Immunology 1993 **78** 132–141

Dendritic cells, the major antigen-presenting cells of the human colonic lamina propria

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Accepted for publication 13 August 1992

SUMMARY

Induction of T-cell responses requires the recognition of antigen in association with class II major histocompatibility complex (MHC) antigens on specialized antigen-presenting cells. It was previously demonstrated that dendritic cells were the major antigen-presenting cell in the mouse intestinal lamina propria whilst macrophages were shown to be suppressive. The aim of this study was to compare the antigen-presenting cell activity of human colonic dendritic cells with macrophages. Colonic mucosa was removed from 46 specimens resected for cancer and other non-malignant conditions and lamina propria cell suspensions obtained by EDTA treatment followed by enzymatic digestion. Lamina propria cell suspensions, depleted of macrophages by adherence to insolubilized human immunoglobulin and carbonyl iron phagocytosis, were enriched for dendritic cells by density gradient centrifugation. Yields represented 0·9% (range 0·7–1·4%) of the starting cell number and the degree of enrichment was 30–50%. Immunocytochemistry demonstrated high levels of class II MHC antigen expression, but low levels or absent expression of macrophage and other markers. The ultrastructural features of the low-density cell fraction were typical of dendritic cells with cytoplasmic extensions or veils and the absence of phagocytic vesicles. Populations of cells enriched for macrophages were obtained by harvesting the human immunoglobulin-adherent cells. These cells were > 70% positive for macrophage markers using immunocytochemistry. The ability of lamina propria cells to induce primary T-cell activation was assayed using allogeneic peripheral blood T cells as responders in the mixed leucocyte reaction (MLR). When antigen-presenting activity was assessed using the MLR, the stimulatory activity was present in the dendritic cell-enriched fraction, with little activity present in the macrophage fraction. These data indicate that dendritic cells, not macrophages, are the major cell population capable of generating a mixed leucocyte reaction in the human colonic lamina propria.

INTRODUCTION

The intestinal mucosa is exposed to a vast array of ingested antigens, many of which can be detected in the lamina propria.[1–4] Once within the lamina propria, antigens may interact with many different components of the immune system but, for recognition by the T-cell antigen receptor, must be 'seen' in association with proteins coded by the major histocompatibility complex (MHC) on specialized antigen-presenting cells.

Cell types in the intestinal lamina propria that express class II MHC antigens, and might therefore act as antigen-presenting cells include dendritic cells,[5] macrophages,[6] B cells,[7] activated T cells,[8] endothelial cells[9] and epithelial cells.[10–13]. Dendritic cells from other sites are particularly potent antigen-presenting cells that initiate primary T-cell responses resulting in the generation of antibody responses and transplantation rejection.[5]

Two lines of evidence suggest that there are dendritic cells present in the intestinal lamina propria: the presence of a population of large irregularly shaped class II MHC-expressing cells which lack macrophage markers detectable at immunohistochemistry,[14–19] and the identification of veiled or non-lymphoid cells in thoracic duct lymph following mesenteric lymphadenectomy.[20–28] Subsequent experiments in the mouse[29] confirmed the presence of antigen-presenting dendritic cells in the Peyer's patches and lamina propria of the small intestine and in the colon. By contrast, intestinal macrophages were shown to

Abbreviations: CD, cluster of differentiation; CMF, calcium- and magnesium-free; CSF, colony-stimulating factor; DNase, deoxyribonuclease; EDTA, ethylenediaminetetraacetic acid; F(ab), F(ab) fraction of the immunoglobulin molecule; FCS, foetal calf serum (heat inactivated); GM-CSF, granulocyte–macrophage colony-stimulating factor; HBSS, Hank's balanced salt solution; HEPES, *N*-2-hydroxylpiperazine-N^1-2-ethane sulphonic acid; HLA, human leucocyte antigen; Ig, immunoglobulin; IL, interleukin; MHC, major histocompatibility complex; MLR, mixed leucocyte reaction; PBS, phosphate-buffered saline; RAM, rabbit anti-mouse immunoglobulin G; SE or SEM, standard error of the mean.

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inhibit the function of dendritic cells *in vitro*.[29] Other workers have also demonstrated that mucosa-associated macrophages have a suppressive effect on immune responses *in vitro* and *in vivo*.[30–34] These findings contrast with reports of the association of antigen-presenting cell activity with macrophages in the human colon.[35]

The aims of these experiments were to characterize the antigen-presenting cell of the human colonic lamina propria, with particular emphasis on clarifying the functional properties of dendritic cells and macrophages, and determining the cell responsible for the generation of primary (rather than secondary) T-cell responses.

This paper demonstrates that dendritic cells are the major antigen-presenting cells of the human colon as measured by the primary allogeneic mixed leucocyte reaction (MLR). By contrast, macrophages had very little activity—that activity which was present may have been due to the presence of small numbers of 'contaminating' dendritic cells.

MATERIALS AND METHODS

Mucosal specimens

Specimens of intestine were obtained from patients undergoing surgery for colorectal cancer (39 patients), Crohn's disease (2), ulcerative colitis (2) and non-malignant/non-inflammatory conditions (4), including diverticular disease (2) and ischaemic colitis (1). The results of histological examination of adjacent tissue were recorded. Mucosa was taken at least 5 cm from tumours. This study was approved by the Woden Valley Hospital Institutional Ethics Committee.

