Immunology 1990 **70** 40–47

Isolation and characterization of antigen-presenting dendritic cells from the mouse intestinal lamina propria

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Accepted for publication 1 February 1990

SUMMARY

A method was developed for the isolation of antigen-presenting dendritic cells, and macrophages, from mouse intestinal lamina propria and Peyer's patches. Peyer's patches, and the lamina propria of both the small and large intestine, contained cells with potent stimulatory activity in the allogeneic mixed leucocyte reaction. These cells were separated from macrophages by fibronectin adherence and further enriched by density centrifugation. The isolated stimulatory cells expressed high levels of class II major histocompatibility complex (MHC) antigens, and resembled splenic dendritic cells in both morphology and function. Macrophages were recovered from the lamina propria but not Peyer's patches. These cells also expressed class II MHC antigens, but failed to stimulate the mixed leucocyte reaction and, instead, induced indomethacin-sensitive suppression.

INTRODUCTION

T-lymphocyte activation requires the presentation of antigen to the responding cell in association with class II major histocompatibility (MHC) gene products (Ia antigens) on the surface of specialized antigen-presenting cells. The aims of this study were to isolate and characterize the antigen-presenting cells from the small intestinal and colonic mucosa. Cell types that express class II MHC antigens, and might therefore act as antigen-presenting cells (at least in a secondary immune response), include dendritic cells (Steinman *et al.*, 1986), macrophages (Unanue, 1984), B cells (Chesnut & Grey, 1985), activated T cells, endothelial cells (Nunez, Ball & Stastny, 1983) and epithelial cells (Bland & Warren, 1986; Mayer & Schlien, 1987). However, there is compelling evidence that in a primary T-cell mediated immune response, dendritic cells play a unique role (reviewed by Steinman *et al.*, 1986).

To generate a T-cell mediated immune response in the intestinal mucosa, antigen must cross the epithelial barrier. The major emphasis in studies of intestinal mucosal immunity has been focused on Peyer's patches and their associated specialized epithelium, which is believed to be the main site of antigen contact and sampling. A number of observations, however, suggest an important role for the non-Peyer's patch epithelium and associated lamina propria lymphoid tissue. Firstly, presumptive antigens have been demonstrated in the lamina propria or within lamina propria macrophages (reviewed by Nicklin, 1987). Secondly, immunohistochemical techniques have identified all of the putative antigen-presenting cells described above in the small intestinal lamina propria. In particular, there are large numbers of class II MHC antigen-bearing macrophages in the intestinal lamina propria of mouse, rat and human (Mayrhofer, Holt & Papadimitriou, 1986; Hume, Perry & Gordon, 1984; Hume, 1985; Janossy *et al.*, 1986; Spencer, MacDonald & Isaacson, 1987; Hume *et al.*, 1987) and some evidence has been presented concerning a population of large irregularly shaped antigen-presenting cells which lack macrophage markers (presumptive dendritic cells) (Mayrhofer, Pugh & Barclay, 1983; Hume *et al.*, 1984; Hume, 1985; Janossy *et al.*, 1986). Thirdly, the lamina propria contains T cells responsive to stimulation by the lectins, phytohaemagglutinin and concanavalin A, and by alloantigens in the mixed leucocyte reaction (MLR) (Mayrhofer, 1984). Finally, the removal of Peyer's patches in rats does not appear to affect normal intestinal antigen handling, in particular the induction of oral tolerance (Enders, Gottwald & Brendel, 1986). Thus, it was considered important to analyse antigen-presenting cell function in the lamina propria and Peyer's patches separately.

This paper describes a method for isolating murine intestinal lamina propria cells and the preliminary characterization of lamina propria macrophages and dendritic cells that have opposing effects on T-lymphocyte activation.

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MATERIALS AND METHODS

Mice

C57BL/6 and BALB/c mice were bred and maintained under specific pathogen-free conditions in the Animal Breeding Estab-

lishment of the John Curtin School of Medical Research, Australian National University, or were purchased from the Animal Production Area of the NCI-Frederick Cancer Research Facility, Frederick, MD and maintained specific pathogen-free at the Department of Cell Biology, M.D.Anderson Hospital and Tumor Institute, Houston, TX. Mice of both sexes aged between 8 and 16 weeks were used.

Media

Calcium- and magnesium-free Hank's balanced salt solution (CMF-HBSS), supplemented with penicillin (100 U/ml) and gentamycin (60 U/ml), was used for isolation of cells from the lamina propria. Where indicated, 0·75 mM EDTA (Fluka, Buchs, Switzerland) was added. RPMI-1640 was supplemented with 2 mM L-glutamine (Sigma, St Louis, MO), penicillin (100 U/ml), gentamycin (60 U/ml) and 10% heat-inactivated fetal calf serum (FCS). For cell culture experiments, 0·01 mM 2-mercaptoethanol was added.

