Direct secretory effect of interleukin-1 via type I receptors in human colonic mucous epithelial cells (HT29-Cl.16E)

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Abstract

The stable differentiated human colonic epithelial cell line, HT29-Cl.16E, was used to study the effects of interleukin-1 (IL-1) on mucin exocytosis. The main findings include: (a) IL-1 stimulated a rapid release of mucin from filter grown HT29-Cl.16E cells, this effect being dose related; (b) this secretory effect was abolished in the presence of the blocking monoclonal antibody M4 specific for IL-1 receptors type I, showing that IL-1 receptors type I mediated IL-1 action; (c) experiments based on chamber cultures showed that these receptors were located on the basolateral membranes of HT29-Cl.16E cells; (d) finally, mRNA for IL-1 receptors type I were detected by reverse transcriptase-polymerase chain reaction in these cells. To extend these findings to the in vivo situation, the rapid stimulatory effect of IL-1 on mucin exocytosis may contribute to the wash out of noxious agents during mucosal inflammation.

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Interleukin-1 (IL-1) is a proinflammatory cytokine, present in the gastrointestinal mucosa, where it is released by lamina propria activated immune cells during inflammation.¹ IL-1 has been found to induce mucin release from explant cultures of mouse duodenum,² this finding suggesting that IL-1 may reinforce the defence of the intestinal mucosa during inflammation. The secretory effect of IL-1 seen in mucosal explants, which are the site of many cellular interactions, could be the result either of a direct effect of IL-1 on intestinal epithelial mucous cells or of an indirect effect through the release of a secretagogue secreted by another cell type. Finally, both direct and indirect pathways may account for the secretagogue action of this cytokine.

On the one hand the indirect pathway is supported by recent in vivo experiments in the rat showing that IL-1 mediated colonic hypersecretion entails mast cell degranulation.³ On the other hand, the recent finding of IL-1 receptors type I on the rat epithelial intestinal cell line IEC-18 strongly supports the hypothesis of a direct action of IL-1 on epithelial cells.⁴ As IEC-18 cells are considered undifferentiated,⁴ however, they are not suitable for

assigning a biological role to the IL-1 receptors type I present at the epithelial cell surface.

The ideal system for (a) providing unambiguous evidence that a direct pathway is implicated in the secretory effect of IL-1 on intestinal epithelial mucous cells, the so called goblet cells, and (b) deciphering this pathway, would be a pure population of epithelial mucous cells maintained in culture. An in vitro model having these characteristics is the so called HT29-Cl.16E cell line, a human homogeneous colonic epithelial goblet cell line, which is a clonal derivative of HT29 cells.⁵ Recent studies based on this cell line used in combination with a sensitive and specific assay for secretory mucins⁶ have clearly shown that HT29-Cl.16E cells have in vitro the same functional characteristics as those of goblet cells in vivo.^{6,7}

Here we show that IL-1 induces a rapid and dose related exocytotic release of mucins from HT29-Cl.16E cells, and that this effect is mediated by IL-1 receptors type I located at the basolateral surface of these cells.

Methods

Cell culture

HT29-Cl.16E cells were seeded on porous nitrocellulose filters (Millipore filters HAHY, porosity 0.45 µm; 2×10⁶ cells per filter) as previously described.⁶ For the experiments aimed at determining the location of IL-1 membrane receptors, HT29-Cl.16E cells were plated on filters mounted in chamber cultures (Millicell culture plate inserts, 0.45 µm porosity, Millipore, 1.2×10⁶ cells per chamber), which delineate an apical (luminal) and a basolateral (serosal) reservoir. The cells were cultured in Dulbecco's modified medium (DMEM, Gibco, Paris, France) supplemented with 10% heat inactivated fetal calf serum (FCS, Gibco). HT29-Cl.16E cells form at confluency homogeneous monolayers of differentiated goblet cells, secreting a mucus gel in the culture medium.⁵

Stimulation of mucin exocytosis by IL-1 and measurement of secretory mucins

Filter grown HT29-Cl.16E cells were metabolically labelled for 24 hours with 4 µCi/ml of D[6-³H]-glucosamine as previously reported.⁶ The cells were then rinsed and incubated for 15 minutes in the presence of IL-1. The incubation medium was then removed by a pipette and the monolayer was rinsed with the

