNC and MNC depleted of macrophages by panning (with antibody 3C10) were compared (Table 2). Proliferation by MNC depleted of macrophages was significantly less than that induced by unfractionated cells.

Clusters

In cultures of intestinal cells and allogeneic T cells incubated in petri-dishes, clusters were observed. After incubation for 6 days, cytospin preparations were made and later stained with monoclonal antibodies using the peroxidase technique. Cells with a dendritic morphology which was strongly HLA-D (201521)-positive were seen (Fig. 2). These cells also stained with antibody RFD1 (which stains dendritic cells) and also with macrophage-specific monoclonal antibodies EMB11, 3C10 and Y1/82A.

Proliferation of lymphocytes within the clusters was shown by the presence of blasts and expression of interleukin-2 receptor (as shown by staining with anti-Tac antibody). Studies with monoclonal antibodies to CD4 and CD8 antigens showed that the proliferating lymphocytes were predominantly CD4-positive, although occasional CD8-positive cells were also seen.

Subpopulations of mononuclear cells

Proportions of T cells, B cells and macrophages in cytospin preparations of unfractionated MNC, fibronectin-adherent and non-adherent cells and MNC after panning with 3C10 was determined using monoclonal antibodies (Table 3).

Fibronectin adherence enriched for macrophages, whereas non-adherent cells and MNC after panning were depleted of these cells. There was no significant difference in the proportion of T cells and B cells in fibronectin non-adherent cells, MNC after panning and in unfractionated MNC.

In unfractionated MNC and fibronectin non-adherent cells, the proportion of cells not staining with antibodies to T cells, B cells and macrophages was about 9%. In fibronectin-adherent and 3C10-depleted cells, this proportion was 27% and 24%, respectively. The CD4/CD8 ratio in the latter two groups of cells was also lower and although the differences in the ratios were not statistically significant, the possibility that adherence to fibronectin and 3C10 depletion was affecting CD4 expression cannot be excluded.

Antigen-presenting activity of MNC isolated from normal and inflamed colon

Antigen-presenting activity of unfractionated MNC isolated from seven normal colons and seven IBD colons (six ulcerative colitis and one Crohn's colitis) was compared (Table 4). Although there appeared to be greater proliferation induced by MNC isolated from inflamed colons, this did not reach statistical significance.

In three colons with ulcerative colitis, with inflammation confined to the distal half of the colon, it was possible to study MNC isolated from inflamed as well as non-inflamed areas of the same colons (Fig. 3). In all three, significantly greater proliferation was induced by unfractionated MNC isolated from inflamed compared to those isolated from non-inflamed areas.

DISCUSSION

In this study, antigen-presenting activity of isolated human intestinal mononuclear cells was studied by their capacity to stimulate allogeneic, resting peripheral blood T cells in a mixed lymphocyte reaction. Cells with potent stimulatory activity have characteristics of adherence to fibronectin and expression of an antigen labelled by antibody 3C10 (CD14). This antibody has been shown to specifically identify human mononuclear phagocytes (Van Voorhis *et al.*, 1983b). It has been shown to label monocytes, alveolar macrophages and macrophages in tissue sections of spleen. Recently, we have shown that macrophages in normal and inflamed colon and terminal ileum are also labelled by this antibody (Mahida *et al.*, 1988).

Studies on human peripheral blood mononuclear cells have shown that dendritic cells are not labelled by the antibody 3C10 (Van Voorhis *et al.*, 1983a,b). Indeed, this antibody has been used (with complement) to deplete monocytes in order to enrich blood dendritic cells (Van Voorhis *et al.*, 1983a,b). Studies have also suggested that circulating monocytes contribute little, if at all, to accessory cell function. Thus when tested as stimulators of mixed lymphocyte reaction and of oxidative mitogenesis, monocyte-depleted peripheral blood mononuclear cells had normal or enhanced stimulatory capacity (Van Voorhis *et al.*, 1983b; Young & Steinman, 1988).

