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Type 1 hypersensitivity reactions in reconstructed tissues using syngeneic cell types

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1 Type 1 hypersensitivity reactions in response to antigen challenge have been measured as short circuit current (SCC) responses in reconstructed tissues consisting of syngeneic cell types.

2 In all instances reconstructed tissues consisted of an epithelial monolayer grown on collagen-coated Millipore filters and a pad of peritoneal cells. Monolayers were formed of either HCA-7 or HCA-7-Col 1 cells derived from a human adenocarcinoma. Peritoneal cells were derived from rats or guinea-pigs sensitized to either ovalbumin or β-lactoglobulin.

3 The SCC responses of the monolayers were dependent upon the 'concentration' of peritoneal cells in the reconstructed tissue. The threshold concentration was 0.4×10^6 cells when rat peritoneal cells are combined with an epithelial monolayer of $0.2\,cm^2$.

4 The SCC responses in response to antigen challenge were selectively inhibited by the H_1-receptor antagonist, mepyramine. Similarly the effects of exogenously applied histamine were antagonised by mepyramine.

5 The responses to antigen challenge were not inhibited by tetrodotoxin in reconstructed tissues. This result is in contrast to that with isolated intestinal epithelia from sensitized animals where tetrodotoxin inhibits the SCC responses to external field stimulation and to challenge with antigens. The consequences of these results for understanding the mechanisms of epithelial Type 1 hypersensitivity reactions are discussed. Suggestions are made to illustrate how the methods developed here may be employed to ask questions about the nature of mediators released and the types of cell responsible in human disease conditions.

Introduction

When epithelial cells are cultured *in vitro* they show the unique characteristic of forming oriented monolayers with the basolateral faces against the substrate and the apical faces contacting the medium. Morphologically they display apical microvilli and tight junctions develop between adjacent cells. Vectorial transport of fluid in the apical to basolateral direction often leads to the formation of fluid-filled hemicysts or domes in which localised areas of cells lift off the substratum. It is difficult to study transepithelial transport of solutes in this configuration and furthermore the composition of the basolateral bathing fluid cannot be controlled. This difficulty can be overcome by growing epithelial monolayers on suitable permeable supports, such as collagen-coated cellulose filters (Millipore). Generally epithelia will form oriented monolayers in these circumstances and can be used to study transepithelial transport by conventional techniques (Handler, 1983).

In recent years there have been a variety of reports of transporting activity in cultured epithelial monolayers using cells derived from kidney, lung, mammary gland, testes, sweat glands and particularly the alimentary canal. For example, human adenocarcinomas of the colon have provided a clutch of cell lines with characteristic and unique properties. These are known as Caco-2 (Grasset *et al.*, 1984), T-84 (Dharmasathaphorn *et al.*, 1985) and HCA-7 (Cuthbert *et al.*, 1985).

Using a totally different approach to the study of epithelial ion transport in the intestine we described the stimulation of electrogenic chloride secretion in isolated epithelia taken from animals sensitized to various antigens. Exposure of tissues to antigen on the basolateral side was shown to produce an immediate hypersensitivity type reaction dependent upon IgG (Cuthbert *et al.*, 1983) or IgE (Baird *et al.*, 1985) antibodies bound to cells of the immune system and

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contained within the lamina propria. It appeared that we might combine these approaches and reconstruct this type of immune response by combining the appropriately immunised cells with cultured epithelial monolayers. We suggest that this approach gives opportunities both for analysis of mechanisms involved in intestinal anaphylaxis and for diagnosis in disease related situations.

Methods

Culture of epithelial monolayers

The methods for the culture of HCA-7 and HCA-Col 1 epithelial monolayers on previous supports has been described elsewhere (Cuthbert *et al.*, 1987).

Sensitization of animals

Rats (Sprague-Dawley 100–150 g) were sensitized to ovalbumin by a single injection (i.p.) of ovalbumin (Sigma Grade V), 1 mg in 0.2 ml saline with 0.2 ml *B. pertussis* vaccine (Burroughs Wellcome) containing 10^{10} organisms. The animals were used 14 days later. Guinea-pigs (Dunkin-Hartley 200–300 g) were sensitized to β-lactoglobulin by providing cow's milk for drinking for three weeks followed by a return to water drinking at least three days before use (see Cuthbert *et al.*, 1983).

Preparation of peritoneal cells

Peritoneal cells were prepared by a method adapted from Atkinson *et al.* (1979). Animals were killed by exposure to CO_2, (100%) and Krebs Henseleit solution (KHS), 10 ml, was injected into the peritoneal cavity. The abdomen was massaged gently for several minutes and then opened with a midline incision. The fluid in the abdomen was withdrawn in siliconised pipettes and placed in siliconised tubes. The cells were recovered by centrifugation, at room temperature, for 2 min at 150 *g*. Cells were counted in a haemocytometer, and diluted appropriately with KHS and used to form the reconstructed tissues. Between $20-30 \times 10^6$ cells were obtained from each rat but less ($7-20 \times 10^6$ cells) from each guinea-pig.

To determine the percentage of peritoneal cells which had the characteristics of mast cells the following protocols were used. Smears of cells were prepared upon glass slides and either air dried or fixed with methanol. They were stained either with (a) acid toluidine blue (b) alcian blue followed by neutral red or (c) Hansels stain.

The number of cells with mast cell characteristics were counted as well as the number of non-mast cells. In 10 separate smears prepared from peritoneal cells from rats sensitized to ovalbumin the percentage of mast cells was 13.9 ± 1.2%. Padawer & Gordon (1956) have shown that the relative contributions of different cell types in rat peritoneal fluid are fairly constant, while the cell concentration varies with the peritoneal fluid volume. Our value is somewhat higher than the percentage reported by others but there may have been a modest hyperplasia following sensitization to ovalbumin.

Similar protocols were carried out with guinea-pig peritoneal cells. Four guinea-pigs sensitized to β-lactoglobulin (βLG) were used to prepare smears. No mast cells were seen in smears from two animals while the other two demonstrated occasional mast cells and basophils, the former being smaller than the mast cells of the rat. It is noted that very few mast cells are seen in normal, non-sensitized guinea-pigs (Padawer & Gordon, 1956) although occasional animals were reported to have up to 20,000 mast cells per ml. We are grateful to Kevin Hickling of Fisons, plc., for carrying out the mast cell identification procedures.

Preparation of ileal and colonic epithelia

Epithelia from guinea-pig or rat colon, or guinea-pig ileum were dissected from the appropriate tissues as described elsewhere (Cuthbert & Margolius, 1982; Cuthbert *et al.*, 1983).

Short circuit current (SCC) recording

Methods for recording SCC from voltage clamped epithelia from rat or guinea-pig intestine have been described previously (Cuthbert & Margolius, 1982). Routinely the SCC was recorded using an epithelial area of 0.6 cm^2. SCC recording from monolayers of cultured cells has also been described (Cuthbert *et al.*, 1985) and in this situation the epithelial area was 0.2 cm^2. Neurones in the lamina propria of intestinal epithelia were stimulated electrically using external field stimulation (EFS). Alternate pins on the Ussing chamber used to locate the tissue in the window were connected together with fine silver wires and the two sets connected to the output of the Grass S-4 stimulator via an isolation transformer. The two sets of wires were insulated from each other by a Parafilm gasket.