Disaggregation of colonic mucosa to produce single-cell suspensions

Full-thickness specimens of mucosa were obtained and transported to the laboratory in ice-cold Hank's balanced salt solution (HBSS) supplemented with penicillin (100 IU/ml) and gentamicin (50 mg/ml). Disaggregation of the tissue was initiated in all cases within 1 hr of resection. Tissue was disaggregated using a method based on modifications[36] of Bull and Bookman's original method.[37,38] Strips of mucosa (2–3 × 0·5 cm) were dissected free from the muscularis and incubated with continuous stirring in Wheaton flasks in calcium–magnesium-free HBSS (CMF-HBSS) containing 20 mM HEPES (pH 7·4), penicillin, gentamicin and 0·75 mM EDTA at 37° for 60 min. The tissue was washed in CMF-HBSS with EDTA for 30-min periods until there was no increase in particulate matter between washes (four to five washes). The strips of tissue were then washed once in CMF-HBSS without EDTA. The tissue was minced finely (2 × 2 mm) and incubated overnight with gentle stirring in RPMI-1640 (Flow Labs, Sydney, Australia) containing 10% heat-inactivated foetal calf serum (FCS) (CSL, Melbourne, Australia), 2 U/ml purified collagenase (CLSPA type, Worthington Biochemical Corp, Freehold, NJ), 5 U/ml DNase II (Calbiochem, San Diego, CA), 100 IU/ml penicillin, 50 mg/ml gentamicin, 100 U/ml nystatin, 20 mM HEPES and 2 mM glutamine. The undigested tissue fragments were then allowed to settle and the digest filtered through four layers of surgical gauze supported in a sterile Buchner funnel. The cells were washed (400 ***g***, 10 min at 4°) and resuspended in RPMI-1640 with 10% FCS. For isolation of lamina propria mononuclear cells and removal of red cells, neutrophils and debris, the cells were layered on to a Ficoll–Paque (Pharmacia Fine Chemicals AB, Uppsala, Sweden) density gradient (1·077 g/ml) and spun at 400 ***g*** for 30 min at 4°. Interface cells were harvested and washed twice. Viability was assessed by the exclusion of 0·1% Trypan blue. Cytospin slides were prepared using 1×10^5 cells in a Shandon cytocentrifuge (22·4 ***g***, 5 min) followed by fixation in methanol and staining in Diff-Quik (Lab Aids, Narrabeen, NSW).

Fibronectin- adherence and binding to human γ-globulin

The isolated lamina propria cell suspension at 5–10 × 10^6/ml was incubated for 2–3 hr on fibronectin-coated gelatinized flasks.[39] Human serum was allowed to clot at 37° and used as a source of fibronectin. Adherent cells were harvested after incubating them in 10 mM EDTA in RPMI containing 10% FCS for 10–15 min.

Bacteriological grade plastic Petri dishes were prepared with normal human γ-globulin (CSL) using a modification of the technique of Young and Steinman.[40] Pooled human γ-globulin (5 ml; 10 mg/ml) was added to 100-mm dishes for 30 min at 20°. The plates were washed and the lamina propria cell suspension added at a final concentration of 5–10 × 10^6/ml. After incubation at 37° for between 30 min and 4 hr, the non-adherent cells were removed and the adherence step was repeated for a further 30–60 min. Adherent cells were harvested by incubating them in 10 mM EDTA in RPMI containing 10% FCS for 10–15 min or by using a rubber policeman.

Carbonyl iron phagocytosis

The lamina propria cell suspension at 2–3 × 10^7/ml was added to 4 mg carbonyl iron powder (Sigma, St Louis, MO). The cells were incubated at 37° for 30 min with occasional mixing. The test-tube containing the cells was placed in a magnet (Dynal AS, Oslo, Norway) for 10 min at 4°. The cells in suspension were transferred to a second tube which was placed in the magnet for a further 10 min at 4°. The non-phagocytic cells were removed, washed and counted.

AET–sheep red cell rosetting of T lymphocytes

A 4% solution of 2-aminoethylisothiouronium bromide (AET) (pH 9·0) was mixed with packed sheep red blood cells (1–10 days old) in a 4:1 quotient (vol/vol) for 20 min at 37°. The mixture was resuspended at a final concentration of 4% sheep red cells and used fresh.

Colonic cell suspensions at a concentration of 10^7/ml were mixed with equal volumes of AET–sheep red cells at 4% and RPMI/FCS (final concentration 15%). The cells were centrifuged at 300 ***g*** at 20° for 10 min and left on ice for a minimum of 1 hr. The cells then were resuspended gently, layered over Ficoll–Paque and centrifuged at 600 ***g*** at 20° for 25 min. The cells at the interface (T-cell depleted) were used in subsequent experiments. When required, the rosetted T cells in the pellet were obtained after lysing the sheep red cells with 0·5 ml sterile water and washing in complete medium.

Monoclonal antibodies (mAb)

Monoclonal antibodies used for cell depletion, immunocytochemistry and flow cytometry included the culture supernatants of hybridoma cell lines listed in Table 1.

