Polarised interleukin 8 secretion by HT 29/19A cells

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Abstract

Interleukin 8 (IL 8) is a neutrophil chemotactic and stimulating cytokine induced by various inflammatory stimuli, including tumour necrosis factor, interleukin 1, and endotoxin. The ability of HT 29/19A enteroctyes to synthesize interleukin 8 was studied. The results show that interleukin 1 is an important stimulus for interleukin 8 synthesis and secretion by HT 29/19A cells, being more potent than tumour necrosis factor. The tumour necrosis factor and interleukin 1 induced interleukin 8 secretion by HT 29/19A cells was seen to be polarised according to the direction of stimulation. These results support the concept that mucosal cells (enterocytes) may play an important part in initiating mucosal inflammation. Furthermore, it is proposed that HT 29/19A cells constitute a tool to study stimuli directed polarised cytokine secretion.

Methods

CELL CULTURE

HT 29/19A cells were grown in 25 cm² cell culture flasks (Costar Cambridge, USA) in Dulbecco’s Modified Eagle’s medium containing 4.5 g/l glucose (Life Technologies Ltd, Paisley Scotland). This medium was enriched with non-essential amino acids (1%) (Life Technologies Ltd, Paisley Scotland), fetal bovine serum (10%), and ciproxin (0.1%).

STIMULATION

Separate experiments were performed with cells grown in wells (Falcor, nr 3047, multiwell, 24 well, Becton & Dickinson, New Jersey) and on microporous filters (Falcor nr 353095, cyclo-pore membrane with 0.45 μm, pore size, diameter 9 mm, Becton & Dickinson, New Jersey) placed in wells. In all experiments, the cells were stimulated after complete confluency was reached. Monolayer confluency was tested on several occasions both morphologically after Giemsa staining and by measuring the electrical resistance over the monolayer (mean resistance (SEM) was 276 (16) Ω×cm², n=19). On each occasion, the electrical resistance was measured for three hours. Permeability studies performed in Ussing chambers showed that the agents used (including tumour necrosis factor and IL 1) did not affect the permeability of horseradish peroxidase, lactulose, and mannitol. Recombinant human tumour necrosis factor was obtained from Cetus Corporation (Emeryville, CA) and IL 1 from Medigenix (Fleurus, Belgium). Phorbol myristate acid and both calcium ionophores, ionomycin, and A23187 were obtained from Sigma Chemical Company Ltd (St Louis, USA). Several experiments were performed in this study. Firstly, cells grown in 24 well plate
wells were stimulated with a combination of phorbol myristic acid (100 ng/ml) and a calcium ionophore (ionomycin, 50 µg/ml or A23187, 200 nM) during 2, 4, 6, and 24 hours, at which time points the amount of IL 8 secreted was measured. In similar experiments tumour necrosis factor (100 ng/ml) and IL 1 (140 pg/ml) were used as the stimulus. Also, HT 29/19A cells were stimulated by graded concentrations of IL 1 (range: 1-1 pg/ml to 140 pg/ml) and tumour necrosis factor (range: 1-1 pg/ml to 140 ng/ml). The data presented represent mean values obtained in three experiments. In a second series of experiments, confluent HT 29/19A cell monolayers grown on microporous filters were stimulated apically or basally with tumour necrosis factor (100 ng/ml), IL 1 (140 pg/ml) or the phorbol myristic acid/A23187 combination. In this experiment the stimuli were added either above (upper compartment) or below (lower compartment) the cells, and the incubation time ranged from 24 to 48 hours. These experiments were performed in duplicate or triplicate and were repeated five to eight times.

IL 8 ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)
IL 8 was measured with a previously published ELISA. In brief, the ELISA wells were coated with anti-IL 8 monoclonal antibody (5 µg/ml). After removal of unbound antibody and blocking of the remaining non-specific binding sites, cell culture supernatants were added to the coated microtitre plate. After incubation, the plate was washed and a detecting antiserum enzyme conjugate of goat polyclonal anti-IL 8 antiseraum was added to the wells. After further incubation and subsequent washings, the bound goat antiserum was detected with p-NPP (1 mg/ml in diethanolamine buffer). The optical density values of the samples were read at 450 nm on an automated ELISA plate reader. The detection limit of this assay in our hands was 15 pg/ml. The measured IL 8 values were corrected for the volume of the supernatant and then expressed as IL 8/ng/10⁶ cells. One well contained about 14x10⁶ cells, one filter contained about 5x10⁶ cells.