Preparation of epithelia monolayer-peritoneal cell sandwiches

A special filter block was constructed to hold Millipore filters (type HVLP, 0.45 μm) so that an area of 0.5 cm^2 was exposed. The filters were fitted into the block after wetting with Krebs-Henseleit solution. Using a plastic rod and gentle pressure a slight depression was made in the Millipore filter. Suspen-

sions with known numbers of peritoneal cells were added to the filter block and the cells allowed to settle on the surface of the filter. Very slight suction was applied to remove the fluid after which a ring of 5 holes was punched with a 19 gauge needle through the pad of peritoneal cells. This was necessary to facilitate the entry of antigens to the cell layer. The filters, bearing a known number of cells, were removed from the filter block and placed on the female half of an Ussing chamber. Epithelial monolayers, also grown on Millipore filters, were placed over the filter containing the peritoneal cells so that the cells were sandwiched between the filter on which they were prepared and the filter carrying the epithelial monolayer. The male half of the Ussing chamber was used then to clamp the sandwich together, the pins in the male half penetrating both filters before passing into holes in the female half-chamber. The reconstructed tissue then consisted of, in order, an epithelial monolayer, Millipore filter, peritoneal cells and a second Millipore filter. Added drugs could reach the apical surface of the monolayer without any intervening structure. In contrast drugs added to the basolateral surface of the cells had to pass through the two Millipore filters and a pad of cells to do so.

Calculation of results

Results are given either as short circuit currents (SCC) which were recorded directly or as the area under the SCC versus time traces. These areas were measured with an Allbrit planimeter. Tests of statistical significance were made by use of Student's t test.

Solutions

Throughout Krebs-Henseleit solutions (KHS) with the following composition was used: (mM) NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.8, KH_2PO_4 1.8, $NaHCO_3$, 25 and glucose 11.1. At 37°C and when bubbled with 95%O_2-5%CO_2 this solution had a pH of 7.4.

Results

The epithelial 'sandwich'

Figure 1 shows the protocols we have used to obtain recordings from sandwiches consisting of an epithelial monolayer of HCA-7 cells and rat peritoneal cells. On the left is shown the response of a piece of rat colon epithelium when challenged with ovalbumin. The upper part of the diagram is the result obtained with tissue from an animal that was sensitized to ovalbumin, while the lower part illustrates results with tissue from a non-sensitized rat. This latter tissue is capable of showing an increase in SCC as shown by the addition of forskolin. A sandwich made from 1.7×10^6 peritoneal cells from the sensitized rat plus an HCA-7 monolayer gave a response on challenge with ovalbumin while a similar one, using five times the number of peritoneal cells, from a non-sensitized rat, failed to respond to ovalbumin but did so to forskolin. From experiments such as these it can be concluded that an interaction of antigen with sensitized immune cells can liberate mediators which affect SCC in cultured epithelial monolayers.

The dependence of the response in the sandwich upon the number of peritoneal cells apposed to the monolayer is given in Figure 2. This is shown both as the actual current generated by a standard amount of antigen or as a fraction of the response to forskolin added after the response to antigen had subsided. Using our configuration the threshold was around 0.4×10^6 cells and the response increased in size up to 8.5×10^6 cells, the practical upper limit we could use.

The nature of mediators

The sandwich technique can be used as a microbioassay for examining the mediators involved in intestinal anaphylaxis responses. This can be achieved by use of appropriate antagonists or, where none are available, use of desensitization procedures. We illustrate both approaches here. Rat peritoneal cells are a classic preparation for the study of histamine release and it might be expected that histamine will be involved in the SCC response. HCA-7 monolayers responded to histamine but only when it was added to the basolateral side. Concentration-response curves were obtained to cumulative additions of histamine to the basolateral bathing fluid (Figure 3). In addition, concentration-response curves were obtained in the presence of mepyramine, 0.1 and 0.01 μM. A near parallel shift in the curves was obtained with mepyramine but the 'dose-ratios' were unexpectedly low. For example with 0.1 μM mepyramine the dose-ratio was around 5, giving an affinity of $4 \times 10^7\ M^{-1}$, some 25 times less than expected for H_1-receptors. However it was necessary to add histamine to the basolateral bathing fluid and both it and the antagonist needed to penetrate the Millipore filter before reaching the receptors. It is possible that the concentrations of agonist and antagonist at the basolateral surface never reach the bath concentration, making calculations of affinity imprecise. We examined the histamine-mepyramine interaction in another cell line, HCA-7-Colony 1, in a few experiments but this proved to be less sensitive to histamine than HCA-7. An approximate value for the affinity of mepyramine from these experiments was $3.6 \times 10^8\ M^{-1}$, again somewhat lower than might be expected for H_1-receptors. We could detect no effect of cimetidine, an H_2-receptor