Table 1. Monoclonal antibodies used

Name	Determinant	Class	Reference
L243	Class II MHC	IgG2a	41
25F9	Mature macrophage	IgG1	42
OKT1	T cell	IgG1	43
OKT3	CD3/T cell	IgG2a	43
OKT4	CD4/T cell	IgG2b	43
OKT8	CD8/T cell	IgG2a	43
OKM1	CD11b/monocyte	IgG2b	43
Leu-M5	CD11c/monocyte	IgG2b	44
Leu-16	CD20/B cell	IgG1	45

All antibodies were derived from the mouse.

Immunocytochemistry

Cytocentrifuge slides were made using $2–10 \times 10^4$ cells in RPMI with 50% FCS on poly-L-lysine-coated glass slides (0·1% poly-L-lysine (Sigma) for 10 min then air dried).

For immunocytochemistry, cytocentrifuge slides were air dried overnight, and blocked with horse serum [4 drops in 10 ml phosphate-buffered saline (PBS)] for 30 min. Primary antibodies (Table 1) were added and incubated at 20° for 30 min. [To inactivate endogenous peroxidase activity, some slides were washed in PBS, dehydrated in increasing concentrations of ethanol in water (50%, 70%, 90%, 100%), treated with 0·1% (vol/vol) hydrogen peroxide in methanol for 10–30 min at 20°. These slides were then rehydrated before labelling with the second antibody.] The slides were washed in PBS then biotinylated sheep anti-mouse immunoglobulin (1/200) (Amersham International, Amersham, U.K.) was added for 30 min. After washing, avidin–biotin–peroxidase complex (Vector Labs, Burlingame, CA) was added for 1 hr and the slides developed for 10 min with 0·5 mg/ml 3,3′-diaminobenzidine (Sigma), 10 mM imidazole (BDH Chemicals, Poole, U.K.) and 0·3% hydrogen peroxide in PBS (pH 7·3). Appropriate positive and negative isotype controls were included. The slides were lightly counterstained in Mayer's haematoxylin (BDH), washed, air dried and mounted. Photography was performed using a Zeiss Axiophot Microscope (Zeiss, Camperdown, Australia).

Flow cytometry

Cells were suspended at a concentration of $2–10 \times 10^6$/ml in a solution containing the primary antibody for 30 min at 4°, washed, resuspended at a concentration of 2×10^7 in fluorescein isothiocyanate (FITC) conjugated affinity-purified sheep anti-mouse immunoglobulin (Silenus, Melbourne, Australia) at a dilution of 1:200 or 1:100 fluorescein-conjugated affinity-purified $F(ab')_2$ fragment goat anti-mouse IgG (heavy and light chain specific) (Cappel, Cooper Biomedical, Cochranville, PA), washed then analysed in a FACScan (Becton Dickinson, Mountain View, CA).

Electron microscopy

Cells were fixed in 2% glutaraldehyde in 0·1 M phosphate buffer, pH 7·4 for 2 hr and post-fixed in 1% osmium tetroxide in 0·1 M phosphate buffer, pH 7·4 for 90 min. For scanning electron microscopy, samples were then dehydrated, critical point dried and gold coated, with micrographs taken on a Hitachi 7000 scanning attachment (Mececo Holdings Pty Ltd, Sydney, Australia). For transmission electron microscopy, samples were 'en bloc' stained in 1% aqueous uranyl acetate for 1 hr, dehydrated and embedded in 'Spurs' resin. Micrographs were taken on a Hitachi 7000 electron microscope (Mececo Holding Pty Ltd).

Antigen-presenting cells assay—the MLR

Responder cells were derived from buffy coats prepared from normal blood donors (Red Cross Blood Transfusion Service, ACT, Australia). The buffy coat was diluted 1:2 with HBSS and underlaid with Ficoll–Paque. The cells were centrifuged at 600 *g* for 30 min at 4° and the interface harvested and washed. The cells were resuspended at $10–20 \times 10^6$/ml in the supernatant of the L243 hybridoma cell line for 30 min at 4°, washed and the antibody-labelled cells removed by panning[46] or complement lysis.

For panning, bacteriological grade 90-mm Petri dishes were pretreated with 5 ml 1/1000 affinity-purified rabbit anti-mouse immunoglobulin G (RAM) (Cappel, Cochranville, PA) and washed before the addition of cells which had been incubated with a saturating amount of mAb (L243). After two cycles of panning, the non-adherent cells were washed, counted and frozen immediately (see below). For complement lysis, complement (Pel-Freez, Rogers, AR or Cedarlane, Hornby, Ontario, Canada) was added at a final dilution of 1:5. Responder peripheral blood mononuclear cells were also prepared from buffy coats using the method of Warren.[47,48] Responder cells were frozen in RPMI containing 20% FCS and 10% DMSO (Malinckrodt, Paris, KY) and kept in liquid nitrogen until the day of use.

MLR cultures were performed in triplicate in round-bottom 96-well plates (Linbro Flow Labs, McLean, VA). A constant number of responder cells (generally 2×10^5/well) was incubated with varying numbers of irradiated stimulators (2500 rads) from the colon. The reaction was carried out in a total volume of 0·2 ml of medium. After 4–6 days, 1 μCi tritiated thymidine (Amersham, Surry Hills, NSW) was added to each well and the incubation continued for a further 16 hr. The plates were freeze thawed and the cells harvested automatically onto glass-fibre discs (Whatman, Maidstone, U.K.) using a cell harvester (Dynatech CH-103). Non-aqueous scintillant (0·5% 2,5-diphenyloxazole in xylene) (5 ml/vial) was added and the assays performed on a Packard Tri-Carb K60 counter.

