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Migration of human intestinal lamina propria lymphocytes, macrophages and eosinophils following the loss of surface epithelial cells

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SUMMARY

Lymphocytes and macrophages are present in the normal intestinal lamina propria, separated from the epithelial monolayer by the basement membrane. There is evidence for movement of mononuclear cells through the lamina propria, entering from the systemic circulation and exiting via lymphatic channels. The goal of our studies was to investigate the capacity of cells to migrate out from the lamina propria into the lumen following the loss of surface epithelial cells. An *in vitro* model was therefore established in which normal human intestinal mucosal samples, denuded of the surface epithelium, were maintained in culture. Electron microscopy showed that during culture, large numbers ($>2\times10^6$/g tissue per 24 h) of cells migrated out of the lamina propria via discrete 'tunnels' which were in continuity with pores (diameter $<4\,\mu$m) in the basement membrane. The emigrating cells were T cells ($68{\cdot}5\pm5{\cdot}1\%$), macrophages ($10{\cdot}5\pm1{\cdot}3\%$) and eosinophils ($7{\cdot}1\pm1{\cdot}3\%$). Our studies have therefore demonstrated, for the first time, the capacity for large numbers of lymphocytes, macrophages and eosinophils to migrate out of the lamina propria, via basement membrane pores. We postulate that such emigration of cells occurs *in vivo* following the loss of surface epithelial cells due to injury, and could represent an important form of host defence against luminal microorganisms and also facilitate wound repair by enhancing restitution by neighbouring epithelial cells, via peptide factors.

Keywords basement membrane lymphocytes macrophages eosinophils intestine

INTRODUCTION

The intestinal mucosa comprises a number of different cell types which are located in the distinct compartments of the muscularis mucosa, the lamina propria and the surface epithelium [1]. Most mucosal T and B cells arise from gut-associated lymphoid tissue (GALT), predominantly Peyer's patches [2]. Lymphocytes primed by antigens in GALT migrate through the lymphatics and mesenteric lymph nodes before entering the systemic circulation. Following specific interactions with endothelial cells, the lymphocytes subsequently migrate into the lamina propria throughout the gastrointestinal tract (as well as other mucosal surfaces). Furthermore, there is evidence for movement of cells within the mucosa after migrating out of the vasculature. Thus intraepithelial lymphocytes migrate from the lamina propria into the epithelial layer and *vice versa* [3], although the pathways used by these cells have remained obscure. Mononuclear cells also emigrate from the mucosa via the lymphatic channels [4]. There is therefore considerable movement of cells into and out of the lamina propria, but it is not known if specific pathways in the extracellular matrix are used for these movements. In addition, there is little information on the ability, and pathways taken by lamina propria cells to migrate into the lumen.

In this study, we demonstrate the migration of large numbers of cells out of the lamina propria of the normal human small and large intestine, following loss of the surface epithelium. This migration occurs via tracts in the extracellular matrix which end as discrete pores in the basement membrane.

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MATERIALS AND METHODS

Resected mucosal tissue

Fresh normal mucosal samples were obtained from human colon ($n=20$) and terminal ileum ($n=2$; right hemicolectomies) resected for tumour. The normal mucosal samples were obtained at least 5 cm away from the tumour and were used to study cell migration after removal of the epithelium.

Mucosal strips were dissected from the submucosa and, after weighing, epithelial cells were detached from the basement membrane using a previously described technique [5]. In brief, mucosal strips were weighed and incubated with 1 mmol/*l* dithiothreitol (Sigma Chemical Co., St Louis, MO) for 15 min at room temperature. Epithelial cells were detached by three 30-min incubations in 1 mmol/*l* ethylenediaminetetraacetic acid (EDTA; Sigma) at 37°C. Between each incubation with EDTA, the mucosal samples were washed with calcium- and magnesium-free Hanks' balanced salt solution (HBSS; GIBCO BRL, Gaithersburg, MD). The mucosal samples, completely denuded of epithelial cells, were subsequently cultured at 37°C in RPMI containing 10% fetal calf serum (FCS; GIBCO) in 60 mm tissue culture dishes (Costar Corp., Cambridge, MA). During culture, cells appeared in suspension (as well as some adherent to the bottom of tissue culture dishes). These cells were collected after each 24-h period by transferring the pieces of mucosal tissue to culture dishes containing fresh medium and incubating the original dishes (containing cells only) at 4°C for 1 h. Following vigorous pipetting to detach adherent cells, isolated cells were counted and viability assessed by their ability to exclude trypan blue (used at a final concentration of 4 μg/ml; Sigma).

Cytospin preparations ($\approx$50 000 cells/slide) were subsequently made, fixed with acetone and stored at -70°C until used for immunohistochemistry. The cells in suspension were also analysed by fluorescein-activated cell sorter (FACS; see below) and for their capacity to proliferate in response to mitogens (see below).

All buffers and media used for isolation of epithelial cells, culture of denuded mucosa and organ culture studies (see below) contained 100 U/ml penicillin G (Britannia Pharmaceuticals Ltd, Redhill, UK), 5 μg/ml gentamicin (Roussel Labs, Uxbridge, UK) and 0·1 mg/ml streptomycin (Evans Medical Ltd, Leatherhead, UK).

Organ culture of colonic biopsies

Nine colonic biopsy specimens were obtained from three patients undergoing colonoscopy, on the same day, for surveillance for polyps. After informed consent, biopsies were taken from the sigmoid region for organ culture studies. Routine histological examination of additional biopsies from the same region were all confirmed to be normal. These studies were approved by the Ethics Committee of Queen's Medical Centre, Nottingham, UK.

Organ cultures were performed as described previously [6]. The colonic biopsies were incubated in RPMI containing 10% FCS at 37°C in a sealed chamber following equilibration with 95% oxygen/5% CO_2. At the end of the 24 h culture period, supernatant from each biopsy was collected, pooled, and the number of cells counted. Cytospin preparations were also made as described above.