Mouse tissues

The small intestine or colon was dissected from C57BL/6 mice. The luminal contents were expressed, and where indicated the Peyer's patches were removed under a dissecting microscope. The intestine was split lengthwise and then cut into 1-cm segments. After brief washing in CMF-HBSS to remove remaining luminal contents, the intestines were placed in a Wheaton stirring flask and washed gently for 2–2·5 hr in CMF-HBSS with EDTA at 37°. During this time, the medium was changed every 10–15 min until no increase in particulate matter was visible in the supernatant. Finally, after a 5-min wash in RPMI, the tissue was finely chopped, weighed and added to an enzyme cocktail containing 1·2 U/ml Dispase II (Boehringer-Mannheim, Tutzing, FRG), 10 U/ml collagenase CLSPA (Cooper Biomedical, Malvern, PA) and 5 U/ml DN'ase Type II (Calbiochem, Behring Diagnostics, La Jolla, CA) in glass Petri dishes siliconized with Coatasil (Ajax Chemicals, Sydney, NSW). Maximal yields were obtained when < 500 mg tissue were digested with 20 ml enzyme mixture. After 2–3 hr incubation at 37° in 5% CO_2 with occasional gentle agitation, the digested tissue was mechanically disrupted by passage through a fine mesh stainless steel sieve. The cell suspension was then passed through six layers of cotton gauze to remove any particulate matter and washed three times. If necessary, the cells could be pelleted in RPMI-1640 containing 20% FCS and kept overnight at 4° for use the next day without loss of antigen-presenting activity.

To obtain macrophages, the cell suspension at $5–10 \times 10^6$/ml was incubated for 2–3 hr on fibronectin-coated gelatinized flasks (Freundlich & Avdalovic, 1983). Human serum from healthy donors, clotted at 37° was used as a source of fibronectin. Non-adherent cells were discarded or pelleted, and the adherent cells further incubated overnight. Any cells which were no longer adherent after overnight incubation were removed by washing with RPMI at 37° and pooled with the other non-adherent cells. Adherent cells were harvested by incubating with 10 mM EDTA in RPMI with 10% FCS for 10–15 min. These cells were washed three times, then counted and viability assessed. Resident peritoneal cells were harvested by peritoneal lavage with CMF-HBSS and treated in parallel.

Splenic dendritic cells were obtained by a modification of a previously described technique (Steinman *et al.*, 1979). Spleens from C57BL/6 mice were minced by pushing through a fine wire grid, washed and resuspended in serum-free media. The cells were seeded at 3×10^6/ml in 185 cm^2 tissue flasks (Nunclon, Roskilde, Denmark) at 50 ml per flask, and allowed to adhere for 2 hr at 37°. Non-adherent cells were removed by washing with HBSS and remaining adherent cells recultured in complete medium [F15 (Gibco, Grand Island, NY), with 5% FCS and 10^{-5}M 2-mercaptoethanol] overnight. Non-adherent or loosely adherent cells were harvested by gentle pipetting and treated with anti-Thy-1.2 antibody (Serotec, Oxford, Oxon, U.K.) and rabbit complement (Low Tox, Cedarlane, Ontario, Canada) with DN'ase 30 mg/ml. Dead cells were removed by separation on Isopaque–Ficoll (Pharmacia, Uppsala, Sweden). To remove surface immunoglobulin- and F_c receptor-bearing cells, the cell suspension was then rosetted with sheep erythrocytes (John Curtin School of Medical Research) coupled by $CrCl_3$ to hyperimmune sheep anti-mouse immunoglobulin (John Curtin School of Medical Research). Following further separation on Ficoll, splenic dendritic cells were obtained. Yields from the spleen were 0·5–1% (Pereira, King & Blanden, 1986). The cells were $> 85\%$ positive for expression of class II MHC antigens by flow cytometry (not shown).

Mesenteric lymph node (MLN) cells to be used as responders were removed from BALB/c mice, pushed through a fine mesh stainless steel sieve, washed and incubated with a monoclonal antibody directed against murine class II MHC antigens (see below) for 30 min at 4°. After three washes, the antibody-labelled cells were removed by panning (Wysocki & Sato, 1978). Briefly, bacteriological grade 90-mm Petri dishes were pretreated with 5 ml 1/1000 rabbit anti-rat heavy and light chain IgG (RAR; Nordic, Tilburg, The Netherlands) in phosphate-buffered saline (PBS) for 45 min at 4°. After removing the unbound antibody, $20–30 \times 10^6$ MLN cells suspended in PBS/5% FCS were added. Plates were kept for 75 min at 4°, the non-adherent cells removed, centrifuged then reapplied to a second RAR-coated dish for another 75 min at 4°. The remaining non-adherent cells were removed, washed, counted and pelleted overnight or used in the MLR. In the initial experiments, Ia^+ cells were not removed.