Method for enrichment of colonic dendritic cells

The colonic cell suspension was incubated on human γ-globulin-coated plastic Petri dishes for 2–4 hr at 37° for two cycles. Non-adherent cells were removed, washed and treated with carbonyl iron (4 mg/10^7 cells) as described above. The remaining cells were incubated in plastic dishes at $5–10 \times 10^6$ cells/ml in RPMI containing 5% heat-inactivated pooled human AB serum overnight. The cells were removed, washed, resuspended at $5–10 \times 10^6$ cells/ml, underlaid with Nycodenz Monocytes (Nyegaard, Oslo, Norway) (density 1·068 g/ml) and centrifuged at 600 *g* for 20 min at 20°. The low density (dendritic cell enriched) cells and high density (T-cell enriched) cells were washed and counted.

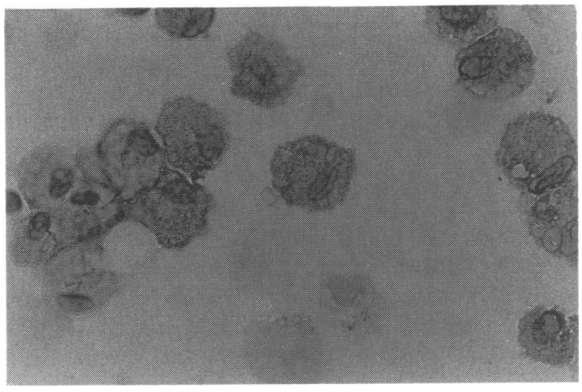

Figure 1. Immunocytochemistry of colonic macrophages. Cytocentrifuge slides of γ-globulin-adherent cells were air dried overnight and stained with L243 (anti-class II MHC) using the avidin–biotin peroxidase technique. Inactivation of endogenous peroxidase using 0·1% H_2O_2 in methanol was not performed (original magnification × 630).

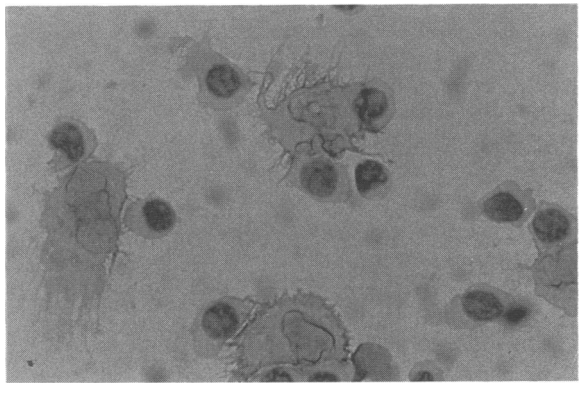

Figure 2. Immunocytochemistry of low-density, non-phagocytic, non-adherent colonic lamina propria cells. The technique was performed as described for Fig. 1. The mAb used was L243 (anti-class II MHC) (original magnification × 1000). Note the irregular nuclear morphology and the tendency to form clusters with lymphocytes.

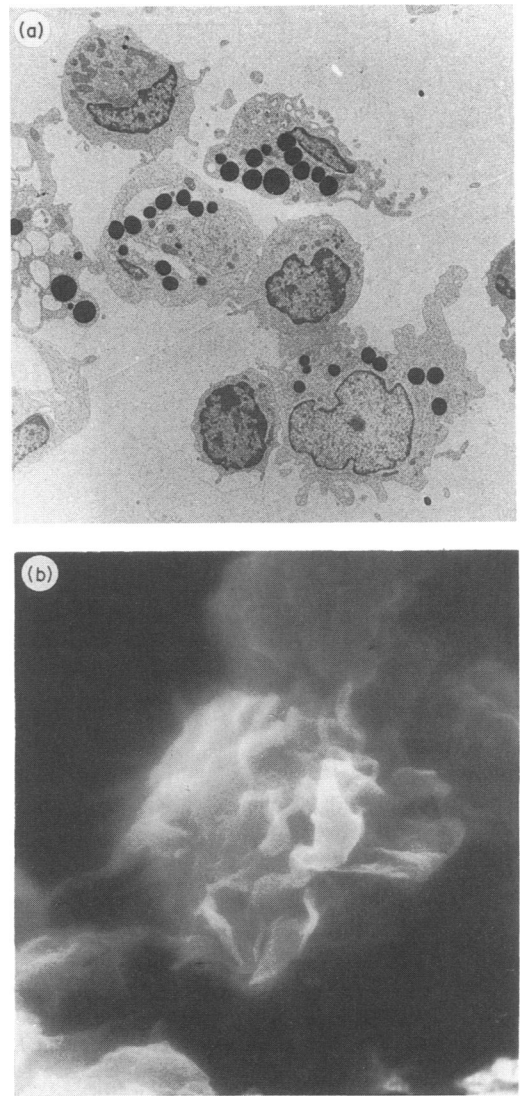

Figure 3. (a) Transmission electron micrograph of low-density cells prepared by density gradient centrifugation (Nycodenz Monocytes, density 1·068 g/ml) of a single-cell suspension of human colonic lamina propria cells (from which macrophages had not been removed). In the low-density fraction there are populations of both macrophages, which contain numerous presumed phagolysosomes (electron-dense and electron-lucent vesicles) of various sizes, and dendritic cells which are smaller, have an irregular nucleus with a peripheral rim of heterochromatin and do not contain phagolysosomes. Original magnification × 3600. (b) Scanning electron micrograph of a human colonic lamina propria dendritic cell demonstrating the characteristic cytoplasmic processes or veils. Original magnification × 8000.