Our study shows that in isolated intestinal mononuclear cells, the stimulatory capacity (of a mixed lymphocyte reaction) is reduced by depletion of macrophages. When clusters of stimulatory intestinal cells and allogeneic T cells were examined, they contained cells with a dendritic morphology in close association with proliferating T cells. Formation of clusters with unprimed T cells has been shown to be a characteristic feature of dendritic cells (Inaba & Steinman, 1986). The cells with dendritic morphology, in our clusters, stained with macrophage-specific monoclonal antibodies 3C10, EMB11 and Y1/82A. Thus the intestinal cells with potent antigen-presenting activity have characteristics of both macrophages and dendritic cells. They are likely to be a distinct, probably complex, population of cells. These cells were also stained with the antibody RFD1, which has been shown to label peripheral blood dendritic cells (Knight *et al.*, 1987) and interdigitating cells of the T-cell zone in lymph nodes, but not monocytes (Poulter *et al.*, 1986). We have shown that intestinal macrophages are labelled by this antibody (Mahida *et al.*, 1988) and that these macrophages are able to phagocytose opsonized zymosan (Y.R. Mahida and D.P. Jewell unpublished observations). Studies on cells with dendritic morphology in T-cell areas of lymphoid tissue have also been shown to express macrophage markers (Hogg *et al.*, 1986). This suggests that the peripheral blood dendritic cells and lymph node interdigitating cells may have a common origin with intestinal macrophages. Studies on cells isolated from mouse intestinal lamina propria suggest that antigen-presenting activity resides in a population of cells that lack characteristics of macrophages (Pavli, Doe & Hume, 1987). Thus there may be species differences in the characteristics of intestinal antigen-presenting cells.

It is likely that subpopulations of intestinal macrophages are specialized to perform different functions. These could include antigen presentation, phagocytosis and secretion of a variety of products. The requirement of intestinal macrophages to act as potent antigen-presenting cells may be because of their pre-

dominant location just below the epithelium where they are likely to come in contact with a wide variety of penetrating luminal antigens and bacterial products.

Recently, there has been a great deal of interest in the capacity of epithelial cells to act as antigen-presenting cells (Bland & Warren, 1986; Mayer & Shilen, 1987). In the study on humans, intestinal epithelial cells were shown to be able to induce a proliferative response in allogeneic T cells. However, this response was lower than that induced by monocytes/dendritic cells (Mayer & Shilen, 1987). We do not believe that contaminating epithelial cells had any significant role in stimulation of allogeneic T cells in our experiments. Epithelial cells (obtained after EDTA treatment) subjected to the same treatment as the mononuclear cells (collagenase digestion, washing and centrifugation on Ficoll–Paque) had a viability of less than 10%. Subsequent culture for 2 hr, or procedures like panning or fibronectin adherence, reduced the viability to less than 1%.

In view of the predominant location of intestinal macrophages just below the epithelium and the results of our study, an investigation of the antigen-presenting activity of intestinal epithelial cells should vigorously exclude any contaminating macrophages.

The second part of this study compared the stimulatory capacity (in a mixed lymphocyte reaction) of intestinal mononuclear cells isolated from normal and inflamed mucosa (from patients with active ulcerative colitis and Crohn's disease). Although there appeared to be greater stimulatory capacity by cells from inflamed mucosa, the difference did not reach statistical significance. This may be because of the wide variation in responses in mixed lymphocyte reactions and the small numbers of specimens studied. In three colectomy specimens from patients with ulcerative colitis, mononuclear cells isolated from inflamed areas of the colon had greater stimulatory capacity compared with cells isolated from the non-inflamed areas of the same colon. Wilders *et al.* (1984) have demonstrated cells resembling veiled cells in mononuclear cell suspensions isolated from inflammatory bowel disease, but not normal, intestine. The increased antigen-presenting capacity in the inflamed mucosa may be required to cope with the increased penetration of luminal antigens, which may occur due to a breach in the epithelial barrier.

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