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spent medium to remove adherent mucins. The collected medium was then dialysed against distilled water, and mucins were measured as ^3H -labelled macromolecules trapped at the stacker-gel/interface of 3% polyacrylamide gels, as previously described.⁶

RNA extraction and reverse transcriptase-polymerase chain reaction (RT-PCR)

Total cellular RNA was extracted from HT29-Cl.16E cells with RNAzol (Bioprobe, Montreuil, France), in a one step procedure according to the manufacturer's instructions. Ten μg of total RNA was reverse transcribed using oligodT₁₂₋₁₈ primer (Clontech). Five μl of the resulting cDNA was submitted to PCR using specific primers for IL-1 receptors type I from Clontech (sense: 5'-ACA CAT GGT ATA GAT GCA GC-3', and antisense: 5'-TTC CAA GAC CTC AGG CAA GA-3'). Amplification was performed for 30 cycles in an automated thermal cycler (Hybaid) (denaturation one minute at 94°C, annealing one minute at 60°C, extension two minutes at 72°C). Appropriate controls were done for each PCR reaction: positive control (control cDNA, Clontech), negative controls (one without RNA, and one without RT to rule out any contamination by genomic DNA). PCR products were run on a 1.6% agarose gel, visualised after ethidium bromide staining, blotted onto nitrocellulose filters (Schleicher and Schuell), and hybridised with a ^{32}P -labelled human IL-1 receptor type I cDNA probe, using a standard Southern blot procedure.

Reagents

The ^3H -glucosamine used was from Amersham, Paris, France (specific activity 20 to 40 Ci/mmol). IL-1 β , referred to as IL-1 if not otherwise stated, was from R&D Systems (Oxford, England), IL-1 α from Boehringer-Mannheim (Meylan, France). The blocking monoclonal antibody M4 directed to IL-1 receptors type I, and the cDNA probe specific for IL-1 receptors type I were kind gifts from Dr Sims (Immunex Corporation, Seattle). Specificity of the M4 antibody has been assessed by immunoprecipitation of metabolically labelled or ^{125}I -IL-1 β cross linked labelled cell lysates,^{8,9} showing unambiguously that only the 80 kDa form (IL-1 receptors type I) was recognised by this antibody.

Results

The addition of IL-1 to filter grown monolayers of HT29-Cl.16E cells elicited a rapid stimulation of ^3H -mucin release within the 15 minute incubation. This secretory effect was dose related for both IL-1 α and IL-1 β (Fig 1). At its maximal effective concentration (10^{-9} M), IL-1 elicited a 1.7 to twofold stimulation of ^3H -mucin release.

The recent availability of monoclonal antibodies that specifically block IL-1 receptors type I,^{8,9} makes it possible to test the

Figure 1: Dose related effect of IL-1 on HT29-Cl.16E mucin exocytosis. Filter grown HT29-Cl.16E monolayers were labelled with ^3H -glucosamine for 24 hours and then challenged for 15 minutes with either IL-1 β (■) or IL-1 α (●). ^3H -mucins were assessed as described in Methods. The results are expressed as per cent stimulation of mucin release over baseline secretion. Values are means (SEM) of nine monolayers from three different experiments, for each cytokine.

implication of these receptors in IL-1 action on HT29-Cl.16E cells. To this end, HT29-Cl.16E monolayers were preincubated for 90 minutes in the presence of M4 (5 $\mu\text{g}/\text{ml}$) and then challenged with IL-1 for 15 minutes. In such conditions, the stimulatory effect of IL-1 was completely abolished by M4 antibody (Fig 2). The finding that the exocytotic

Figure 2: Inhibition of IL-1 induced secretory response by M4 monoclonal antibody directed to IL-1 receptor type I. HT29-Cl.16E monolayers were pre-treated (M4) or not (Control), for 90 minutes with 5 $\mu\text{g}/\text{ml}$ M4 antibody, before being challenged for 15 minutes with 10^{-10} M IL-1 β or with 10^{-6} M vasoactive intestinal peptide (VIP). ^3H -mucins were assessed as described in Methods. The results are expressed as dpm per million cells. The stimulation of the exocytotic response elicited by IL-1 β and VIP was statistically significant compared with baseline secretion ($p < 0.001$), and the inhibitory effect of M4 on IL-1 β stimulatory effect was statistically significant ($p = 0.004$ between IL-1 β and IL-1 β +M4). Values are means (SEM) of nine monolayers for IL-1 and IL-1+M4 (three independent experiments), and of three monolayers for VIP and VIP+M4.

response to vasoactive intestinal peptide remained unchanged in the presence of M4 antibody (Fig 2) is a clear indication that the antibody added to the incubation medium did not exert in itself a non-specific inhibition of the exocytotic response.