An alternative method involved the treatment of the colonic cell suspensions with AET–sheep red cell rosettes to remove T cells before incubating on human γ-globulin-coated Petri dishes. The subsequent steps are as described above.

RESULTS

Cell yields and the development of the methods

Cell yields after Ficoll–Paque were $15 \pm 12 \times 10^6$/g tissue. Recovery represented 69 ± 15% of the number applied. The amount of tissue obtained from surgical specimens ranged from 5 to 21 g. The final yield of dendritic cell-enriched populations was always < 2% of the starting cell number.

Initial attempts to enrich for MLR stimulatory cells involved overnight adherence to fibronectin-coated gelatinized flasks and density gradient centrifugation. It was evident that this method did not allow the effective separation of human colonic macrophages from dendritic cells. Examination of cytospin preparations and electron micrographs showed that the low-density, non-adherent cells contained low, but significant (5–10%), numbers of macrophages. Similarly, the fibronectin-adherent cell fraction contained dendritic cells.

The technique described in Materials and Methods was monitored for its effectiveness in removing macrophages and enriching for dendritic cells by examining cytospin preparations and immunocytochemical slides (assessing the proportion of class II MHC$^+$ or 25F9$^+$ cells, see below). In later experiments, flow cytometry was also used.

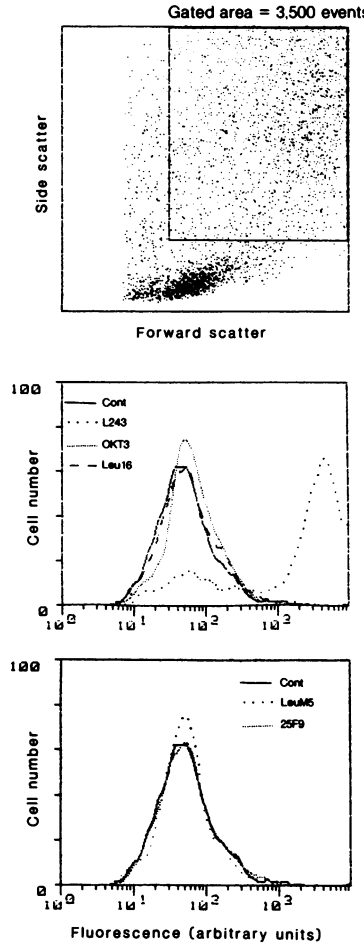

Figure 4. Flow cytometry of human colonic γ-globulin-adherent cells: analysis of a gated population of large cells. Adherent cells were incubated with no antibody (control) or the specified antibody, then washed and labelled with FITC-conjugated sheep anti-mouse IgG and analysed by flow cytometry (5000 events). The abscissae represent forward scatter (linear scale, arbitrary units) or fluorescence intensity (logarithmic scale, arbitrary units) as indicated. The ordinate axes represent side scatter (linear scale, arbitrary units) and cell number (linear scale, arbitrary units). The gated population comprised > 75% of the total numbers analysed. These cells expressed high levels of class II MHC antigens but did not express any other markers. These are the same cell populations as illustrated in Fig. 1.

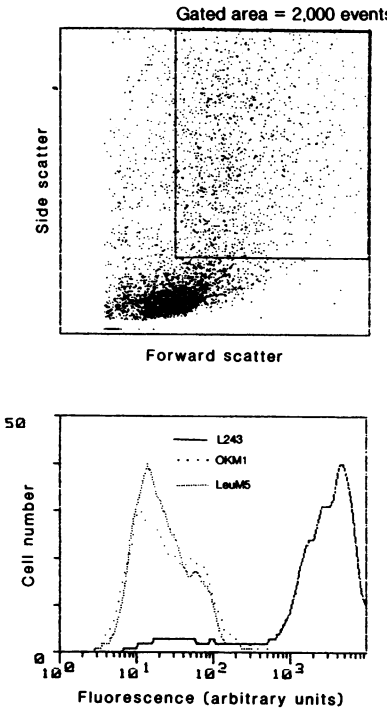

Figure 5. Flow cytometry of human colonic low-density non-adherent cells: analysis of a gated population of large cells. Low-density non-phagocytic non-adherent human colonic lamina propria cells were incubated with no antibody (control) or the specified antibody. Fluorescence labelling was performed as described in Fig. 3. The gated population comprised 40% of the total cell number analysed. All these cells expressed high levels of class II MHC antigens.