Cell depletion experiments were performed using the 'panning' technique as described above. Antibodies used for cell depletion, immunocytochemistry and flow cytometry included the culture supernatants of hybridoma cell lines secreting monoclonal antibodies to: class II MHC (Ia) (TIB 120; Bhattacharya, Dirf & Springer, 1981); anti-dendritic cell antibody (33D1; Nussenzweig *et al.*, 1982); F4/80 antigen (F4/80; Austyn & Gordon, 1981; Hume *et al.*, 1983); Thy-1 (AT83; a gift from Dr R. Ceredig, John Curtin School of Medical Research); CD4 (GK-1.5; Dialynas *et al.*, 1983); CD8 (53–6.72; Ledbetter & Herzenberg, 1979); CD11b (M1/70; anti-C3bi receptor; Springer *et al.*, 1979); CDw32 (2.4G2; anti-Fc receptor; Unkeless, 1979); and CD44 (the phagocyte glycoprotein, Pgp-1; IM7; Hughes, Colombatti & August, 1983).

Mixed leucocyte reaction

Cultures were performed in round-bottomed 96-well plates (Linbro Flow Laboratories, McLean, VA). A constant number of responder MLN cells (2×10^5) was incubated with varying numbers of stimulator cells (from the intestine or spleen) that had previously been treated with mitomycin C (Sigma; 5×10^6 cells/ml were incubated with mitomycin C at a final concentra-

tion of 25 mg/ml for 45 min at 37°, then washed three times) or 3000 rads irradiation. The reaction was carried out in a total volume of 0·2 ml of medium (see above). After 4 days, 1 μCi [^{3}H]thymidine ([^{3}H]TdR; Amersham Australia, Surrey Hills, NSW or New England Nuclear, Boston, MA) 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.

Cell separation

Disaggregated lamina propria or Peyer's patch cells at concentrations of 10–20 × 10^6/ml were layered onto 2–3 ml Nycodenz (Nyegaard, Oslo, Norway) and centrifuged at 600 *g* for 15 min at 20°. Cells at the interface or in the pellet were washed and resuspended in RPMI for further experiments.

Immunocytochemistry

Cytocentrifuge slides were made using 2–10 × 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]. Specimens for cytology were stained using Diff-Quik (Lab Aids, Narrabeen, NSW). For immunocytochemistry, the cytocentrifuge slides were air-dried overnight, and then blocked with horse serum (four drops; Flow Labs, Woodcock Hill, Herts, U.K.) in 10 ml PBS for 30 min. Primary antibodies (see above) were added and incubated at 20° for 30 min. The slides were washed in PBS then biotinylated sheep anti-rat immunoglobulin (1/200; Amersham, Amersham, Bucks, 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, Dorset, U.K.) and 0·3% hydrogen peroxide in PBS (pH 7·3). The slides were lightly counterstained in Mayer's haematoxylin (BDH), washed, air-dried and mounted. Photography was performed using a Zeiss Axiophot Microscope.

Cytofluorometry

Cells were suspended at a concentration of 2–10 × 10^6/ml in the primary antibody for 30 min at 4°, washed, resuspended at a concentration of 2 × 10^7 in 1/100 fluorescein isothiocyanate-labelled sheep anti-rat immunoglobulin (Silenus, Melbourne) or 1/100 fluorescein-conjugated affinity-purified F(ab′)$_2$ fragment goat anti-rat IgG (heavy and light-chain specific; Cappel, Cooper Biomedical, Cochranville, PA), then analysed in a FACS IV flow cytometer (Becton-Dickinson, Sunnyvale, CA) or a FACScan (Becton-Dickinson, Mountain View, CA).

Electron microscopy

Cells were fixed in 3% glutaraldehyde/2% paraformaldehyde in 0·1 M cacodylate buffer (pH 7·4) and processed for scanning and transmission electron microscopy, as described by Bucana *et al.* (1983). The cells were examined with a JEOL 1200X scanning electron microscope or a JEOL 1200EX transmission electron microscope.

RESULTS

Recovery of lamina propria and MLN cells

Disaggregation of intact small intestinal mucosa resulted in yields of 17 ± 4 × 10^6 per mouse (mean ± SD, $n = 204$ mice) with 78 ± 16% viability as determined by trypan blue exclusion. Three to five per cent of the starting cell population was recovered following adherence to fibronectin. When the lamina propria and Peyer's patches were disaggregated separately, the average yield was 11·5 ± 4 × 10^6/mouse ($n = 56$) for Peyer's patches (< 0·1% fibronectin-adherent cells) and 7 ± 3 × 10^6/mouse ($n = 59$) for the lamina propria (10% fibronectin-adherent). The yield of cells harvested at each stage of the separation process is given in Table 1. The yield from the colon was 7 × 10^6 cells/mouse ($n = 18$) (10% fibronectin-adherent) and from mesenteric lymph nodes was 39 × 10^6/mouse. Following panning to remove cells expressing class II MHC antigens (TIB 120), the average yield of mesenteric node cells was 52 ± 7%. Cytofluorometric analysis of this MHC II-depleted population demonstrated a homogeneous population of T lymphocytes with CD4:CD8 = 70:30 (not shown).