Immunohistochemistry

Cytospin preparations were stained using the Vectastain ABC-peroxidase kit (Vector Labs, Burlingame, CA) and the mouse MoAbs Y1/82A (CD68; gift from Professor D. Y. Mason, John Radcliffe Hospital, Oxford, UK), RFT11 (CD2), RFB7 (CD20; obtained from the Royal Free Hospital, London, UK) and anti-CD14 antibody (from SeraLab, Oxford, UK). After incubation with the primary antibody, biotinylated goat anti-mouse IgG was applied followed by avidin–biotinylated horseradish peroxidase (HRP) complex. Peroxidase activity in the latter was developed with diaminobenzidine. Endogenous peroxidase activity within the cells had previously been blocked (using H_2O_2 and methanol) in those cytospin preparations that were incubated with MoAbs.

The proportion of positively stained cells in each cytospin preparation was determined by analysing at least 200 cells. Cytospins were also stained with toluidine blue to determine the proportion of eosinophils present.

FACS analysis

Peripheral blood (obtained from five healthy individuals; mean age 53 years) was used to study circulating lymphocytes using labelled MoAbs and FACS analysis as described previously [7]. Lamina propria cells in suspension were studied at a concentration of 1×10^6/ml (in 10% FCS/RPMI).

After incubation with mouse serum (final dilution 1:100) at 4°C for 30 min, 100 μl of cell suspension (10^5 cells) were incubated with 5 μl of labelled mouse MoAb solutions for 30 min, on ice, in the dark. The MoAbs, conjugated with FITC or PE, for FACS analysis were used in pairs or individually (see below). The cells were subsequently washed twice with PBS pH 7·0 containing 0·1% sodium azide before fixing with FACS Fix (0·5% formaldehyde in sheath fluid (6·38 mmol/*l* NaCl, 0·5 mmol/*l* sodium tetraborate, 16·2 mmol/*l* boric acid and 0·5 mmol/*l* EDTA; from Sysmex, Hamburg, Germany)). Analysis by two-colour flow cytometry was performed using FACScan (Becton Dickinson Immunocytometry Systems, Mountain View, CA).

The antibody pairs Hle-1/Leu-M3 (CD45/CD14), Leu-4/Leu-12 (CD3/CD19), Leu-4/Leu-3a (CD3/CD4), Leu-4/Leu-2a (CD3/CD8), Leu-3a/Leu-2a (CD4/CD8) were obtained from Becton Dickinson. Individual FITC- or PE-labelled MoAbs used were CD25, CD4, CD8, CD18, CD45RO, CD45RA (from Dako Ltd, High Wycombe, UK), CD11a and CD49d (from Immunotech, Marseilles, France).

Lymphocyte proliferation

Cells obtained following culture of denuded mucosa were centrifuged (at 400 ***g***) and resuspended in fresh 10% FCS/RPMI to a final concentration of $0{\cdot}5\times10^6$/ml. Proliferation assays were performed in 96-well microtitre plates (Nunc A/S, Roskilde, Denmark) using 200 μl (10^5 cells) of the cell suspension per well. Mitogens used were phytohaemagglutinin (PHA; Sigma), pokeweed mitogen (PWM; Sigma) and concanavalin A (Con A; Sigma). PHA and PWM were used at final concentrations of 1 μg/ml and 10 μg/ml and Con A at 2·5 μg/ml and 25 μg/ml. Assays were performed in quadruplicate and cells cultured for a total of 72 h, the last 18 h in the presence of ^{3}H-thymidine (0·8 μCi/well; Amersham International plc, Aylesbury, UK). Cells were harvested using Filtermate Packard Cell Harvester (Packard, Pangbourne, UK) and uptake of ^{3}H-thymidine determined using Top Count Microplate Scintillation Counter (Packard).

Electron microscopy

Samples of denuded intestinal mucosa were obtained for scanning and transmission electron microscopy (SEM and TEM, respectively), soon after removal of epithelial cells and also following culture for varying periods of time.

Denuded mucosal tissue samples were fixed in 2·5% gluteraldehyde (in 0·1 M cacodylate buffer pH 7·4) and processed according to standard procedures [8]. A Jeol 1200 EX transmission electron microscope and a Joel-35 scanning electron microscope (Joel, Welwyn Garden City, UK) were used. The diameter of pores present in the basement membrane of denuded mucosal samples were measured from SEM images using Kontron Videopran Image

analysis equipment (Contron Elektronik, Watford, UK).

Statistical analysis

Data are expressed as mean (± s.e.m.) and were analysed by one way analysis of variance and paired or unpaired *t*-tests, as appropriate.

RESULTS

Studies with mucosa denuded of epithelial cells

Following treatment with 1 mmol/*l* EDTA, all the epithelial cells were consistently removed to leave an intact lamina propria and muscularis mucosa (Fig. 1). Studies by SEM showed the presence of numerous discrete pores in the basement membrane (Fig. 2). These pores were present in denuded mucosa of the colon and the terminal ileum, where they were distributed over the villus surface as well as the crypt region. Their diameter varied from 0·2 to 2·8 μm in the terminal ileum and 0·2 to 3·3 μm in the colon, and many of the pores appeared to be in continuity with tunnels in the lamina propria.

SEM studies of denuded mucosa after culture

SEM studies of the denuded mucosal tissue following culture over varying time periods showed numerous cells migrating through the pores in the basement membrane (Fig. 3a,b). Higher power views showed cells at various stages of migration, initially extending processes before squeezing through the discrete orifices in the basement membrane (Fig. 4a–c).

Cells continued to migrate out of the denuded mucosa in cultures for 8 days, but this migration was predominant over the first 3 days. After this period, 'empty' pores were often seen by SEM. Despite culture of denuded colonic mucosa for 8 days, the discrete pores in the basement membrane remained intact (pore diameter after culture for 8 days: 0·88–7·1 μm).