STATISTICAL ANALYSIS
Differences between means were calculated with the Mann-Whitney U test, using the SPSS package (Chicago, Illinois). All reported significance levels represent two tailed p values.

Results
IL 1 AND TUMOUR NECROSIS FACTOR STIMULATE IL 8 SECRETION
After 24 hours of stimulation with tumour necrosis factor and IL 1, HT 29/19A cells secreted IL 8, whereas non-stimulated cells produced virtually no IL 8. A time course experiment with tumour necrosis factor and IL 1 resulted in a time dependent increase of IL 8 during the first four hours after addition of the stimulus (Fig 1). IL 1 and tumour necrosis factor induced IL 8 synthesis in a dose dependent way, IL 1 being a more potent stimulus than tumour necrosis factor (Fig 2). The optimal dose for IL 1 was 4-4 pg/ml, for tumour necrosis factor 1-1 ng/ml. The IL 8 concentrations measured at these optimal stimulation doses were 1-13 (0-03) IL 8/ng/ml (for IL 1) and 1-53 (0-23) IL 8/ng/ml (for tumour necrosis factor).

POLARISED IL 8 SECRETION
Stimulation of HT 29/19A cell monolayers with tumour necrosis factor (100 ng/ml) and IL 1 (140 pg/ml) at both the apical and basolateral side (Fig 3) induced a polarised IL 8 secretion that was higher at the side where the stimulus had been added (Fig 4). Hence, addition of the stimulus (either tumour necrosis factor or IL 1) to the upper compartment caused secretion of IL 8 that was significantly higher to the apical direction. Conversely, stimulation from the lower compartment resulted in a preferential secretion of IL 8 to the basolateral direction. Tumour necrosis factor added to the upper compartment caused IL 8 secretion that was 5-49 (2-69) IL 8/ng/10⁶ cells to the upper compartment, and 1-41 (0-88) IL 8/ng/10⁶ cells to the lower compartment (p=0-01). Tumour necrosis factor applied in the lower compartment caused IL 8 secretion that amounted 7-96 (1-72) IL 8/ng/10⁶ cells to the lower compartment and 3-58 (1-45) IL 8/ng/10⁶ cells to the upper compartment (p<0-01). The amount of IL 8 secreted after IL 1 application to the upper compartment was 1-69 (0-72) IL 8/ng/10⁶ cells to the lower compartment and 2-39 (0-9) IL 8/ng/10⁶ cells to the upper compartment (p=0-25). IL 1 applied to the lower compartment, however, induced a much larger amount of IL 8 to be secreted to the lower compartment (8-42 (1-56) IL 8/ng/10⁶ cells) than to the upper compartment (1-78 (0-66) IL 8/ng/10⁶ cells) (p<0-01). At the concentrations of 140 ng/ml (tumour necrosis factor) and 100 pg/ml (IL 1), tumour necrosis factor was a more powerful inducer of IL 8 secretion than IL 1 when applied in the upper compartment, 5-49 (2-69) > 2-39 (0-1) IL 8/ng/10⁶ cells in the upper compartment (p<0-01). When applied below, no significant differences in IL 8 secretion to the lower compartment were seen between tumour necrosis factor (7-96 (1-72) IL 8/ng/10⁶ cells) and IL 1 (8-42 (1-56) IL 8/ng/10⁶ cells). The polarised stimulus directed pattern of IL 8 secretion by
monolayer, the electrical resistance was measured. The results of these experiments suggest that the cytokines used do not change junctional permeability or cell viability (mean resistance (SEM) was 276 (16) Ω×cm²) and did not change.