Effective removal of macrophages required at least two procedures. The use of human γ-globulin to immobilize the macrophages permitted their harvesting and use in subsequent experiments. Adherence to plastic did not remove as great a proportion of macrophages, whilst adherence to fibronectin resulted in populations of macrophages containing significant numbers of dendritic cells. Macrophages not adherent to γ-globulin-coated dishes were depleted by carbonyl iron phagocytosis and overnight plastic adherence.

Light and electron microscopy

Macrophages had a characteristic morphology—they were large cells with oval or round nuclei and basophilic cytoplasm which contained varying numbers and sizes of intracellular vesicles (Fig. 1). Dendritic cells were smaller and their nuclei were oval or pleiomorphic whilst the cytoplasm contained few, if any, inclusions (Fig. 2).

Transmission electron microscopy of macrophages showed numerous electron-dense and electron-lucent vesicles (Fig. 3a). Dendritic cells were smaller, had an irregular nucleus with a peripheral rim of heterochromatin and did not contain many vesicles (Fig. 3a). Scanning electron microscopy of dendritic cells demonstrated the characteristic cytoplasmic extensions or veils (Fig. 3b).

Immunocytochemistry

Adherent cells (macrophage enriched)

The majority (> 90%) of fibronectin- or plastic-adherent cells displaying the morphology of classical macrophages had detectable class II MHC antigens when studied by immunocytochemistry (Fig. 1). The mature macrophage marker, 25F9, also labelled virtually all the macrophages (not shown). The other macrophage markers used, OKM1 and Leu-M5, were either not detectable on intestinal macrophages or present on small numbers (< 5%) only. Immunohistochemical studies of small intestinal macrophages[29] and immunocytochemistry of populations of macrophages isolated from the colon[36] demonstrated similar findings.

Low-density non-phagocytic non-adherent cells (dendritic cell enriched)

Cells displaying dendritic cell morphology were labelled with the antibody to class II MHC antigens (Fig. 2). The mAb, Leu-

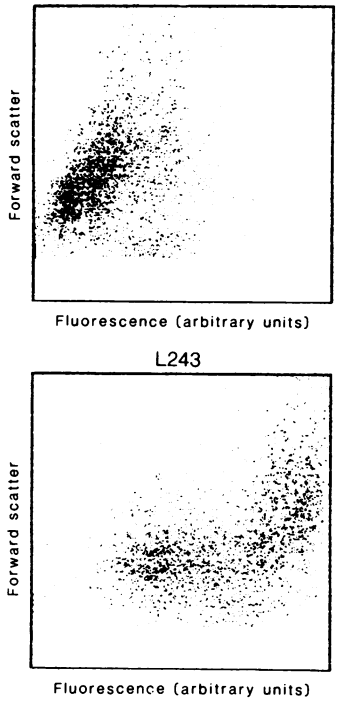

Figure 6. Flow cytometry of human colonic low-density non-adherent cells: dot plot of fluorescence against forward scatter (cell size). The experiment was performed as described in Fig. 4. The abscissae represent fluorescence intensity (logarithmic scale, arbitrary units) and the ordinate axes forward scatter (linear scale, arbitrary units). In the top graph, the cells were labelled with an isotype-matched control as described in Fig. 3, and 5000 events analysed. The bottom graph represents labelling with L243 (anti-class II MHC). The events with high fluorescence intensity spanned a wide range of sizes from very large (which may represent cell clusters) to a size approximately that of a lymphocyte. The smaller cells (lymphocyte size) expressed intermediate levels of L243 when compared to the isotype-matched control.

M5, directed against the p150,95 antigen (CD11c) weakly labelled dendritic cells in three of eight experiments in which it was used (not shown). None of the other antibodies used stained dendritic cells.

Flow cytometry

Adherent cells

When the human γ-globulin-adherent cells were analysed using forward and side scatter, two populations were demonstrated (Fig. 4). The larger cells expressed high levels of class II MHC antigens but did not have detectable monocyte/macrophage, T- or B-cell antigens. These cells had much higher levels of background fluorescence (non-specific primary and secondary antibody staining and autofluorescence) than the smaller cells (data not shown). The population of smaller cells consisted of mainly T and B cells and comprised $<25\%$ of the total cell number.

Low-density non-phagocytic non-adherent cells

The low density non-adherent non-phagocytic cells also consisted of two populations when analysed using forward and side scatter characteristics (Fig. 5). When fluorescence was plotted against size or forward scatter (Fig. 6), it was apparent that the highly fluorescent cells were spread over a wide size range. In addition, almost all of the remaining small cells expressed intermediate levels of class II MHC determinants.

Analysis of a gated population of large cells (Fig. 5) demonstrated high level expression of class II MHC antigens, with no detectable macrophage, T- or B-cell marker expression. This population comprised 40% of the total cell number. When the smaller population was analysed (data not shown), the cells expressed T-cell markers (CD3 and CD4) with intermediate and high levels of class II MHC antigens. Markers expressed on macrophages and B cells were undetectable or expressed only on small numbers of cells.