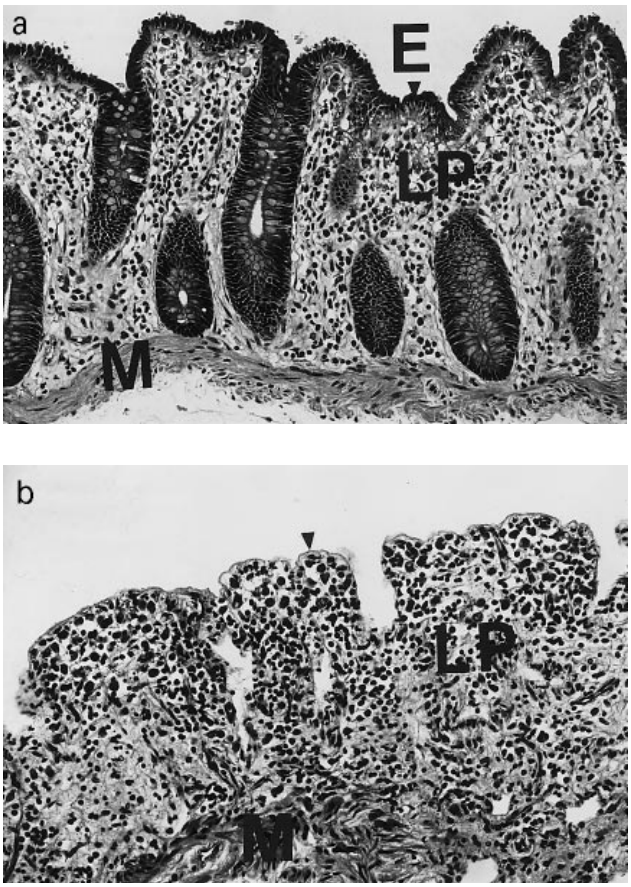


Fig. 1. Photomicrographs of haematoxylin and eosin-stained sections of normal colonic mucosa before (a) and after (b) treatment with EDTA. In (b) the epithelial cells have been removed to leave an intact lamina propria (LP) and muscularis mucosa (M). A basement membrane (arrow) is present on the superficial surface of the lamina propria.

TEM studies of denuded mucosa after culture

Studies by TEM showed that cells of different phenotype (lymphocytes, macrophages and eosinophils) were 'lining up' in the lamina propria matrix before migrating out through the basement membrane pores. To migrate into the medium, cells squeezed through the pores in the basement membrane (Fig. 5).

The presence of discrete pathways ('tunnels') in the lamina propria matrix was evident after cells had migrated out into the medium to leave empty tracts (Fig. 6a,b). Such empty tunnels were seen on numerous occasions, especially after prolonged culture of the mucosa.

In studies on all the mucosal tissue samples following culture for 1–8 days, lamina propria cells were only seen to migrate out over the surface through the discrete pores in the basement membrane, and not from the cut margins or the undersurface of the mucosa.

Characterization of cells migrating out of denuded normal colonic lamina propria

Studies by phase contrast microscopy showed that following culture of denuded normal colonic and ileal mucosa, cells began to appear in suspension. They were seen within the first hour of culture and progressively increased in number. At 24 h, $2{\cdot}7 \pm 0{\cdot}3 \times 10^6$ cells/g tissue (mean ± s.e.m.) (viability 93 ± 3·3%) were present in suspension. Following re-culture of the denuded mucosal samples in fresh medium (after removal of the cells in suspension) cells continued to migrate out of the lamina propria; cells/gram tissue: 24–48 h period of culture, $2{\cdot}3 \pm 0{\cdot}3 \times 10^6$ (viability 85 ± 4·2%); 48–72 h period of culture, $2{\cdot}6 \pm 0{\cdot}5 \times 10^6$ (viability 83 ± 3·8%).

Thus over the 72-h period of culture, a total of more than 6×10^6 cells/g tissue had migrated out of the lamina propria into the medium. The numbers of cells obtained during culture of the two ileal specimens were (per gram of tissue): 0–24 h, −5 and $4{\cdot}5 \times 10^6$ cells; 24–48 h, 2·8 and $1{\cdot}2 \times 10^6$ cells; and 48–72 h, 0·7 and $0{\cdot}6 \times 10^6$ cells, respectively.

Phenotypic studies

The phenotype of the cells that had migrated out of the normal colonic lamina propria and into the medium was evaluated using cytospin preparations and FACS. Cytospin preparations of cells were analysed after immunohistochemical staining using MoAbs specific for different cell types (Table 1). These studies showed that the cells migrating out of the lamina propria into the medium were predominantly T cells. The proportion of B cells in suspension was significantly lower in 0–24 h mucosal cultures (3·2 ± 1·6%) compared with 24–48 h cultures (8·3 ± 2·4%; $P < 0{\cdot}01$).