As several studies have shown that most neuroendocrine receptors are restricted to the basolateral membrane facing the internal medium, it was important to examine the location of the receptors for the cytokine IL-1. For this reason, HT29-Cl.16E monolayers were cultured in chambers, which permits the incubation of the cytokine either at the apical (mucosal) or basolateral (serosal) pole of the cells. In such conditions, IL-1 stimulated ³H-mucin exocytosis when added to the basolateral reservoir mean (SEM) 153% (13%) of baseline secretion, n=4 monolayers), whereas it had no effect when added to the apical reservoir (97% (5%) of baseline secretion, n=4 monolayers).

Finally, the presence of mRNA coding for IL-1 receptors type I was determined using RT-PCR. Figure 3 shows a representative experiment where RT-PCR performed on RNA extracted from confluent HT29-Cl.16E monolayers generated a 300 bp product (lane 5), the specificity of which was confirmed by Southern blot analysis, using an IL-1 receptor type I cDNA probe. Figure 3 also shows that IL-1 receptors type I are not only present in the mucous secreting cells HT29-Cl.16E, but also in the parental cell line HT29 (lane 4) and in another clonal derivative, HT29-Cl.19A (lane 3), which is an electrolyte secreting cell line.⁵

Discussion

This study based on a human cell line extends the finding in a mouse model that IL-1 is able to induce mucin hypersecretion from the intestinal mucosa.² In addition, this study provides the first unambiguous demonstration that IL-1 directly stimulates mucin exocytosis from colonic epithelial cells via IL-1 receptors type I located at their basolateral membranes.

Figure 3: RT-PCR analysis of IL-1 receptor type I expression in HT29 and its clonal derivatives. RNA was extracted from confluent HT29-Cl.16E cells, HT29-Cl.19A, or the parental cell line HT29, and RT-PCR performed as detailed in Methods, with oligonucleotides specific for IL-1 receptor type I. The PCR product was run in ethidium bromide stained agarose gel (A), which was then subjected to Southern blot analysis using a cDNA probe directed to IL-1 receptor type I (B). At the extreme left of the gel: molecular weight markers (Φ X174/HaeIII DNA fragments); lane 1: negative control (no RNA), lane 2: negative control (no reverse transcription); lane 3: HT29-Cl.19A; lane 4: HT29; lane 5: HT29-Cl.16E; lane 6: positive control cDNA fragment (Clontech).

Our experimental evidence includes (a) the finding that IL-1 triggers a dose dependent exocytotic response from a pure population of mucous cells in culture devoid of any other contaminating cell type, (b) the strong inhibitory action of blocking monoclonal antibodies specific for IL-1 receptors type I on the stimulatory effect of IL-1, and (c) the polarised action of IL-1.

Intestinal goblet cells are structurally polarised epithelial cells whose main function is to protect the intestinal mucosa by secreting a mucus gel at their apical pole serving to protect the mucosa and to wash out pathogens present in the intestinal lumen. A number of studies based on in vivo or in vitro models have shown that the mucin exocytotic function of goblet cells may be modulated by various agents including neuroendocrine mediators acting via specific receptors located at the basolateral surface of the cells.¹⁰ In addition to being in itself a secretagogue for goblet cells, IL-1 may bring a number of soluble mediators (neurocrine and inflammatory mediators) in the vicinity of epithelial cells through mast cell degranulation and nervous stimulation. It can be thus suggested that epithelial goblet cells may be the target for potentiating interactions between IL-1 and some neurocrine agents or inflammatory mediators on mucus secretion. Further experiments are needed to explore this hypothesis.

Finally, the finding of a direct control of mucin exocytosis by immune mediators reveals new possibilities for the pharmacological manipulation of an epithelial important secretory function.

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