Characteristics of mucosal cells stimulating the MLR

The expression of various surface markers on stimulator cells was assessed by panning. Panning alone, which depletes surface immunoglobulin-bearing cells because the second antibody used cross-reacts with mouse immunoglobulin, resulted in removal of 41 ± 6% of the mucosal cells; pretreatment with anti-Ia, 57 ± 14%. Other antibodies used in combinations (Lyt-2, L3T4, F4/80, Pgp-1 and anti-mouse immunoglobulin) resulted in removal of 76 ± 3%. Removal of Ia$^+$ cells resulted in a significant decrease in MLR stimulatory ability (Fig. 1), with a 90–95% reduction in peak MLR stimulation (10·9 versus 113·5 × 10^3 c.p.m. at maximal stimulator cell number). Treatment with other antibodies did not reduce the maximal MLR response. In general, removal of the T (Lyt-2, L3T4, Thy-1-positive) or B (surface Ig-positive) cells enriched for stimulator cells to the expected extent (given the proportion of lamina propria cells expressing T- or B-cell markers). Overnight plastic- or fibronectin-adherence decreased the proportion of macrophages to < 2% as judged by morphology. Removal of macrophages resulted in a significant increase in MLR stimulatory activity of the remaining non-adherent lamina propria cells (Fig. 2). These results suggest that the MLR stimulator cells in the intestinal lamina propria express class II MHC antigens but lack the characteristics of macrophages, B cells or T cells.

In each of 10 experiments, separated lamina propria cells

Table 1. Cell yields

	Yields % starting cell number (range)		
	Whole intestine	Lamina propria alone	Peyer's patches
Fibronectin adherent	3 (3–5)	10 (6–13)	< 0·1
Non-adherent	41 (32–57)	30 (22–44)	55 (22–90)
High density* (> 1·068 g/ml)	22 (15–31)	15 (9–20)	27 (12–37)
Low density (< 1·068 g/ml)	3 (2–6)	2 (1–3)	2 (1–3)

*The cells were predominantly small lymphocytes.

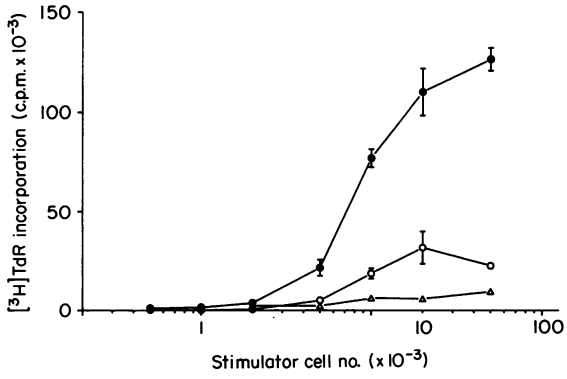

Figure 1. MLR stimulation by lamina propria cells. The abscissa represents increasing numbers of unfractionated small intestinal lamina propria cells (●) or lamina propria cells that were fibronectin-adherent (△) or depleted of cells expressing class II MHC antigens (Ia) by panning (○). Lamina propria cells from C57BL/6 mice were added to 2×10^5 mesenteric lymph node cells, depleted of Ia-bearing cells, from BALB/C mice. After 4 days, 1 μCi of [^{3}H]TdR was added to each microculture. After a further 16 hr incubation, the cells were freeze-thawed and harvested onto glass fibre discs. Unstimulated responder cells incorporated < 1000 c.p.m. The values are the means ± SEM of triplicate assays. All experiments were performed at least three times and representative results are illustrated.

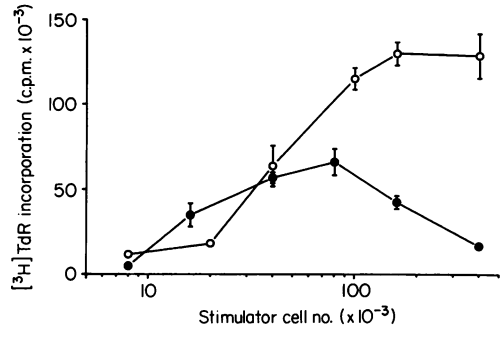

Figure 2. The effect of removal of cells adherent to fibronectin on MLR stimulation. The experiment was performed as described in Fig. 1. (●) Unfractionated small intestinal lamina propria cells; (○) non-adherent cells.

stimulated maximal MLR responses at 5–10-fold lower stimulator:responder ratios than Peyer's patch cells from the same animals (Fig. 3). At high stimulator:responder ratios, the lamina propria cells were suppressive.