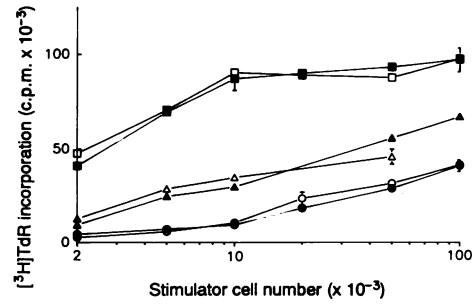

Figure 7. MLR stimulation: comparison of high- and low-density non-adherent intestinal lamina propria cells with fibronectin-adherent cells in the presence and absence of indomethacin. The abscissa represents increasing numbers of low-density (squares) and high-density (circles) non-adherent cells and fibronectin-adherent cells (triangles) that were incubated in the presence (closed symbols) and absence (open symbols) of indomethacin (2 μg/ml). Intestinal lamina propria cells were added to 2×10^5 peripheral blood cells from an unrelated donor. After 4 days, 1 μCi of [^{3}H]thymidine was added to each microculture and the cells freeze thawed and harvested onto glass-fibre discs after a further 16 hr. Unstimulated responder cells and control stimulator cells incorporated <100 c.p.m. The values are the means ± SEM of triplicate assays. The absolute yields of high-density, adherent and low-density cells in this experiment were 50×10^6, 2×10^6 and $2{\cdot}5 \times 10^6$ respectively. Assuming relative activities of 1:10:50 from the graph, this indicates that 26, 10 and 64% of the total activity was present in the respective fractions.

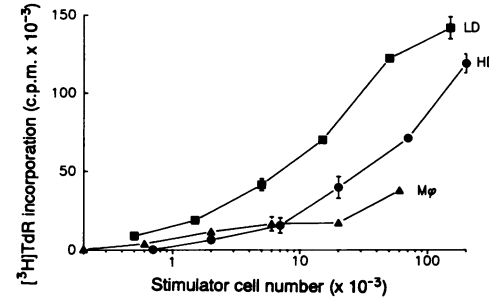

Figure 8. MLR stimulation: comparison of high- and low-density lamina propria cells with γ-globulin adherent cells (macrophages). The experiment was performed as described in Fig. 7. Increasing numbers of high-density (●), low-density (■) and γ-globulin adherent (▲) cells were added to 2×10^5 allogeneic peripheral blood cells obtained after panning with L243 (anti-class II MHC).

The assay system—the allogeneic MLR

The MLR was used as the assay system because of its ability to detect *primary* T-cell activation.[49,50] Gene transfer experiments have confirmed that the primary MLR is a valid and physiological model for studying primary T-cell activation. Studies of the interactions between antigen, class II MHC gene products and the clonotypic T-cell receptor demonstrate that the *same* T-cell receptor can recognize both (1) antigen complexed to self-MHC, and (2) allogeneic MHC molecules.[51,52]

MLR responder cells

The peripheral blood responder lymphocytes consisted of a homogenous population of T cells with a $CD4^+ : CD8^+ = 70:30$ (data not shown). There was no detectable expression of class II MHC antigens on the cells treated with L243 (anti-class II MHC) and either complement lysis or panning. Class II MHC-bearing cells were removed from the responder cell population because the presence of antigen-presenting cells in the responder cell population may result in cell proliferation, even in the absence of added allogeneic stimulatory cells.

MLR stimulation

In the initial experiments using fibronectin adherence, greater MLR stimulatory activity was always found in the low density, non-adherent cells (Fig. 7). There was, however, significant activity in the adherent fraction. This suggested either that there was significant contamination with dendritic cells (as suggested by their presence in cytospin and immunocytochemical slides) and/or that macrophages had MLR stimulatory activity. In order to differentiate between these two possibilities, the effect of adding macrophages to dendritic cell-enriched populations was studied and the activities of the separate populations was directly compared.

When added to dendritic cell-enriched populations, human colonic macrophages neither inhibited nor potentiated MLR stimulation, either in the presence or absence of indomethacin (data not shown).

The direct comparison of dendritic cell-enriched populations (30–50% dendritic cells, < 5% macrophages) with macrophage-enriched populations (> 70% macrophages), and high density cells (< 5% dendritic cells) (Fig. 8) showed greatly enhanced MLR stimulatory activity in the dendritic cell population. A small cross-contamination with dendritic cells can explain the weak activity of the macrophage-enriched population but the converse does not hold true.

DISCUSSION

Cells with the morphology of veiled cells were first described in the human intestinal lamina propria in tissue obtained from patients suffering from inflammatory bowel diseases.[53,54] These cells, which were neither phenotyped nor functionally characterized, may have been tissue dendritic cells. They were found in greater numbers in inflamed bowel than in normal controls. Similar findings were reported more recently; immunohistochemical studies suggested that in ulcerative colitis the invasion of the rectal mucosa with dendritic cells paralleled active inflammation.[55]

Dendritic cells have been obtained from human tissues including peripheral blood, synovial fluid, tonsils, thymus and the lung.[5] The isolation of dendritic cells in the human is associated with many of the problems encountered in the mouse (e.g. low cell numbers and the absence of specific cell surface markers), but additional problems are encountered using human tissues. For example, human dendritic cells are fibronectin adherent, at least in the short term,[56] so this property does not permit their separation from macrophages. In addition, the function and viability of human peripheral blood dendritic cells appears to be sensitive to the toxic effects of complement.[40] Colonic dendritic cell function was affected by complement so this method could not be used for enrichment. The purest populations of human dendritic cells reported to date are obtained by highly sophisticated techniques, including cell sorting by negative selection using a broad range of mAb.[57,58]