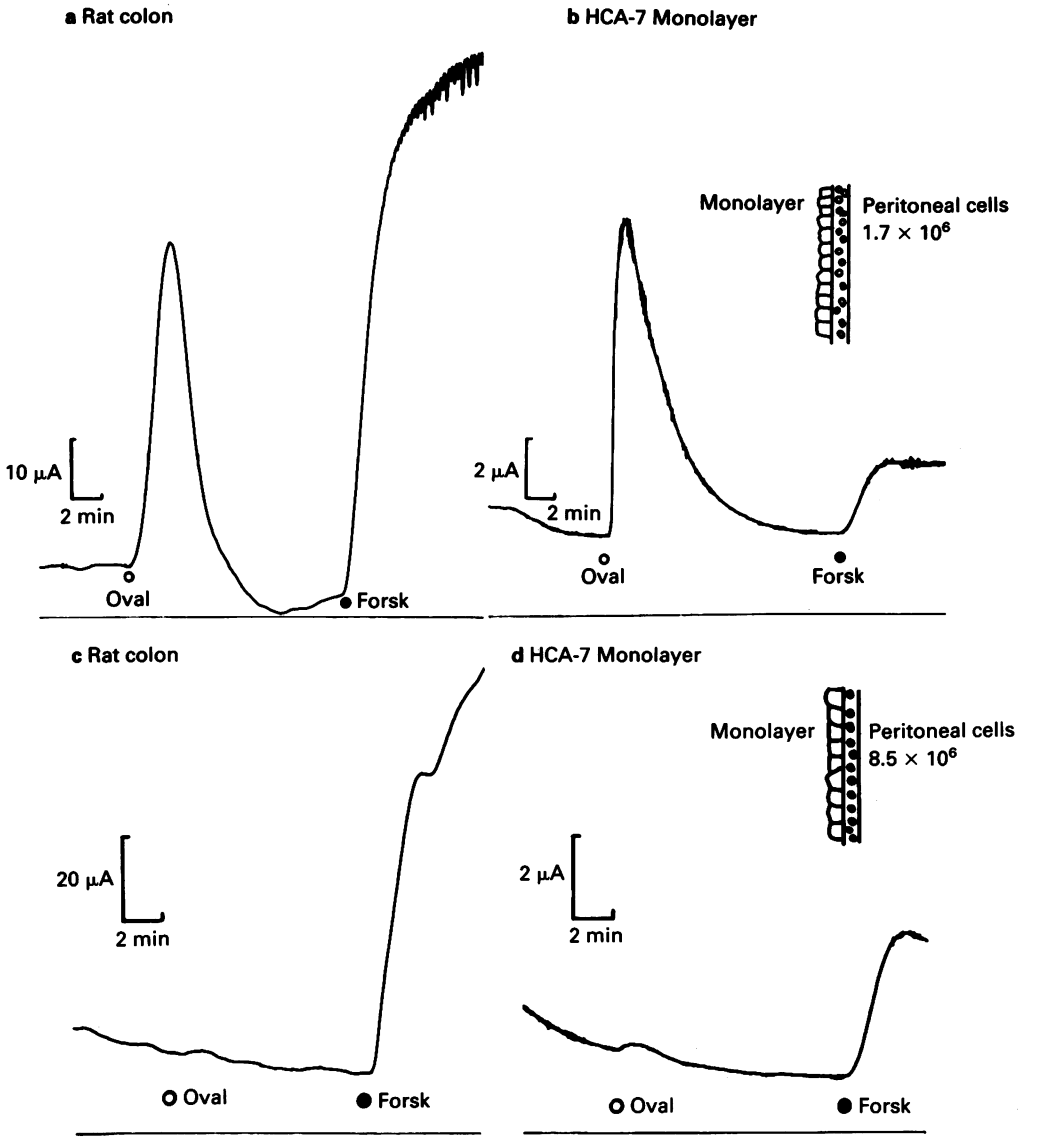

Figure 1 Illustrative examples of the epithelial monolayer-rat peritoneal cell sandwich technique. Shown are SCC records from rat colon epithelium (0.6 cm^2) on the left (a and c) and from HCA-7 monolayers plus peritoneal cells (0.2 cm^2) on the right (b and d). Traces in (a) and (b) were derived from colon and peritoneal cells from a rat sensitized to ovalbumin (oval), while the equivalent tissues used to obtain the traces in (c) and (d) were derived from a non-sensitized animal. Challenge with ovalbumin (20 $\mu g\ ml^{-1}$) was applied to the basolateral side of the epithelia. Note tissues from the non-sensitized rat failed to respond to challenge with ovalbumin, even though 5 times the number of peritoneal cells were used. All tissues responded to forskolin, 10 μM (Forsk). Horizontal lines indicate zero SCC.

antagonist, on responses to histamine even when used at a concentration of 100 μM. We conclude that H_2-receptors play no part in the response of the epithelia to histamine.

Mepyramine significantly reduced the response to challenge with ovalbumin in HCA-7-peritoneal cell sandwiches. At 1.0 μM the response to challenge was virtually abolished suggesting that histamine accounted for the whole of the SCC response. However there was some reduction in the forskolin response at this

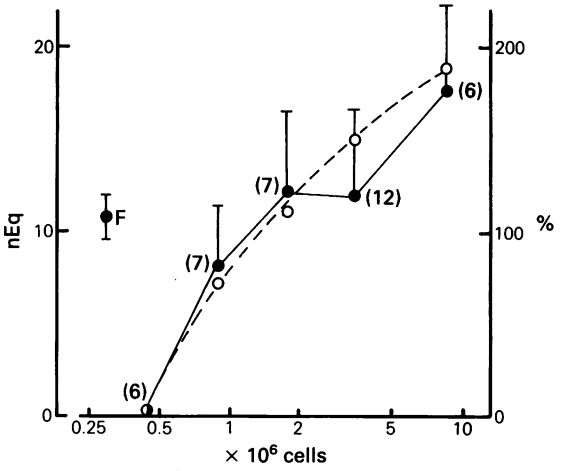

Figure 2 'Concentration'-response relationship for the HCA-7 monolayer-peritoneal cell sandwich. The area under the SCC versus time curve following challenge with ovalbumin (20 μg ml^{-1}) was integrated and the results expressed as nEq(●). Additonally the results are expressed as a percentage of the response to forskolin (10 μM) (SCC versus time curve integrated during 10 min following addition) (○). The point marked F shows the mean response to forskolin. Means are shown with number of observations given in parentheses; vertical lines show s.e. HCA-7 monolayers were 0.2 cm^2.

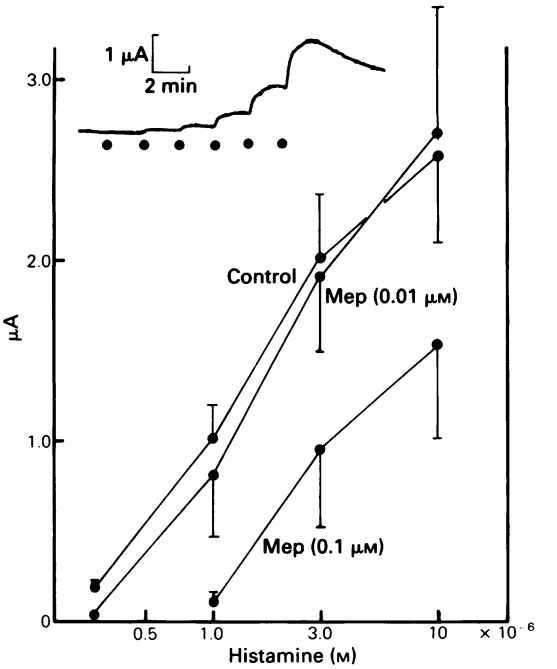

Figure 3 Concentration-response curve for histamine (applied basolaterally) on HCA-7 monolayers (0.2 cm^2) in the presence and absence of mepyramine. Means and standard errors for between 6 and 11 observations are given. Inset shows a typical cumulative dose-response curve to histamine in the presence of mepyramine (Mep), 0.1 μM. Histamine concentrations were 0.1, 0.3, 1.0, 3.0, 10.0 and 30 μM.

mepyramine concentration although this was not statistically significant. At the lower mepyramine concentration (0.1 μM) where forskolin responses were unaffected there was incomplete blockade of the response to antigen challenge (Figure 4).

Among the various mediators liberated from mast cells are kinins, but we have also shown that HCA-7 monolayers show pronounced desensitization to these agents, responses taking several hours to recover (Cuthbert *et al.*, 1987). We used this fact to examine whether kinins were involved in the response to ovalbumin challenge in monolayer-mast cell sandwiches. An example is given in Figure 5 where it can be seen that a substantial response to ovalbumin challenge was obtained even in the presence of lysyl-bradykinin (LBK), but after the response to the peptide had declined. We have not used this approach quantitatively for there is a yet easier method to examine the release of kinins. HCA-7 monolayers are sensitive to kinin added to the apical side of the monolayer, unlike the situation with most other agonists, including histamine. We have shown that when the peritoneal cells are apposed to the apical side of the monolayer there is no SCC response upon challenge (Figure 7). Taken together it appears that no significant amounts of kinin are released from rat peritoneal cells under our conditions.

The effects of tetrodotoxin

A major stimulus for these studies was finding that tetrodotoxin (TTX) inhibited the Type 1 hypersensitivity reaction to βLG in intestinal epithelia from sensitized guinea-pigs. This result is shown in Figure 6 which illustrates responses with two paired epithelial intestinal preparations from a guinea-pig sensitized to βLG. Preparations were stimulated transmurally at 30 min intervals after which they were challenged with βLG. In one preparation only TTX was added after the second period of transmural stimulation. Both the response to field stimulation and βLG were inhibited by TTX, with some recovery of the former after removal of the TTX. The combined results from 6 paired experiments of this kind are also given. While the effect of TTX on transmural stimulation is expected its effect on the chemical stimulus was not so. The effect of TTX on the response to βLG in sensitized intestinal epithelia was concentration-dependent as shown in Table 1.