Lymphocytes migrating out from the lamina propria were also

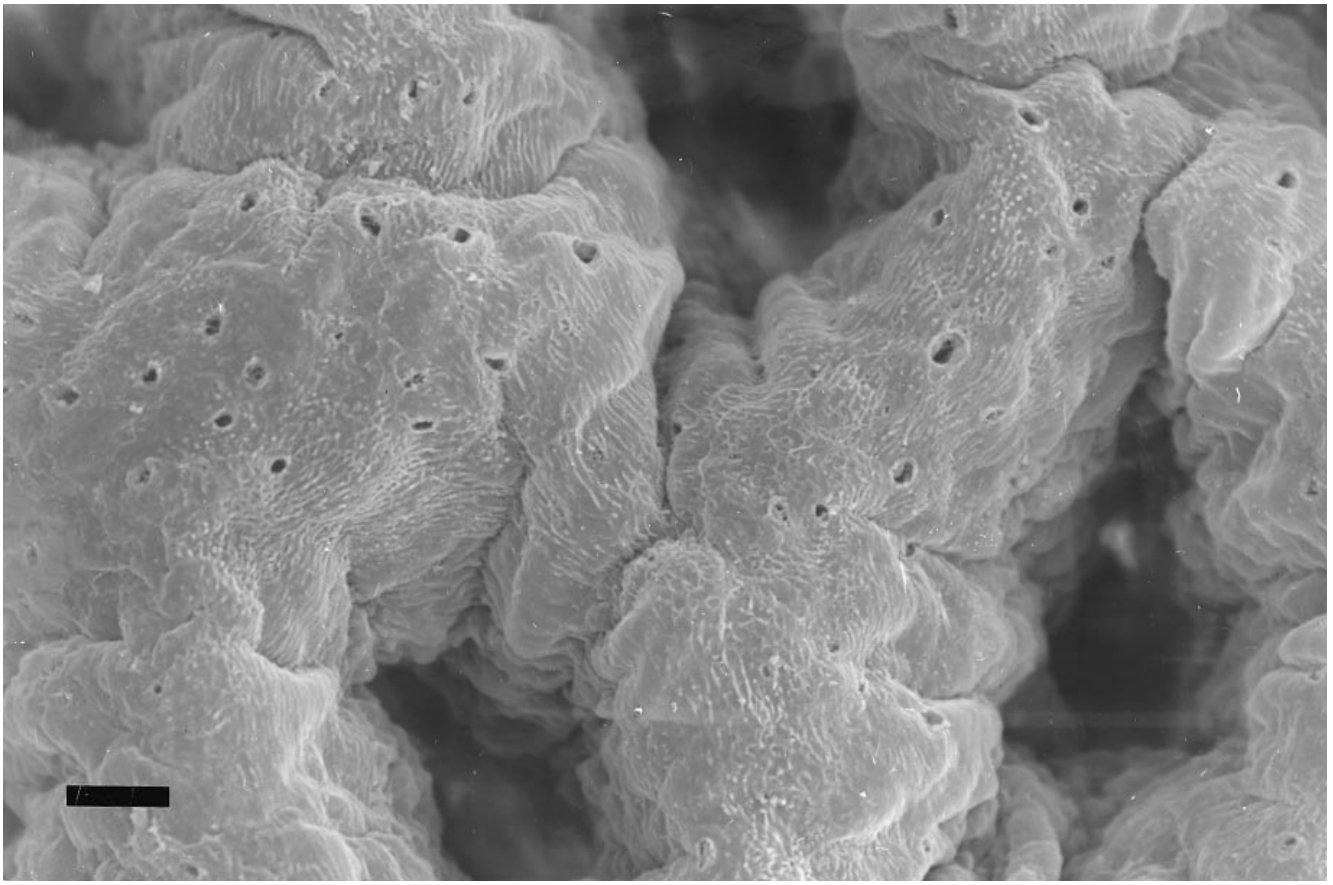


Fig. 2. Scanning electron micrograph of normal colonic mucosa, following removal of epithelial cells. The tissue sample was fixed immediately after detachment of epithelial cells with EDTA. Discrete, uniformly distributed pores are present in the basement membrane. The diameter of the pores varied from 0·2 to 3·3 μm in the colon. Bar = 10 μm.

characterized by FACS analysis and compared with peripheral blood lymphocytes. Lamina propria cells obtained from six subjects during the 0–24 h and 24–48 h culture periods and circulating lymphocytes from five controls were analysed after applying a similar gate.

T cells migrating out of the lamina propria were found to be predominantly $CD4^+$. Paired analyses showed a significant increase in the proportion of B cells ($CD19^+$, 14·2 ± 5·0% *versus* 3·7 ± 1·1%; $P < 0·05$) and a significant decrease in the proportion of T cells ($CD3^+$, 80·4 ± 6·0% *versus* 91·5 ± 2·2%; $P < 0·05$) in cells obtained from 24–48 h mucosal cultures compared with those from 0–24 h cultures (Table 2), findings consistent with the results of analysis by cytospin preparations (Table 1). The reduction in the proportion of T cells ($CD3^+$) in 24–48 h cultures was largely the result of a lower number of $CD4^+$ cells. There were no significant differences in the proportions of $CD8^+$ and CD4, CD8 double-positive T cells obtained from the 0–24 h and 24–48 h periods of mucosal cultures. The total number of cells migrating into the medium at the two

Table 1. Phenotype of cells migrating out of the lamina propria during culture of denuded normal colonic mucosal samples (obtained from 10 resection specimens)

Duration of culture (h)	Percent T cells ($CD2^+$)	Percent B cells ($CD20^+$)	Percent macrophages ($CD68^+$)	Percent macrophages ($CD14^+$)	Percent eosinophils
0–24	68·4 (± 5·1)	3·2 (± 0·6)	10·5 (± 1·3)	3·7 (± 0·1)	8·2 (± 1·5)
24–48	65·3 (± 3·6)	8·3 (± 1·0)*	8·6 (± 1·6)	3·0 (± 0·3)	7·4 (± 1·4)

Cytospin preparations were made of cells obtained after 0–24 h to 24–48 h periods of culture and the proportions of different cell populations determined after immunohistochemical staining using monoclonal antibodies. Results are expressed as mean (± s.e.m.) % positive cells.

* $P < 0·01$ compared with 0–24 h culture.