Adherent cells as inhibitors of the MLR

Removal of cells adherent to fibronectin (macrophages) did not abrogate any stimulatory activity, but abolished the high-dose suppression (Fig. 2). Suppression by lamina propria adherent cells was also reversible by indomethacin (2 μg/ml; Fig. 4). The action of indomethacin was presumed to involve suppression of eicosanoid synthesis since prostaglandin E_2 (PGE_2) has been shown to inhibit lymphocyte activation (reviewed by Hume & Wiedemann, 1980). It was confirmed that addition of PGE_2 resulted in impairment of the MLR generated by both Peyer's patch and lamina propria cells (not shown).

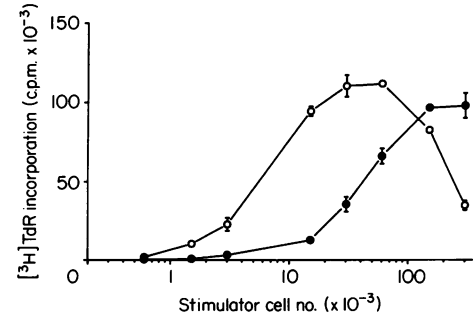

Figure 3. Comparison of MLR stimulation by Peyer's patch or lamina propria cells. The experiment was performed as described in Fig. 1. (○) Unfractionated small intestinal lamina propria cells; (●) unfractionated Peyer's patch cells.

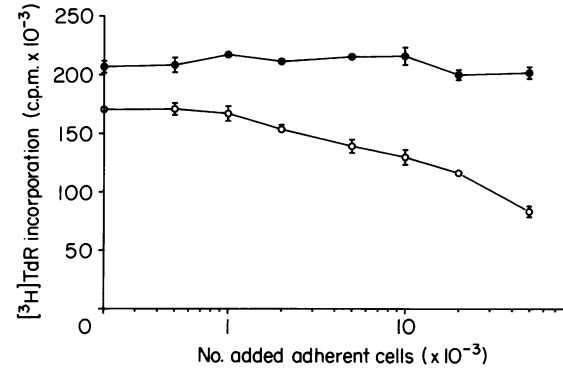

Figure 4. The effect of indomethacin on suppression of the MLR by lamina propria macrophages. The experiment was performed as described in Fig. 1. Increasing numbers of lamina propria macrophages (enriched by fibronectin adherence) were added in the presence (●) or absence (○) of indomethacin (2 μg/ml) to 1×10^5 non-adherent lamina propria cells and 2×10^5 purified mesenteric lymph node responder cells.

The majority of the adherent cells were classical macrophages morphologically, with numerous inclusions, basophilic cytoplasm and an oval nucleus (Fig. 5a). In contrast to peritoneal macrophages, they expressed very low levels of the macrophage-specific marker F4/80, 2.4G2 (the Fc receptor) and M1/70 (the C3bi receptor) (not shown). The low level of F4/80 antigen expression does not appear to be due to the enzymes used in the digestion, since peritoneal cell expression of F4/80 antigen was unaffected by the enzyme cocktail (not shown).

Since fibronectin-adherence did not deplete MLR stimulators from the lamina propria, it appeared unlikely that the adherent cells would act as stimulators. This was found to be the case (Fig. 1). In the presence of indomethacin, some stimulator activity was revealed (Fig. 6), but only at high stimulator:responder ratios. One potential reason for poor stimulatory activity could be the absence of class II MHC antigens. However, cytofluorometric analysis showed that the cells did express class II MHC antigens but at lower levels than dendritic cells (Fig. 7). The observed stimulation is probably attributable to a contaminating population of dendritic cells that was identified in small numbers in the adherent cell population.