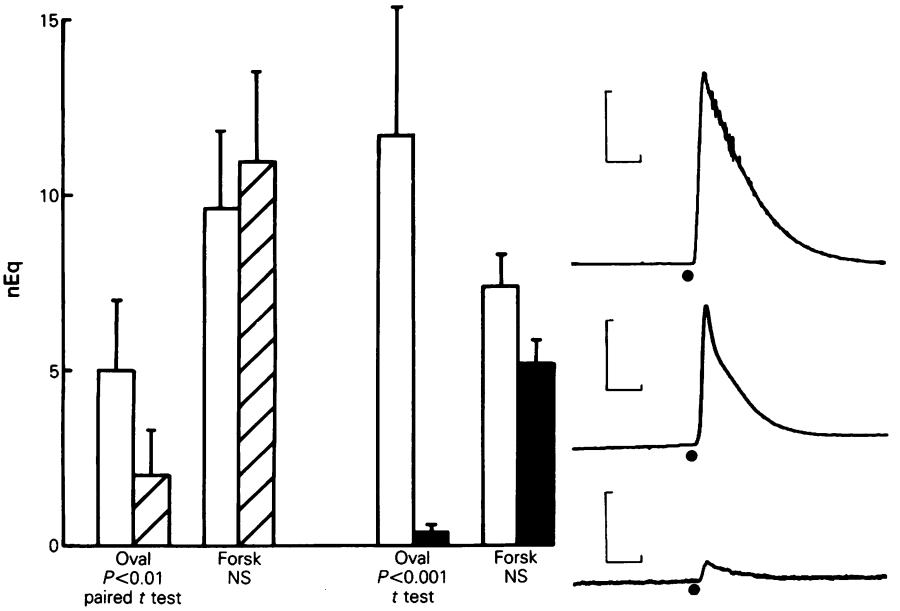

Figure 4 Effect of mepyramine on response to ovalbumin in HCA-7 monolayer-peritoneal cell sandwiches. HCA-7 monolayers were used between 4 and 9 days following plating. Each monolayer was combined with 3.4×10^6 mast cells from rats sensitized to ovalbumin. Rat colon preparations challenged with ovalbumin were used to confirm that animals were sensitized. All experiments were paired, consisting of two identical monolayers plus 3.4×10^6 cells from the same sensitized rat. One of each pair was equilibrated on both sides with mepryramine, following which both preparations were challenged with ovalbumin 20 g ml^{-1} (Oval). Twenty minutes later, when the response to ovalbumin had disappeared, preparations were treated with forskolin, 10 μM (Forsk). The area under the SCC versus time curves for the ovalbumin challenge and for the first 10 min of the sustained forskolin response are plotted (as nEq). Hatched columns are results with 0.1 μM mepyramine, solid columns with 1.0 μM mepyramine while open bars represent controls. Means 4–6 observations are given; vertical lines show s.e. Mepryamine caused a significant reduction in the response to ovalbumin challenge both at 0.1 μM ($P < 0.01$, paired t test) and 1.0 μM ($P < 0.001$, t test). Responses to forskolin were not significantly altered by mepyramine. In some experiments a single control was paired with two test preparations one each exposed to 0.1 μM and 1.0 μM mepyramine. The inset shows the responses to ovalbumin challenge in one of the these experiments. The records show from the top: control, mepyramine 0.1 μM test and mepyramine 1.0 μM test records. Calibrations are 2 μA and 2 min and (●) indicates ovalbumin addition.

There are various ways to interpret these data. Either there are mediators liberated from the immunocytes within the lamina propria, which affect neuronal elements too and which then secondarily affect the epithelial cells by release of transmitter or alternatively the interaction of sensitized immunocytes with antigen is a TTX sensitive process. Using the epithelial monolayer sandwich technique we were able to test the second hypothesis directly, albeit with peritoneal cells.

In five separate experiments (Figure 7) there was no indication that TTX inhibited the response to antigen challenge in the monolayer-peritoneal cell sandwich. Indeed the responses to antigen challenge were consistently enhanced in the presence of TTX, although the increase was not statistically significant. The experiment in which the enhancement was most marked is illustrated in Figure 7. We have no explanation for this phenomenon, as TTX did not affect responses to histamine. Assuming that peritoneal and mucosal mast cells behave the same way with respect to TTX then it must be concluded that in normal epithelia there is neuronal involvement in intestinal anaphylaxis.

Other procedures

In the experiments with reconstructed sensitized tissues we have used amounts of antigen which preclude a further response on a second exposure. However, with the reconstructed systems the possibility exists of disassembly and reconstruction with a second batch of immune cells. We found that reassembly with a further aliquot of rat peritoneal cells sensitized to ovalbumin

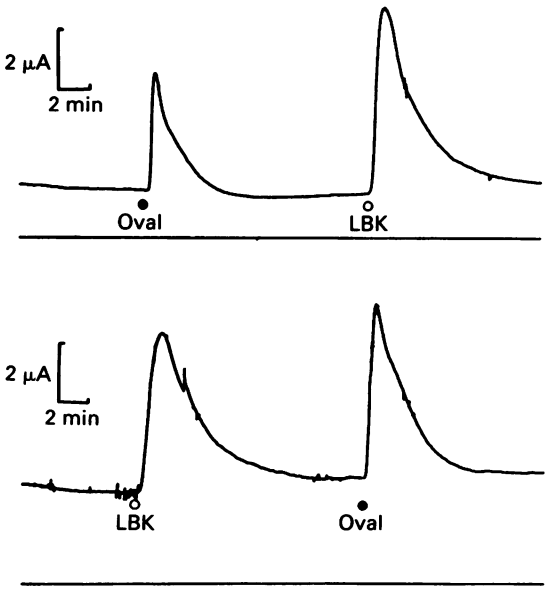

Figure 5 Effects of ovalbumin (Oval, 20 μg ml^{-1}, ●) and lysylbradykinin (LBK, 0.1 μM, ○) on HCA-7 monolayer-mast cell sandwiches. Results are shown from a paired experiment in which each monolayer (0.2 cm^2) was sandwiched with 3.4×10^6 peritoneal mast cells from a rat sensitized to ovalbumin. Horizontal lines indicate zero SCC.

resulted, upon challenge, in a second, but less brisk response. This result indicates that the epithelium has a totally passive role as far as the antigen-antibody reaction is concerned; the epithelial response being simply a monitor of the release of mediators. The loss of sensitivity during the second hypersensitivity reaction is most probably due to long lasting desensitization of HCA-7 monolayers, as we have shown occurs with some agonists (Cuthbert *et al.*, 1987).

To make sure of the secondary role of the epithelium we attempted to transfer sensitivity passively using immune serum. Serum from guinea-pigs sensitized to β-LG was added to solutions bathing HCA-7 monolayers and then removed. Subsequent exposure to the antigen failed to cause any alteration in the SCC of HCA-7 monolayers, indicating that either the monolayers had no receptors for IgG antibodies, or, if they do, then subsequent reaction with βLG had no effect on the electrogenic ion transporting behaviour of the epithelial cells. The same immune serum was able to passively sensitize guinea-pig colon epithelium (Baird *et al.*, 1987).

Finally, since most secretagogues affect epithelial transport only when added to the basolateral face of the tissue release of agents which do affect transport from the apical side could be detected simply by apposing the immune cells to the apical face of the monolayers (Figure 7).

Other systems

The majority of the experiments described earlier were carried out with a combination of HCA-7 monolayers and rat peritoneal cells sensitized to ovalbumin. We found similar results could be obtained using HCA-7-Colony 1 monolayers combined with ovalbumin-sensitized rat peritoneal cells.