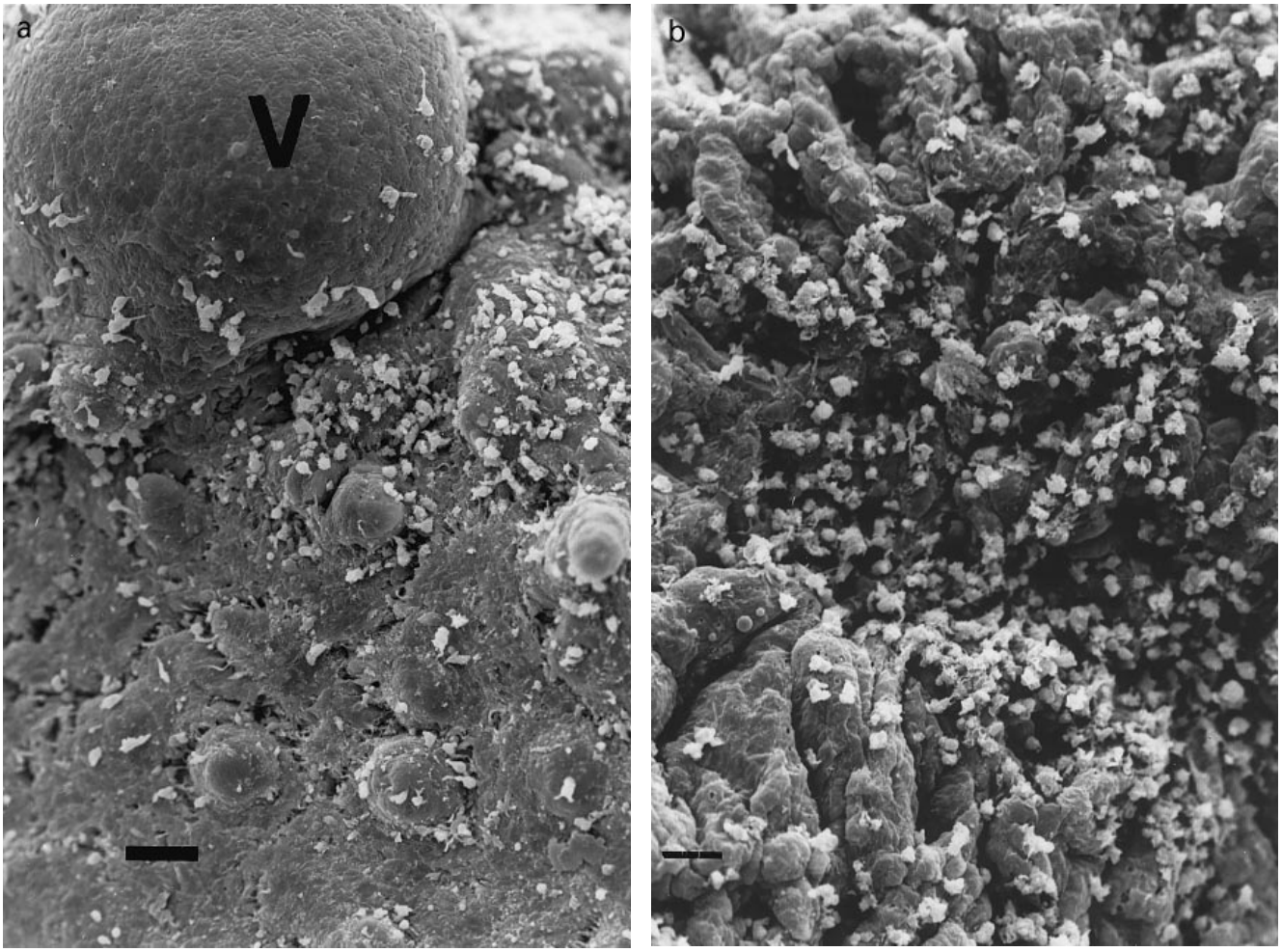


Fig. 3. Scanning electron micrographs of denuded normal terminal ileal (a) and colonic (b) mucosa, following culture for 24 h. Numerous cells are in the process of migrating out through the discrete pores in the basement membrane of denuded villi (V) and the colonic mucosal surface. Bar = 50 μm (a) and 30 μm (b).

different time intervals was not significantly different (per gram tissue: $2{\cdot}4 \pm 0{\cdot}2 \times 10^6$ *versus* $2{\cdot}3 \pm 0{\cdot}3 \times 10^6$), and therefore the differences in proportions of T and B cells seen above reflect changes in the absolute numbers.

When compared with peripheral blood lymphocytes, significantly greater proportions of CD4, CD8 double-positive, CD16/56, CD3 double-positive, $CD25^+$ and $CD45RO^+$ lymphocytes were present in cell suspensions obtained from cultured denuded mucosal samples (Table 2). The proportion of $CD4^+$ lymphocytes expressing CD49d was also greater in cells obtained from denuded colonic mucosa. In contrast, peripheral blood lymphocytes comprised a greater proportion of cells that were $CD16/56^+$, $CD3^-$ (natural killer cells).

Cell proliferation studies

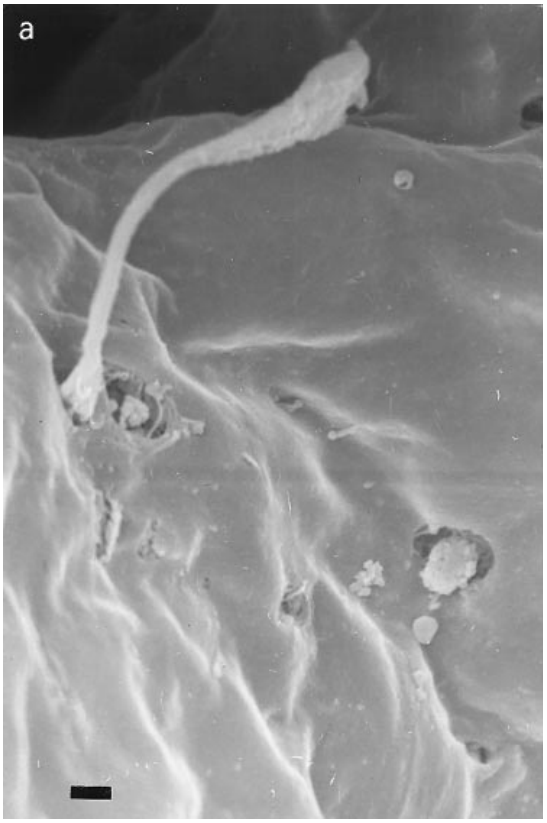
In order to demonstrate that cells migrating out of the lamina propria into the culture medium were functionally active, lymphocyte proliferation assays were performed using three different mitogens, PHA, Con A and PWM. For these studies, cells were resuspended in fresh medium without separation of the mononuclear cells on a density gradient. All three mitogens induced proliferation in a dose-dependent fashion: medium only, 135 ± 33·7 ct/min; 1 μg/ml PHA, 599 ± 80 ct/min; 10 μg/ml PHA, 9744 ± 258 ct/min; 1 μg/ml PWM, 3962 ± 48 ct/min; 10 μg/ml PWM, 5918 ± 523 ct/min; 2·5 μg/ml Con A, 3915 ± 46·3 ct/min; 25 μg/ml Con A, 12 245 ± 297 ct/min.