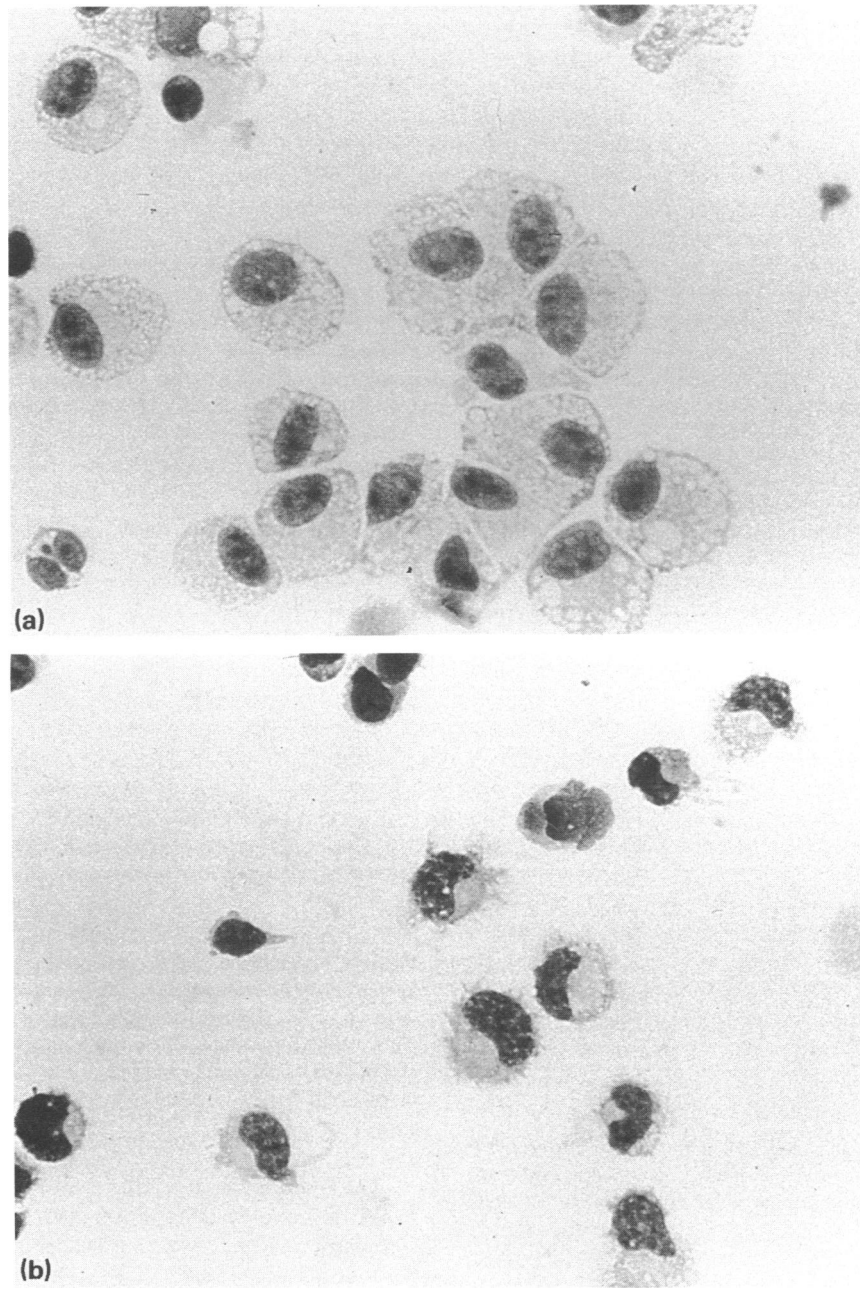

Figure 5. Cytocentrifuge slides of (a) intestinal lamina propria macrophages (×378); and (b) low density lamina propria cells (×378).

Enrichment of MLR stimulatory dendritic cells

The lamina propria and Peyer's patch cells that had been depleted of fibronectin-adherent macrophages were further enriched for MLR stimulatory activity by density gradient centrifugation. The yield of cells harvested at the Nycodenz interface (density < 1·068 g/ml) was 2·7% of the starting cell number from both sites (Table 1). Dendritic cells were smaller than intestinal macrophages and had oval, irregular or pleiomorphic nuclei and a basophilic cytoplasm (Fig. 5b). Characterization was best done by phase-contrast microscopy, immunofluorescence or by immunocytochemistry, where they were identified by their morphology and the expression of class II MHC antigens. Like splenic dendritic cells, they expressed very high levels of class II MHC proteins with low, but detectable, levels of F4/80, Pgp-1 and the dendritic cell-specific marker 33D1 (not shown). Other markers, in particular surface immunoglobulin, were negative (not shown). The majority of the remaining cells in the intestinal low-density fractions (20–35% of the total cell number at cytofluorometry) were Thy-1$^+$. At electron microscopy, the cells had the cytological features described previously for lymphoid dendritic cells. The nucleus was irregular with a peripheral rim of heterochromatin and small nucleoli. The cytoplasm contained well-developed mitochondria and scattered smooth vesicles but no evidence of phagolysosomes. The surface of the cells observed with the

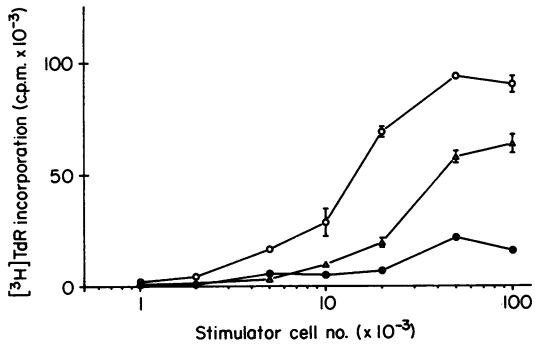

Figure 6. A comparison of MLR stimulatory activity of non-adherent and adherent lamina propria cells. The experiment was carried out as described in Fig. 1. Increasing numbers of non-adherent (to fibronectin; ○) or fibronectin-adherent lamina propria cells, with (△) or without (●) indomethacin (2 μg/ml) were added to purified mesenteric lymph node responder cells.