Although it is generally agreed (Padawar & Gordon, 1956) that guinea-pig peritoneal mast cells are rare, we decided to conduct experiments with peritoneal cells from guinea-pigs sensitized to βLG by milk drinking. As we have shown earlier, ileal epithelia from such animals respond to βLG challenge with an

Table 1 Effect of tetrodotoxin on the response to challenge with β-lactoglobulin (βLG) in ileal epithelia from sensitized guinea-pigs

	Tetrodotoxin (nM)			
	0	10	100	1000
Resting SCC (μA)	92.4 ± 14.9	93.0 ± 6.6	105.8 ± 17.7	98.0 ± 15.9
Fall in SCC by TTX (μA)	−0.2 ± 1.5	8.0 ± 2.7	24.6 ± 6.9	19.8 ± 4.1
Response to βLG (μEq)	0.173 ± 0.031	0.164 ± 0.011	0.069 ± 0.011	0.089 ± 0.023
Difference from control	—	NS	$P<0.01$	$P<0.05$

Guinea-pigs were sensitized to βLG by milk drinking. Challenge was with βLG, 0.55 μM applied to the basolateral side of the ileal epithelium. Tetrodotoxin (TTX) was added to the solutions bathing both sides of the tissue. Each value is the mean ± s.e. for 5 observations.

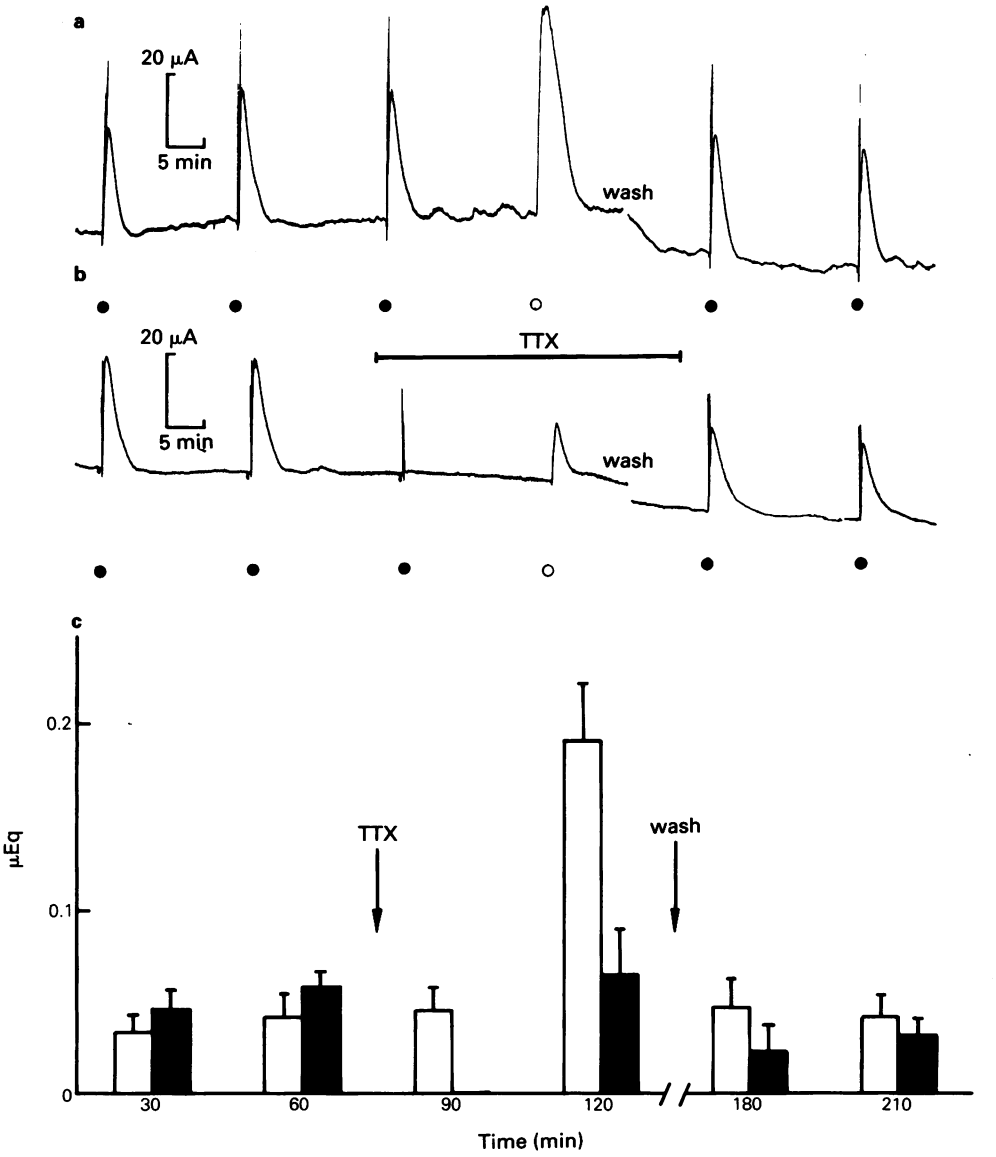

Figure 6 Effects of tetrodotoxin (TTX) on electrical field stimulation (EFS) and response to β-lactoglobulin (βLG) in ileal epithelium from guinea-pigs sensitized to βLG by milk drinking. SCC records from two paired preparations are shown, the upper (a) being the control. TTX, 0.1 μM, was added at 75 min and washed away at 150 min. The parameters of EFS were 5 Hz, 20 ms at 5 v for 20 s every 30 min (●) except at 120 min when βLG (1.1 μM) (○) was added. Note that TTX abolishes the response to field stimulation and reduces the response to βLG. Data from a series of experiments using paired preparations are given below the traces (c) where open columns refer to controls and closed columns to preparations to be treated with TTX at 75 to 150 min. Mean values for n observations are given ($n = 4$ for EFS and 6 for βLG responses); vertical lines show s.e. TTX significantly ($P < 0.01$) reduced the response to βLG.