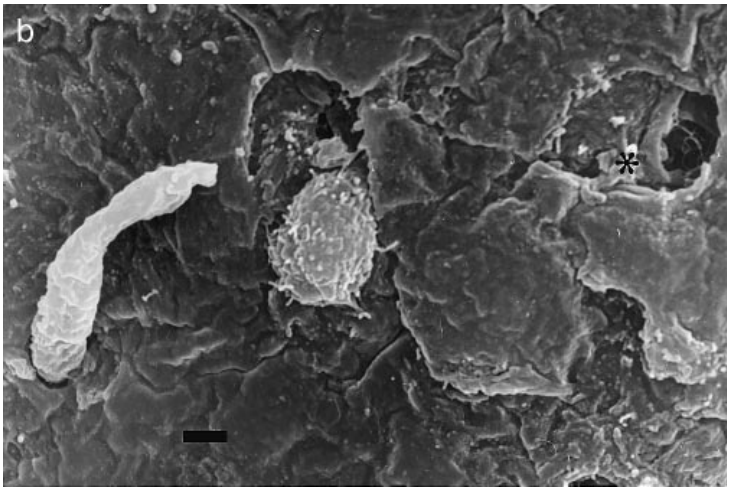
Studies with untreated mucosa

To investigate the capacity of lamina propria cells to emigrate in the presence of an intact epithelium, organ culture studies of nine normal colonic biopsies were performed simultaneously. The total weight of the biopsies was 71 mg, and after 24-h culture, a total of $3{\cdot}9 \times 10^3$ cells were present in the pooled supernatant ($54{\cdot}9 \times 10^3$/g tissue). Immunohistochemical studies on cytospin preparations showed that there were 3·8% $CD2^+$ and <1% $CD68^+$ cells (macrophages) and eosinophils. The rest of the cells had morphological appearances of epithelial cells on toluidine blue-stained preparations.

DISCUSSION

In the gastrointestinal mucosa, a distinct layer of matrix, in the form of basement membrane, underlies the epithelium and separates it from the lamina propria. It is composed of type IV collagen,





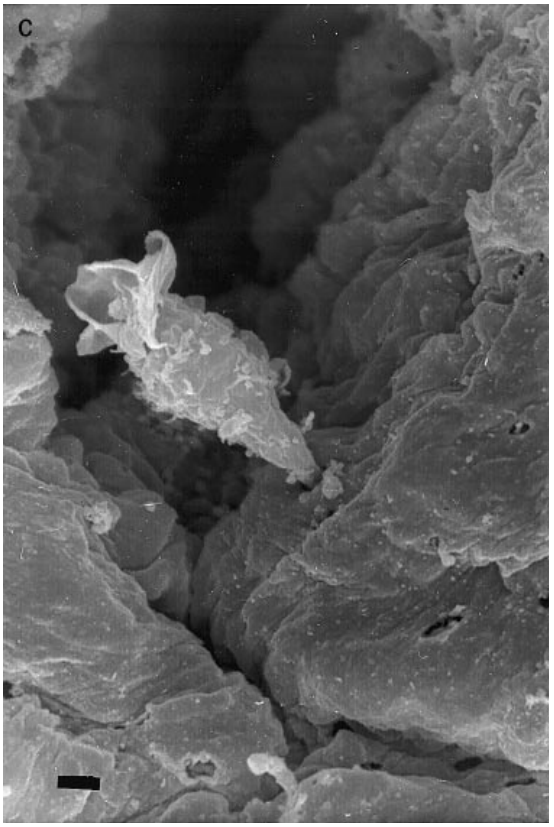


Fig. 4. Scanning electron micrographs of cells at various stages of migration out of denuded normal colonic mucosa during culture. Cells initially extend processes through discrete pores in the basement membrane (a,b) before migrating out through them (c; cell is emigrating through a pore in the crypt region). (b) An empty tunnel can be seen extending beyond a pore (*). Bar = 1 μm (a) and 2 μm (b,c).

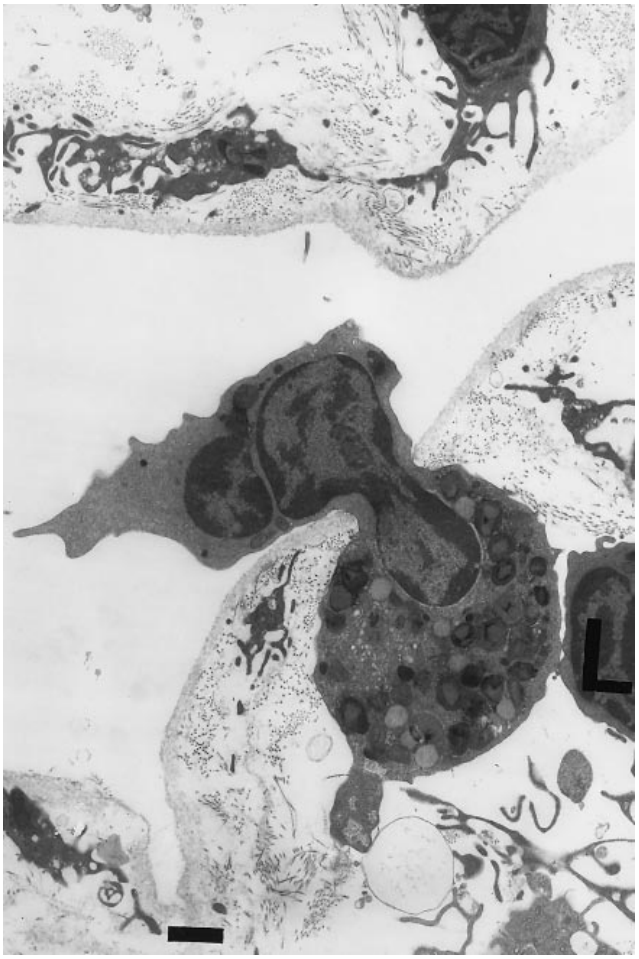


Fig. 5. Transmission electron micrograph of denuded normal colonic mucosa following culture for 24 h. A cell, probably an eosinophil, is squeezing through a pore in the basement membrane and a lymphocyte (L) is lining up behind it to emigrate next. Bar = 1 μm.

fibronectin, laminin, nidogen and heparan sulphate proteoglycan. In addition to providing support to the epithelial cells, it also appears to modulate epithelial proliferation and differentiation [9]. The lamina propria contains extracellular matrix closely associated with a variety of cell types, of which lymphocytes and macrophages are the most prominent. These cells are derived from the circulation and, *in vivo*, some may migrate from the lamina propria into the epithelium [3]. Migration of intra-epithelial lymphocyte (IEL) precursors through the lamina propria would require that the cells traverse the basement membrane, and it is likely that these cells cross the basement membrane via the discrete pores demonstrated in this study, and which have also been observed previously [10–12].