The separation of mucosal dendritic cells from intestinal macrophages is difficult because of their shared properties. These include fibronectin adherence (dendritic cells are weakly adherent), low density and the expression of class II MHC antigens (dendritic cells > macrophages). This distinction could be made, however, using several criteria. Firstly, macrophages have a characteristic morphology with oval or round nuclei and basophilic cytoplasm which contains varying numbers and sizes of presumed phagolysosomes. By contrast, the nuclei of dendritic cells were oval or pleiomorphic whilst the cytoplasm contained only occasional small vesicles. Secondly, when cytospin preparations were examined, the majority of the macrophages expresed the antigen labelled by the antibody, 25F9 (not shown). (This marker was not readily detectable at flow cytometry because it labels a predominantly intracellular, rather than a cell surface, antigen.[15]) The 25F9 antigen was not detectable on dendritic cells using either method. Thirdly, macrophages could be distinguished from dendritic cells by their electron microscopic features (Fig. 3a, b); and finally, dendritic cells were not adherent to human γ-globulin and did not take up colloidal iron.

Particular importance was placed on the effective depletion of mononuclear phagocytes from the colonic cell suspensions. This was because of the demonstration of a suppressive effect of mouse intestinal lamina propria macrophages,[29] and the observation that cell populations need to be depleted of monocytes to see the rapid development of large cell aggregates in the human MLR.[59] In addition, there is still some controversy about the antigen-presenting cell function of the human intestinal macrophage. Mahida *et al*[35] reported the association of antigen-presenting cell activity with intestinal macrophages. These cells were obtained using fibronectin adherence so it is likely that there was a significant proportion of 'contaminating' dendritic cells. Our results showed that low-density, fibronectin-adherent cells were indeed potent stimulators of the MLR (Fig. 7), but that there were, on morphological grounds, low, but functionally significant, numbers of dendritic cells. The addition of macrophage-enriched cells to a constant number of the low-density dendritic cell-enriched population, resulted in neither suppression nor enhancement of MLR stimulatory activity, either in the presence, or the absence, of indomethacin. These findings suggest that human intestinal macrophages play no role

in MLR stimulation in spite of their high level of class II MHC antigen expression. This observation contrasts with previous observations in the murine system where macrophages have a suppressive role.[29] This may represent a true species difference, but is more likely to be due to variations in the effects of the disaggregation processes on the function of the macrophages. When direct comparisons were made between macrophage-depleted, low-density cells (30–50% dendritic cells, generally $<5\%$ macrophages) and human γ-globulin-binding cells ($>70\%$ macrophages), markedly greater MLR stimulatory activity was associated with the macrophage-depleted dendritic cell-enriched population (Fig. 8). The levels of maximal proliferation and stimulator–response curves of the dendritic cell-enriched populations are comparable to those using populations of human tonsillar dendritic cells.[57]

Dendritic cells are extremely effective in antigen presentation suggesting that their endocytic apparatus is specialized to perform that particular task.[60] In contrast, the bulk of phagocytic activity in the macrophage appears to result in antigen degradation.[61] Suggestions that macrophages regurgitate partially digested antigens for presentation by dendritic cells or that antigens are derived from extracellular proteolysis are not supported by the current data.[62] The evidence shows that dendritic cells and macrophages subserve differing functions *in vitro*. The anatomical distribution of these cells *in vivo* is also consistent with different functions in the process of antigen handling [P. Pavli, manuscript in preparation].

Human colonic lamina propria dendritic cells had potent MLR stimulatory activity upon initial isolation (Fig. 8). These observations are consistent with those of Spalding *et al.*[63] who demonstrated that murine splenic and Peyer's patch dendritic cells had equivalent T-cell stimulatory ability in the oxidative mitogenesis assay and with our work on murine intestinal lamina propria dendritic cells.[29] In constrast, dendritic or Langerhans' cells of the epidermis are weak stimulators of the MLR upon initial isolation[64] but in the presence of granulocyte–macrophage colony-stimulating factor (GM-CSF) and interleukin-1, (IL-1), *in vitro* rapidly mature into potent MLR stimulators.[65,66] IL-1 also amplifies proliferative responses to limiting doses of dendritic cells.[67] Tissue variations in cytokine production may account for the differences in maturity of dendritic cells at different sites; both IL-1 and GM-CSF are produced constitutively by cells of the human intestinal lamina propria.[68] Alternatively, the functional maturation of intestinal dendritic cells into potent MLR stimulating cells may occur during the disaggregation process.

In summary, dendritic cells are the major stimulators of the MLR in the human colonic lamina propria, and have potent antigen-presenting cell activity upon initial isolation. Intestinal macrophages, in spite of expressing high levels of class II MHC antigens and evidence of avid phagocytosis, have no effect on MLR stimulation *in vitro*. Their role may be to degrade invasive antigens, rather than to recruit T-cell immune responses.

ACKNOWLEDGMENTS

P.P. was the recipient of the Gastroenterological Society of Australia Philip Bushell Senior Research Fellowship. We would like to thank Ms L. Maxwell who performed the electron microscopy, Dr H. Warren for the supply of reagents and for helpful discussions, and the surgeons of Royal Canberra and Woden Valley Hospitals who provided access to the tissues used.

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