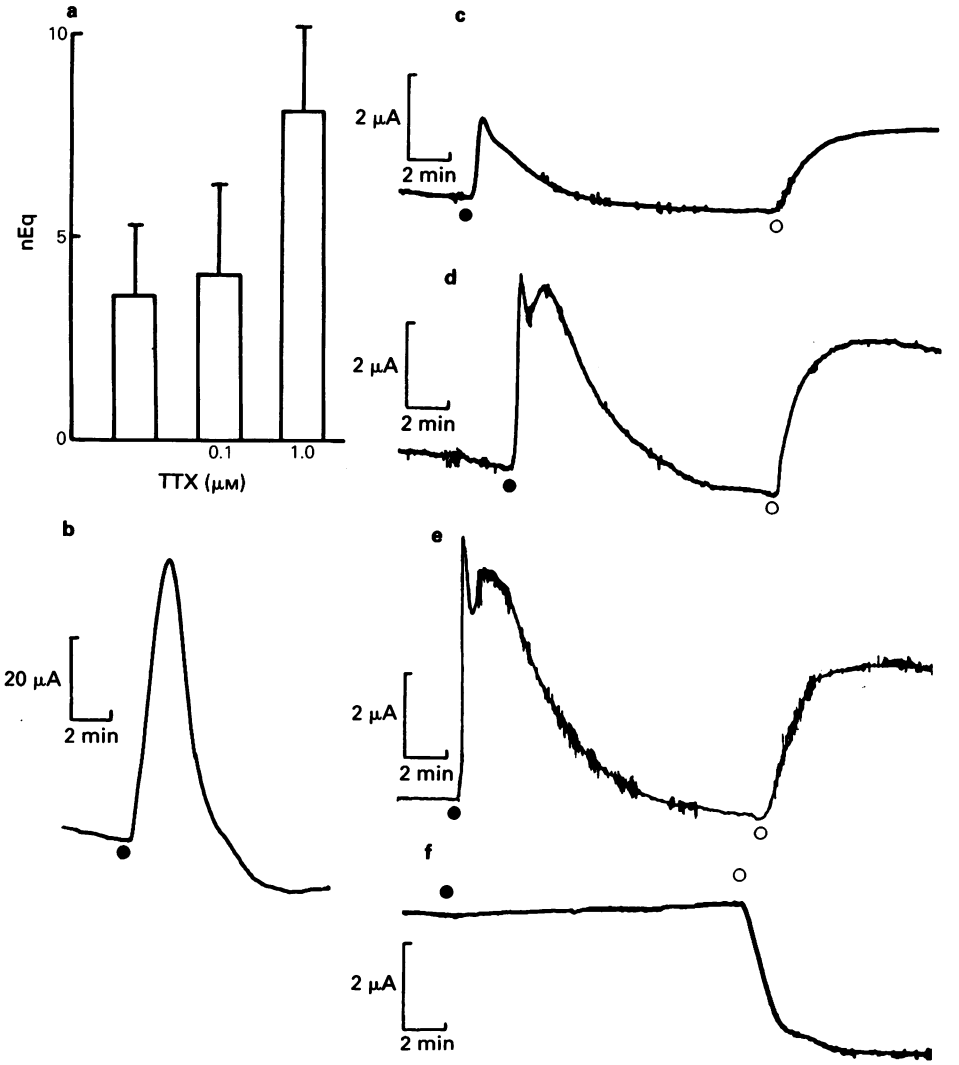

Figure 7 Effects of tetrodotoxin (TTX) on the response of HCA-7 monolayers with rat peritoneal cells sensitized to ovalbumin. In all instances 3.4×10^6 peritoneal cells were used and challenge was with ovalbumin 20 $\mu g\ ml^{-1}$. The sandwiches were preincubated with TTX, on both sides, for 20 min before challenge. Histogram shows the responses in nEq, obtained by integrating the SCC versus time curves for control preparations and in the presence of TTX. The five SCC records illustrate a single experiment: (b) shows the response of a rat colon epithelium (0.6 cm^2) challenged with ovalbumin, 20 $\mu g\ ml^{-1}$ (●) showing that the animal from which the peritoneal cells were derived was appropriately sensitized; (c–f) are records obtained from sandwiches with HCA-7 monolayers (0.2 cm^2) each with 3.4×10^6 peritoneal cells. Each was challenged with ovalbumin (20 $\mu g\ ml^{-1}$) (●) and forskolin, 10 μM (○). (c) is the control, (d) and (e) were preincubated with 0.1 and 1.0 μM TTX, respectively. In (f) the peritoneal cells were sandwiched on the apical surface of the monolayer. It was convenient to mount this preparation in the opposite way so that the current records are reversed. Note this preparation did not respond to ovalbumin but did to forskolin.

inappropriate chloride secretion, presumably resulting from an interaction of βLG with sensitized immunocytes in the lamina propria.

One experiment using guinea-pig peritoneal cells combined with an HCA-7 monolayer is illustrated in Figure 8 and is typical of several experiments. The responses are very small compared to those obtained with rat peritoneal cells, for example using 8 million cells the response was less than 2 nEq, equivalent to less than 0.5 million rat peritoneal cells (Figure 2). Apart from demonstrating a 'concentration-dependence' (Figure 8) for guinea-pig peritoneal cells, the responses were too small to allow systematic study.

Discussion

Immune responses are not thought to be mediated by epithelial cells but by associated lymphoid tissue which in the intestine makes up 25% of the mucosal mass (Kagnoff, 1984). We have shown in earlier publications that Type 1 reactions between cells of the immune system contained within the lamina propria and appropriate antigens leads to a stimulation of chloride secretion by the epithelial cells (Cuthbert *et al.*, 1983; Baird *et al.*, 1985; 1987). In these studies, tissues were sensitized either with oral antigens, such as βLG, or by infection with nematode parasites. The ensuing hypersensitivity reactions triggered by antigens were mediated by IgG or IgE antibodies respectively.

In this paper we have extended the study of immediate hypersensitivity reactions in guinea-pig intestinal epithelia sensitized to βLG. We have shown that TTX inhibits the epithelial chloride secretory response in a concentration-dependent manner (Figure 6, Table 1). At the same time TTX inhibited the SCC responses to EFS. While this latter effect is due to inhibition of neural effects of secretion (Hubel, 1984) the action of TTX on the antigenic stimulus is unexplained. There are at least two possibilities which may provide an explanation; first, mediators, released by antigen-antibody reactions, presumably at mucosal mast cells, might secondarily affect nerve cell bodies in the lamina propria which in turn release neurotransmitters which then cause epithelial chloride secretion. Secondly, the antigen-antibody reaction at the mast cell surface may involve a voltage-sensitive, electrical excitatory event, sensitive to TTX. In trying to decide between these possibilities the idea of the epithelial monolayer-mast cell sandwich was conceived.

We chose the rat peritoneal cells for our preliminary study as this preparation has been widely used to study histamine release in response to chemical and immunological stimuli (Kazimierczak & Diamant, 1978). We have used ovalbumin as a sensitizing antigen as it has been shown that this yields epithelia which show anaphylaxis upon challenge (Russell & Castro, 1985), a finding we have confirmed (Figure 1).

Using HCA-7 monolayers combined with rat peritoneal cells we demonstrate there is a concentration-dependence with a threshold around 0.4×10^6 cells, although we have not established the concentration of cells causing the maximal response. The concentration-dependence curve (Figure 2) was smoother when results were related to the maximal response to forskolin, presumably because it takes into account varying transport capability of different epithelial monolayers. We have also shown that the SCC effect following antigen challenge was significantly inhibited by mepyramine, supporting the well known fact that histamine is released from sensitized rat peritoneal cells with antigenic stimuli.

We have commented earlier about the apparently low value for the affinity of mepyramine in HCA-7 monolayers. As histamine effects can only be elicited when added to the basolateral side of the epithelia, both it and mepyramine were added on that side only. These agents had to diffuse through the Millipore filter to reach the basolateral surface, furthermore diffusion paths between cells may have allowed the agents to find ways into the apical bathing solution. In these circumstances neither the agonist nor antagonist concentrations at the basolateral surface may have achieved that in the bulk bathing fluid.

We conclude therefore that the responses in our reconstructed epithelia made with rat peritoneal cells are due to histamine release from mast cells. However rat peritoneal mast cells liberate other agents in response to antigen challenges, for example 5-hydroxytryptamine (5-HT), leukotrienes and kinins, or at least kininogenases (Proud *et al.*, 1982). HCA-7 and HCA-7-Colony 1 monolayers are insensitive to 5-HT. Although 5-HT stimulates chloride secretion in guinea-pig isolated ileal epithelium it is likely that the effect is indirect (Keast *et al.*, 1985) which may explain, in part, inhibition of the response to βLG challenge by TTX.