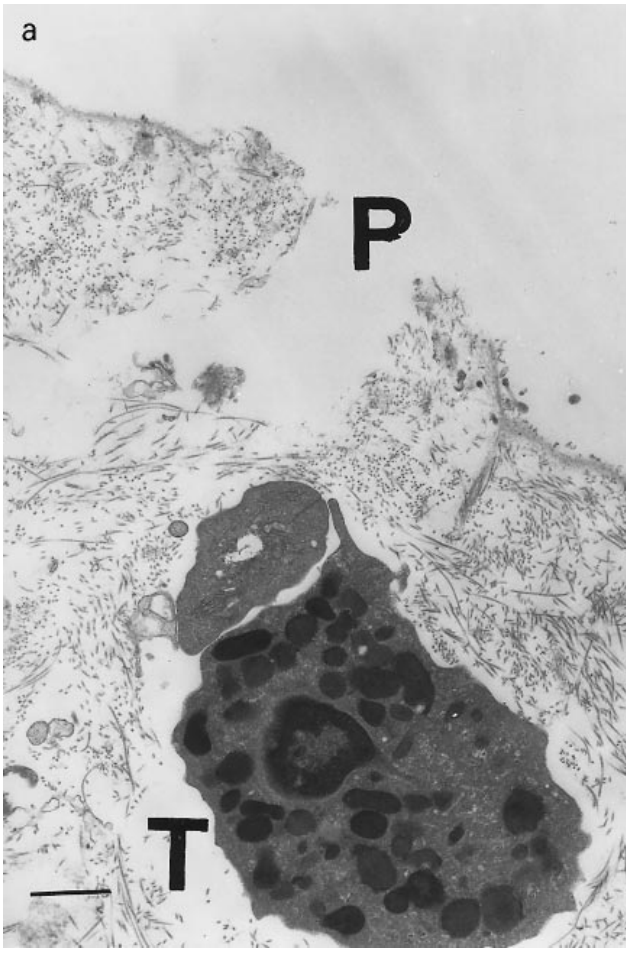
We are not aware of any previous study that has demonstrated the migration of a large number of lymphocytes, macrophages and eosinophils out of the lamina propria, through the basement membrane pores, following removal of the surface epithelium. During culture of denuded mucosal samples, cells migrate up tunnels in the lamina propria that are in continuity with basement membrane pores. Our organ culture studies using normal colonic biopsies with an intact epithelium suggest that the lamina propria cells only emigrate following loss of surface epithelial cells. In addition, they also support the findings by electron microscopy that the lamina propria cells migrate via basement membrane pores and not from the cut edge or the undersurface of the mucosa. In separate studies we have also observed [13] lamina propria cells migrating out of basement membrane pores following *Clostridium difficile* toxin A-induced detachment of epithelial cells.

Migration of cells out of the lamina propria was selective, as demonstrated by the presence of very few B cells during the first 24 h of culture of denuded mucosal samples. The large numbers of T cells that migrated out via basement membrane pores largely reflect the population present in the lamina propria [14–16]. We postulate that the migration of large numbers of lamina propria T cells, macrophages and eosinophils occurs *in vivo* following loss of injured surface epithelial cells. Such a response may represent an important type of intestinal host defence. Continuous migration of cells through the basement membrane pores would provide a physical barrier to penetration by luminal bacteria and their products. Such protection would be of considerable importance in the colon, which in humans contains 10^{10}–10^{12} bacteria per gram of contents [17]. Macrophages are prominent just below the surface epithelium [18,19] and, together with eosinophils [20], would be capable of mediating the host response to luminal bacteria. The expression of CD14 by a significant proportion of lamina propria macrophages would facilitate these responses [21,22]. Lymphocytes and macrophages have been demonstrated in the intestinal lumen of rabbits, with evidence that they had migrated from the mucosa [23]. These luminal cells remained active and immunocompetent, despite the large resident microbial flora. Indeed, a number of viable cells were seen closely associated with bacteria, suggesting that they may mediate antimicrobial activity [23]. T cells 'armed' with secretory IgA antibodies have been shown to be capable of direct bacterial killing [24]. Large numbers of lymphocytes and macrophages have also been found in the intestinal lumen of animals infected with *Giardia lamblia* [25], and migration of significant numbers of lymphocytes, from Peyer's patches into the lumen, has also been demonstrated following *in vivo* antigenic stimulation [26].

Peptide factors secreted by lamina propria macrophages and lymphocytes in response to luminal bacteria and their products may also be important in wound repair by facilitating restitution by neighbouring epithelial cells. *In vitro* studies using epithelial cell lines have demonstrated the capacity of lymphocyte and macrophage-derived peptide factors to induce intestinal epithelial cell restitution. These factors include transforming growth factor-beta (TGF-β), fibroblast growth factors (FGF), interferon-gamma and IL-1 and IL-2 [27–30].

Cells migrating out of the lamina propria are likely to utilize specific receptors for interaction with the extracellular matrix. A number of such receptors on T cells have been identified, and include members of the β_1 or very late antigen (VLA) integrin subfamily, CD44, CD26 and CD73 [31]. Most T cells emigrating from the denuded normal colonic mucosa express CD49d (α_4-integrin) which, in association with the β_1 subunit (CD29, together designated VLA-4), has been shown to mediate adhesion to the extracellular matrix fibronectin as well as vascular cell adhesion molecule-1 (VCAM-1) [32]. The α_4-integrins can also associate with another β subunit, β7, and the resulting heterodimer mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1 [33].