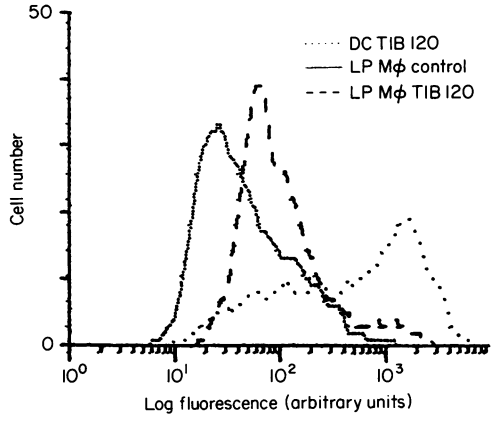

Figure 7. Flow cytometric analysis of class II MHC antigen expression by fibronectin-adherent cells (LP and Mϕ) low density lamina propria dendritic cells (DC). Fibronectin-adherent cells (intestinal lamina propria macrophages) and low density cells (dendritic cell-enriched) were incubated with no antibody (control) or TIB120 (anti-class II MHC), then washed and labelled with FITC-conjugated $F(ab')_2$ goat anti-rat IgG and analysed by flow cytometry. Intestinal lamina propria macrophages expressed much lower levels of class II MHC antigens when compared to lamina propria dendritic cells.

scanning electron microscope showed characteristic cytoplasmic processes often observed in cells from afferent lymphatics and referred to as veils (Knight, 1984).

These cells had 20–50 times greater MLR-stimulatory activity than the initial, unfractionated lamina propria cell suspensions (not shown). Isolated dendritic cells from the lamina propria or Peyer's patches were not distinguishable in terms of the number of cells required to induce a maximal MLR (not shown). The low-density cells from the lamina propria of either the small or large intestine were greatly enriched for MLR stimulatory activity (Fig. 8). The function of dendritic cell-enriched intestinal cell populations was compared with that of

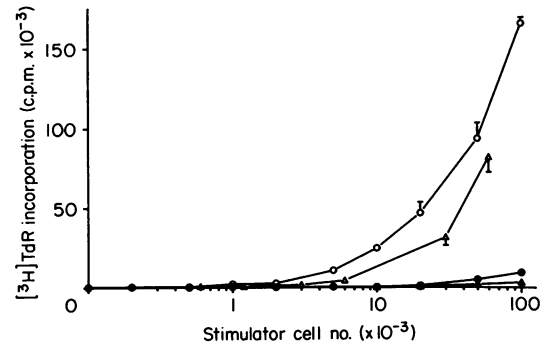

Figure 8. Fractionation of lamina propria dendritic cells from small or large intestine. Fibronectin non-adherent cells from the small intestinal (circles) or large intestinal (triangles) lamina propria were separated into low-density (< 1·068 g/ml, open symbols) or high-density (> 1·068 g/ml, closed symbols) fractions by centrifugation over a Nycodenz density gradient. Increasing numbers of cells were added to 2×10^5 purified mesenteric lymph node responder cells and the experiment was performed as described in Fig. 1.

splenic dendritic cells. MLR stimulatory activity was identical (not shown). When equal numbers of splenic and lamina propria dendritic cells were mixed together, there was no evidence of either enhanced or suppressed activity. Treatment of splenic dendritic cells with the enzyme cocktail used for disaggregation of the lamina propria for 2–3 hr did not alter their MLR stimulatory activity (not shown).

DISCUSSION

This study demonstrates the presence of MLR stimulatory cells in both the Peyer's patches, as described previously (Spalding *et al.*, 1983), and in the non-Peyer's patch lamina propria. These cells resemble the lymphoid dendritic cell first described by Steinman & Cohn (1973) in their surface phenotype (Ia^+, absence of typical macrophage, T-cell and B-cell markers), their physical properties (low density, weakly or non-adherent, non-phagocytic) and their cytological and ultrastructural features (absence of secondary lysosomes, pleiomorphic nucleus). The MLR stimulator cells differed further from macrophages in failing to adhere to fibronectin. Macrophages have a specific magnesium-dependent receptor which binds fibronectin (Bevilacqua *et al.*, 1981). This property allowed the separation and recovery of both cell types from the lamina propria and a comparison of their function. Intestinal macrophages could be differentiated from dendritic cells by assessing morphology, function (macrophages are phagocytic, adherent overnight in culture, and suppress MLR stimulation), and the expression of class II MHC antigens (dendritic cells express very high levels; Fig. 8).

The lamina propria contained relatively large numbers of Ia^+ macrophages (10% of disaggregated lamina propria cell suspensions). Consistent with immunohistochemical observations (Hume *et al.*, 1983, 1984; Witmer & Steinman, 1984), and with previously described yields from disaggregation experiments (MacDonald & Carter, 1982), a paucity of macrophages in the Peyer's patch lymphoid tissue was found. Isolated lamina propria macrophages did not express detectable F4/80 antigen, in contrast to their presumptive counterparts *in vivo* (Hume *et al.*, 1984). This may simply be a question of the sensitivity of the

avidin–biotin–immunoperoxidase method in comparison with immunofluorescence. An additional problem in assessing low-level expression of markers on these cells was high autofluorescence and non-specific binding of both first and second antibodies. In spite of the constitutive expression of Ia on lamina propria macrophages, these cells were weak stimulators of the MLR and were suppressive, in part, by an indomethacin-sensitive mechanism. Prostaglandin-producing macrophages have also been implicated in immunosuppression in the lung (Holt *et al.*, 1985). Other studies have demonstrated opposing actions of dendritic cells and macrophages on T-cell responses *in vivo*. For example, hapten-specific tolerance was observed when haptenated, Ia^+ macrophages were injected intravenously. This macrophage-induced tolerance could be overcome by subsequent injection of haptenated dendritic cells (Britz *et al.*, 1982).