We have not made a systematic study of all possible mediators here as our main aim is to describe a feasible method that might be used with sorted cells of human origin. However, the virtually complete eradication of the response by mepyramine suggests that histamine is a major mediator released from rat peritoneal mast cells. It seems unlikely that kinins *per se* are liberated when ovalbumin reacts with peritoneal mast cells as no response was obtained from the apical side of the monolayer and further a cross-desensitization protocol failed to provide any evidence for kinin release. These same procedures could be used to examine for released kallikrein by adding kininogen to the bathing fluid.

A functional interaction between mast cells and neurones was proposed by Lewis (1927) and recently

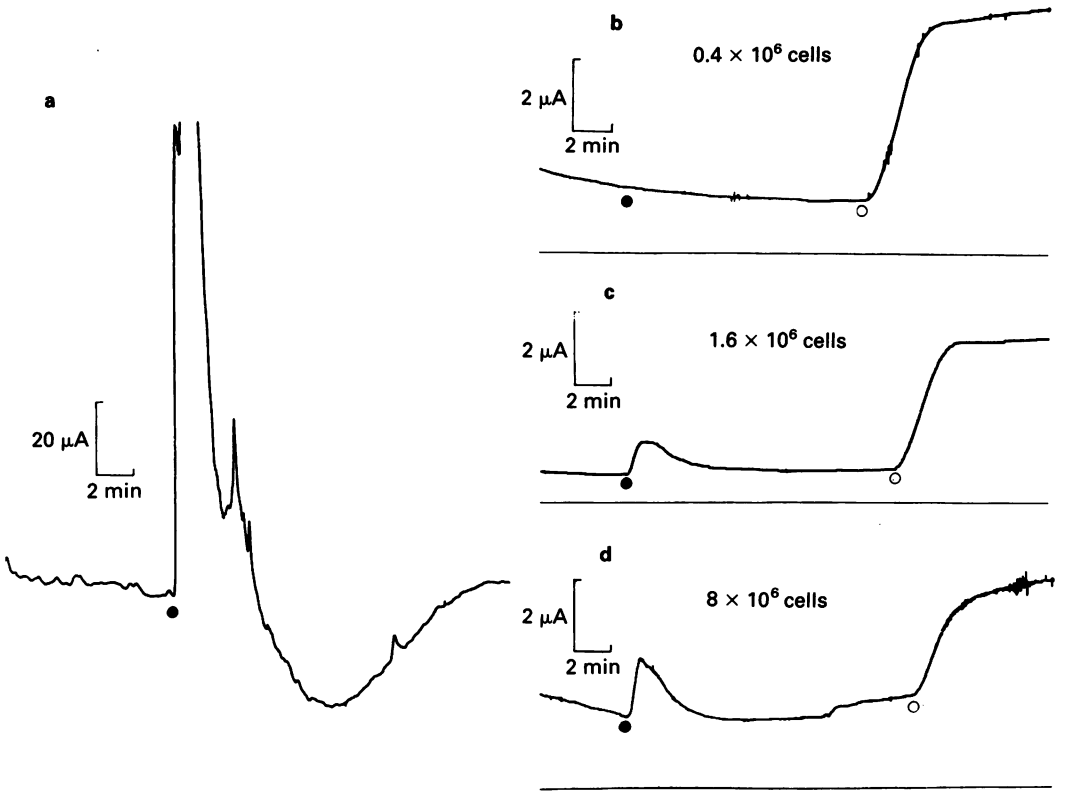

Figure 8 SCC from HCA-7 monolayers sandwiched with peritoneal cells from a guinea-pig sensitised to β-lactoglobulin (βLG) by milk drinking: (a) shows the response of a guinea-pig colon epithelium (0.6 cm^2) challenged with βLG (20 $\mu g\ ml^{-1}$); (b), (c) and (d) show three sandwich preparations made with 0.4, 1.6 and 8.5 × 10^6 peritoneal cells respectively. Each preparation was challenged with βLG (20 $\mu g\ ml^{-1}$) (●) and forskolin (10 μM) (○).

reviewed by Foreman & Piotrowski (1984). In the model system without neurones there was no evidence that TTX could inhibit the epithelial hypersensitivity reaction upon antigenic challenge, rather there was an indication that responses were potentiated by TTX. This result differs in a fundamental way from the result with the guinea-pig epithelium with intact lamina propria. The Schultz-Dale reaction of the longitudinal muscle strip is unaffected by TTX (Dale & Zilletti, 1970) indicating that intrinsic neuronal nets are not involved in mast cell-mediated contraction. However, γ-aminobutyric acid (GABA) does reduce the response to antigen challenge, an effect which is blocked by TTX, indicating that inhibitory neurones can interact with the system (Luzzi *et al.*, 1985). We can find only a single reference for an interaction of TTX with rat peritoneal mast cells, where it was shown that the release of histamine by exaprolol was inhibited by TTX, 1 μM, (Nosal *et al.*, 1982). We suggest the available evidence indicates that the effect of TTX on the hypersensitivity reaction favours the view that neuronal elements in the lamina propria are involved.

Our results with guinea-pig peritoneal cells sensitized to βLG confirm the generally held view that the peritoneal fluid in this species contains few mast cells. However, we have not shown the nature of the mediator(s) or the cell-type from which it is derived. It is clear, however, that feeding with cows' milk is able to sensitize some cellular components in the peritoneal fluid as well as immunocytes in the lamina propria of the gut. It will be necessary to use sorting procedures to concentrate the reactive cells in order to determine both the nature of the mediators and the cell types involved.

While this paper was being prepared Lazarus *et al.* (1986) described an interaction of mast cells mediators derived from chopped sensitized lung fragments challenged with ragweed antigen and canine tracheal epithelium. In this study supernatants were added to the fluid bathing the epithelium. The direct coupling of

the immunoctyes with the epithelium that we have described has some advantages, particularly if very labile mediators are released. More importantly our method can be developed for use with biopsy material and therefore has the potential of being used to examine individual human allergic conditions.

There remains, however, a further consideration which relates to the nature of the mast cells in the lamina propria of the intestine. In the rat, for example, it is known that antigen challenge in sensitized epithelia liberates histamine from mucosal mast cells (Perdue *et al.*, 1984) but, more importantly, mucosal mast cells have different properties from those found in the peritoneum (Befus *et al.*, 1982). Currently, methods are becoming available for the isolation of mast cells from a number of tissue sources and these could also be used in the sandwich configuration we have described. Theoretically, we would need to repeat our experiments using monolayers derived from guinea-pig ileal epithelium and mucosal mast cells from the same source to answer definitively the question we have posed. However, we consider our method offers unique opportunities to investigate some disease-related conditions. For example, it is possible now to culture epithelial monolayers from human airway epithelia from autopsy material (Widdicombe *et al.*, 1985; Welsh, 1986) or from nasal polyps removed at surgery (Yankaskas *et al.*, 1985). Similarly, pulmonary mast cells and basophil leucocytes, both of which release histamine in response to anti-human IgE (Leung *et al.*, 1986) are among the diverse types of cells obtained by human bronchoalveolar lavage. Development of cell separation techniques would not only allow identification of cell types involved in an epithelial interaction, but the method also provides an assay system for the mediators released. Such *in vitro* test systems using human tissues could be a useful adjunct to those used in patients to detect local allergic reactions, such as the nasal challenge test (Proud *et al.*, 1983). Finally our method provides an alternative way of investigating what is being increasingly recognised as an important pathophysiological mechanism, namely the immunological regulation of epithelial function (Castro, 1982).