The migration of lymphocytes and macrophages out of the lamina propria during culture provides a simple model for the



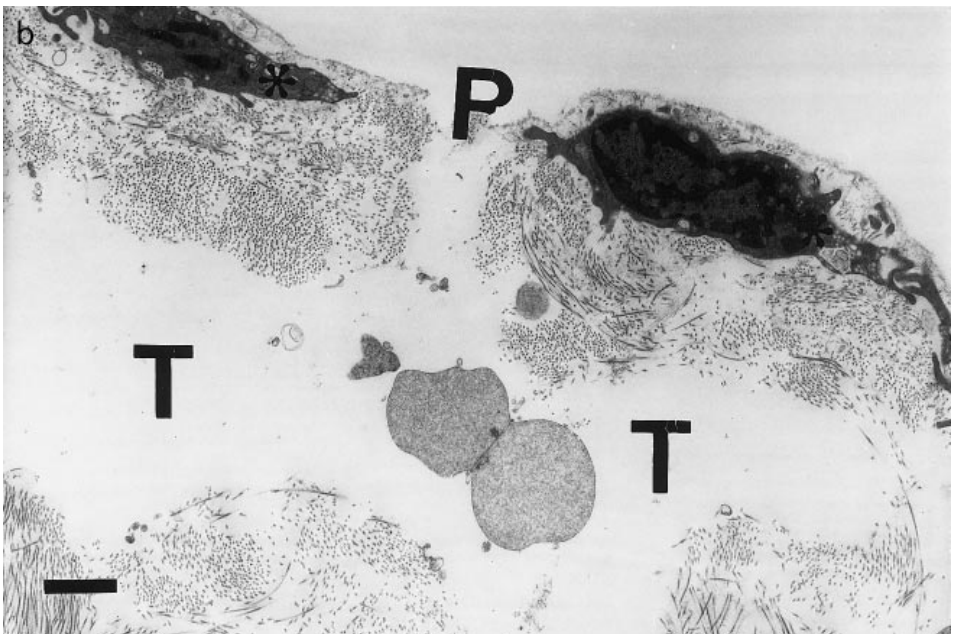


Fig. 6. Transmission electron micrographs of denuded normal colonic mucosa following culture for 48 h (a) and 96 h (b). (a) A cell lies in a tunnel (T) in the lamina propria, close to a pore (P) in the basement membrane. Another tunnel is devoid of cells and is in continuity with the pore. (b) Three tunnels (T) in the lamina propria end at a pore (P) in the basement membrane. Mesenchymal cells (*) are also present immediately underneath the basement membrane. Bar = 1 μm (a,b).

Table 2. FACS analysis of lymphocytes migrating out of the lamina propria during culture of denuded mucosal samples from six normal colons

Cell phenotype	Intestinal lamina propria lymphocytes obtained during culture of denuded mucosa		Peripheral blood lymphocytes
	0–24 h culture	24–48 h culture	
$CD19^+$	3·7 (± 1·1)	14·2 (± 5)*	11·0 (± 1·5)
$CD3^+$	91·5 (± 2·2)	80·4 (± 6)*	79·4 (± 1·5)
$CD4^+$	58·7 (± 5·3)	49·1 (± 5·7)*	54·4 (± 4·1)
$CD8^+$	22·9 (± 3·7)	22·8 (± 3·8)	22·2 (± 4·1)
$CD4^+$ and $CD8^+$	10·1 (± 3·6)†	8·4 (± 2·6)†	1·4 (± 0·3)
$CD16/56^+$ and $CD3^+$	7·8 (± 1·4)††	7·0 (± 2·3)†	1·4 (± 0·2)
$CD16/56^+$ and $CD3^-$	3·5 (± 0·7)††	2·1 (± 0·6)††	9·5 (± 1·6)
$CD25^+$	9·0 (± 2·0)†	7·4 (± 2·2)	2·6 (± 0·1)
Percent of $CD4^+$ lymphocytes expressing CD11a	98·6 (± 0·4)	ND	99·8 (± 0·1)
Percent of $CD8^+$ lymphocytes expressing CD11a	95·6 (± 0·6)	ND	99·7 (± 0·1)
$CD18^+$	98·4 (± 0·4)	ND	95·7 (± 1·4)
Percent of $CD4^+$ lymphocytes expressing CD49d	88·5 (± 1·8)†	ND	77·3 (± 4·3)
Percent of $CD8^+$ lymphocytes expressing CD49d	89·1 (± 1·3)	ND	95·2 (± 1·1)
CD45RO:CD45RA ratio	11·6 (± 3·2)††	ND	0·8 (± 0·1)

Results are expressed as mean (± s.e.m.) % positive cells. Number of cells obtained per gram tissue: 0–24 h, $2{\cdot}4 \pm 0{\cdot}2 \times 10^6$; 24–48 h, $2{\cdot}3 \pm 0{\cdot}3 \times 10^6$.

† $P < 0{\cdot}05$; †† $P < 0{\cdot}01$ compared with peripheral blood lymphocytes.

* $P < 0{\cdot}05$ compared with 0–24 h cultures.

isolation of these cells for detailed phenotypic and functional studies, without the use of collagenase. The proportions of lymphocytes and macrophages obtained are comparable to those analysed after enzymatic digestion and centrifugation on Ficoll–Paque [14,34–37].

In conclusion, we have shown that following removal of the surface epithelium, large numbers of lymphocytes, macrophages and eosinophils migrate out of the intestinal lamina propria. This migration, which occurs via tunnels in the extracellular matrix that end as discrete pores in the basement membrane, probably represents an important form of host defence elicited following injury and loss of epithelial cells. *In vitro* culture of intestinal mucosa denuded of epithelial cells also provides a simple model for the isolation of functionally viable lamina propria lymphocytes, macrophages and eosinophils.

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