Cells resembling dendritic cells have been isolated from afferent lymph draining the mesenteric nodes of the rat (MacPherson & Steer, 1980; Mason, Pugh & Webb, 1981; Pugh, MacPherson & Steer, 1983; Mayrhofer *et al.*, 1986) and other species. These so-called veiled cells, or non-lymphoid cells, express high levels of class II MHC antigens and are potent antigen-presenting cells *in vitro* and *in vivo* (MacPherson & Steer, 1980; Mason *et al.*, 1981; Lechler & Batchelor, 1982; Pugh *et al.*, 1983; Mayrhofer *et al.*, 1986). Like lamina propria dendritic cells, they lack most definable macrophage markers (MacPherson & Steer, 1980). Veiled cells in the rat are bone marrow-derived and have extremely high rates of turnover (Pugh *et al.*, 1983). If lamina propria dendritic cells are the precursors of similar cells in mouse afferent lymph, we would expect their turnover to be similarly rapid. This point has yet to be addressed.

There was consistently 5–10 times greater MLR stimulatory activity in the unfractionated lamina propria cell suspensions compared to Peyer's patches. However, the yield of dendritic cells from the two sources was similar (Table 1) and maximal MLR stimulation occurred with similar numbers of purified stimulator cells from both the lamina propria and Peyer's patches (not shown). In the unfractionated cell suspensions, the difference may represent a genuine decrease in Peyer's patch dendritic cell activity due to a deficiency of macrophage-derived factors. This possibility is supported by unpublished data (P. Pavli and D. A. Hume) showing that macrophages can increase the sensitivity of the MLR to Peyer's patch cells. This effect may be mediated by macrophage production of interleukin-1 (IL-1), which is not produced by dendritic cells (Koide & Steinman, 1987) but which amplifies dendritic cell function (Koide, Inaba & Steinman, 1987).

When compared to splenic dendritic cells, lamina propria dendritic cells had similar MLR stimulatory activity (not shown). These observations are consistent with those of Spalding *et al.* (1983) who demonstrated that splenic and Peyer's patch dendritic cells had an equivalent ability to stimulate T-cell proliferation in the oxidative mitogenesis assay. In contrast, dendritic or Langerhans' cells of the epidermis are weak stimulators of the MLR upon initial isolation (Inaba *et al.*, 1986), but in the presence of granulocyte–macrophage colony-stimulating factor (GM-CSF) and IL-1 *in vitro* rapidly mature into potent MLR stimulators (Witmer-Pack *et al.*, 1987; Heufler, Koch & Schuler, 1988). Tissue variations in basal levels of cytokine production may account for the differences in maturity of dendritic cells at these two sites. GM-CSF is produced constitutively by cells of the human intestinal lamina propria (W. E. Pullman, 1988), but levels have not been compared with those of the epidermis. An alternative explanation is that the isolation procedures resulted in different effects on dendritic cell maturation.

This study has demonstrated the presence of two populations of cells, with opposing effects on T-cell activation, in the intestine. Furthermore, these cells are present in definite compartments. The lamina propria contains a large population of macrophages, which may have a suppressive effect on T-cell activation, with small numbers of stimulatory dendritic cells. In contrast, the luminal antigen-sampling Peyer's patches contain dendritic cells with very few macrophages. The significance of these observations in relation to the control of intestinal immune responses and, in particular, the induction of oral tolerance, needs to be addressed.

ACKNOWLEDGMENTS

We wish to thank Drs William Allan and Rhod Ceredig of the John Curtin School of Medical Research, Mr Phillip Barron and Dr Hilary Warren of Woden Valley Hospital, Canberra and Drs Joann Trial, Cora Bucana and Kim Jessup of M.D.Anderson Hospital, Houston, TX for technical advice, supply of reagents and helpful discussion. We also thank Mr Mike Dennis for the electron microscopy and Mrs M. Schofield for secretarial assistance. We thank Professor I. J. Fidler for his encouragement and support of the work carried out in the Department of Cell Biology, M.D. Anderson Hospital, and Tumor Institute, Houston, TX.

This work was supported, in part, by the National Health and Medical Research Council of Australia and, in part, by the Amoco Foundation. P. Pavli is currently the Gastroenterological Society of Australia Philip Bushell Senior Research fellow.

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