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References

ATKINSON, G., ENNIS, M. & PEARCE, F.L. (1979). The effect of alkaline earth cations on the release of histamine from rat peritoneal mast cells treated with compound 48/80 and peptide 401. *Br. J. Pharmac.*, **65**, 395–402.

BAIRD, A.W., BARCLAY, W.S., BLAZER-YOST, B.L. & CUTHBERT, A.W. (1987). Affinity purified IgG transfers immediate hypersensitivity to guinea-pig colonic epithelium *in vitro*. *Gastroenterology*, **92**, 635–642.

BAIRD, A.W., CUTHBERT, A.W. & PEARCE, F.L. (1985). Immediate hypersensitivity reactions in epithelia from rats infected with Nippostrongylus brasiliensis. *Br. J. Pharmac.*, **85**, 787–795.

BEFUS, A.D., PEARCE, F.L., GAULDIE, J., HORSEWOOD, P. & BIENENSTOCK, J. (1982). Mucosal mast cells. Isolation and functional characteristics of rat intestinal mast clls. *J. Immunol.*, **128**, 2475–2480.

CASTRO, G.A. (1982). Immunological regulation of epithelial function. *Am. J. Physiol.*, **243**, G321–G329.

CUTHBERT, A.W. & MARGOLIUS, H.A. (1982). Kinins stimulate net chloride secretion by the rat colon. *Br. J. Pharmac.*, **75**, 587–598.

CUTHBERT, A.W., McLAUGHLAN, P. & COOMBS, R.R.A. (1983). Immediate hypersensitivity reaction to β-lactoglobulin in the epithelium lining the colon of guinea-pigs fed cows' milk. *Int. Archs Allergy appl. Immun.*, **72**, 34–40.

CUTHBERT, A.W., KIRKLAND, S.C. & MacVINISH, L.J. (1985). Kinin effects on ion transport in monolayers of HCA-7 cells, a line from a human colonic adenocarcinoma. *Br. J. Pharmac.*, **86**, 3–5.

CUTHBERT, A.W., EGLEME, C., GREENWOOD, H., HICKMAN, M.E., KIRKLAND, S.C. & MacVINISH, L.J. (1987). Calcium and cyclic AMP mediated chloride secretion in human colonic epithelia. *Br. J. Pharmac.*, (in press).

DALE, M. & ZILLETTI, L. (1970). The Schultz-Dale response of the longitudinal muscle strip preparation of guinea-pig ileum. *Br. J. Pharmac.*, **39**, 542–555.

DHARMASATHAPHORN, K., McROBERTS, J.A., MANDEL, K.G., TISDALE, L.D. & MASUI, H. (1984). A human colonic tumour cell line that maintains vectorial electrolyte transport. *Am. J. Physiol.*, **246**, G204–G208.

FOREMAN, J.C. & PIOTROWSKI, W. (1984). Peptides and histamine release. *J. Allergy clin. Immunol.*, **74**, 127–131.

GRASSET, E., PINTO, M., DUSSAULX, E., ZWEIBAUM, A. & DESJEUX, J.F. (1984). Epithelial properties of human colonic carcinoma cell line Caco-2: electrical properties. *Am. J. Physiol.*, **247**, C260–C267.

HANDLER, J.S. (1983). Use of cultured epithelia to study transport and its regulation. *J. exp. Biol.*, **106**, 55–69.

HUBEL, K.A. (1984). Electrical stimulus-secretion coupling in rabbit ileal mucosa. *J. Pharmac. exp. Ther.*, **231**, 577–582.

KAGNOFF, M.R. (1981). Immunology of the digestive system. In *Physiology of the Gastrointestinal Tract.* ed. Johnson, L.R. pp. 1337–1359. New York: Raven Press.

KAZIMIERCZAK, W. & DIAMANT, B. (1978). Mechanisms of histamine release in anaphylactic and anaphylactoid reactions. *Prog. Allergy*, **24**, 295–365.

KEAST, J.R., FURNESS, J.B. & COSTA, M. (1985). Investigations of nerve populations influencing ion transport that can be stimulated electrically, by serotonin and by a nicotinic agonist. *Naunyn Schmiedebergs Arch. Pharmac.*,

331, 260–266.

LAZARUS, S.C., McCABE, L.J., NADEL, J.A., GOLD, W.M. & LEIKAUF, G.D. (1986). Effects of mast cell-derived mediators on epithelial cells in canine trachea. *Am. J. Physiol.*, **251,** C387–C394.

LEUNG, K.B.P., FLINT, K.C., BROSTOFF, J., HUDSPITH, B.N., JOHNSON, N. McI. & PEARCE, F.L. (1986). Some properties of mast cells obtained by human bronchalveolar lavage. *Agents & Actions*, **18,** 110–112.

LEWIS, T. (1927). *The Blood Vessels of the Human Skin and their Responses.* London: Shaw & Sons.

LUZZI, S., FRANCHI MICHELI, S., SPUGNESI, S., ROSI, E., CIUFFI, M. & ZILLETTI, L. (1985). Inhibition of anaphylactic histamine release *in vitro* by GABA. *Agents & Actions*, **16,** 160–162.

NOSAL, R., PECIVOVA, J. & DRABIKOVA, K. (1982). Evidence for intracellular histamine liberation in isolated rat mast cells. *Agents & Actions*, **12,** 601–607.

PADAWER, J. & GORDON, A.S. (1956). Cellular elements in the peritoneal fluid of some mammals. *Anat. Rec.*, **124,** 209–222.

PERDUE, M.H., FORSTNER, J.F., ROOMI, N.W. & GRANT GALL, D. (1984). Epithelial response to intestinal anaphylaxis in rats: goblet cell secretion and enterocyte damage. *Am. J. Physiol.*, **247,** G632–G637.

PROUD, D., SCHULMAN, E.S., MacGLASHAN, D.W., PIERCE, J.V. & NEWBALL, H.H. (1982). Anaphylactic release of a kininogenase from purified human lung mast cells. *Clin. Res.*, **30,** 165A.

PROUD, D., TOGIAS, A., NACLERIO, R.M., CRUSH, S.A., NORMAN, P.S & LICHTENSTEIN, L.M. (1983). Kinins are generated *in vivo* following nasal airway challenge in allergic individuals with allergen. *J. clin Invest.*, **72,** 1678–1685.

RUSSELL, D.A. & CASTRO, G.A. (1985). Anaphylactic-like reaction of small intestinal epithelium in parasitised guinea-pigs. *Immunology*, **54,** 573–579.

WELSH, M.J. (1986). The apical-membrane chloride channel in human tracheal epithelium. *Science*, **232,** 1648–1650.

WIDDICOMBE, J.H., WELSH, M.J. & FINKBEINER, W.E. (1985). Cystic fibrosis decreases the apical membrane chloride permeability of monolayers cultured from cells of tracheal epithelium. *Proc. natn. Acad. Sci. U.S.A.*, **82,** 6167–6171.

YANAKASKAS, J.R., COTTON, C.U., KNOWLES, M.R., GATZY, J.T. & BOUCHER, R.C. (1985). Culture of human nasal epithelia cells on collagen matrix supports. *Am. Rev. Resp. Dis.*, **132,** 1281–1287.

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