**STIMULATION OF IL 8 SECRETION WITH PHORBOL MYRISTIC ACID/CALCIUM IONOPORE**

After stimulation with phorbol myristic acid/ ionomycin or phorbol myristic acid/A23187 combinations, HT 29/19A cells secreted large amounts of IL 8. After four hours of stimulation with phorbol myristic acid/ ionomycin and phorbol myristic acid/A23187 the IL 8 concentration in the supernatant increased to 3-01 (0-51) ng/ml and 1-69 (0-05) ng/ml respectively. After six hours the IL 8 concentrations further increased to 5-72 (0-20) ng/ml and 5-9 (0-16) ng/ml, and after 24 hours to 5-86 (0-03) ng/ml and 9-64 (2-71) ng/ml (a 13 to 21-fold increase). Surprisingly, in experiments with cells grown on microporous filters the combination of phorbol myristic acid/A23187 induced only IL 8 secretion when applied in the upper compartment. No IL 8 secretion was induced when phorbol myristic acid/A23187 was added in the lower compartment (Fig 4). To discover if phorbol myristic acid/A23187 applied to the lower compartment was adsorbed by the microporous filter, we cultured HT 29/19A cells in wells and stimulated the cells for 24 hours with phorbol myristic acid/A23187, which was added in a microporous filter insert that previously had been coated with HT 29/19A matrix proteins. In this system phorbol myristic acid/A23187 induced an IL 8 secretion from the HT 29/19A cells, showing that phorbol myristic acid/A23187 was not absorbed to the microporous filter. The combined action of phorbol myristic acid/calcium ionophore did not modify the electrical resistance of the monolayers.

**Discussion**

Our results show that IL 8 is secreted by HT 29/19A cells after stimulation with tumour necrosis factor and IL 1. These data complement and extend previous findings showing that primary cultures of human airway epithelial cells as well as human bronchial epithelial cell lines can secrete IL 8 upon stimulation with various stimuli. After stimulation with tumour necrosis factor and IL 1, the amount of IL 8 released progressively increased during the first four hours, suggesting that IL 8 was continuously produced and not merely released from internal storage. This finding is in line with other results showing that human bronchial epithelial cell lines express IL 8 in response to the inflammatory mediator tumour necrosis factor primarily by increasing the rate of transcription of the gene. The IL 8 concentration in both experiments decreased slightly, possibly because of degradation of IL 8. The decrease seen in the IL 8 concentration may have also resulted from a cytotoxic effect, because IL 1 in concentrations higher than 140 pg/ml was toxic for HT 29/19A cells. Appreciable IL 8 induction was seen when
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The combined action of phorbol myristic acid/calcium ionophore resulted in IL 8 secretion by the HT 29/19A cells. Phorbol myristic acid/calcium ionophore as a stimulus showed a time dependent IL 8 secretion similar to tumour necrosis factor. The phorbol myristic acid/calcium ionophore combination, however, was only effective in stimulating IL 8 release when applied to the upper compartment. Experiments performed to check if phorbol myristic acid/A23187 applied in the lower compartment was restricted by the filter.

tumour necrosis factor and IL 1 were added in concentrations that can be found in the mucosa of patients with ulcerative colitis. It remains unknown whether these cytokines trigger the same intracellular signalling pathways. It was recently shown, however, that viral infection can stimulate IL 8 secretion from bronchial epithelial cells in an IL 1 independent manner. This finding supports the concept that IL 8 secretion may be triggered by several pathways.

Surprisingly, after addition of the stimuli (tumour necrosis factor, IL 1) to the upper (apical) or lower (basolateral) compartments, HT 29/19A cells could 'sense' the direction from which they were stimulated and secreted IL 8 predominantly into that direction. The mechanisms that cause such polarised IL 8 secretion are incompletely understood. In the process of transport of membrane bound or secretory proteins, reorganisation of the cytoskeleton is considered to play an important part. It is well known that cytokines, such as tumour necrosis factor, may cause a rapid change in the cytoskeletal organisation in various cells. Cytoskeletal reorganisation that results from intercellular contact of immune cells, such as T cell-B cell contact, may direct cytokines intracellularly specifically to the area of the cell-cell contact. Our preliminary results, however, seem to refute an important role of actin filaments in the sorting of IL 8, as cytochalasin B, a drug that interferes with the actin filaments did not perturbate its sorting (data not shown). Another hypothesis is that IL 1 or tumour necrosis factor may cause changes in the apical to basolateral area ratio upon binding to their specific receptors. In fact the possibility exists that such changes may occur because it has recently been shown that an exogenous stimulus -- that is, a different growth support -- can induce a modification of the apical to basolateral surface area ratio of cultured MDCK cells. Consequently the secretory rate of IL 8 would be increased at the side showing the maximal surface area.

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Presumably, IL 8 plays an important part in inflammatory bowel disease, in particular in ulcerative colitis, which is histologically characterised by large amounts of infiltrating neutrophils. Increased IL 8 mRNA concentrations were detected in mucosal biopsy specimens of patients suffering from inflammatory bowel disease. The IL 8 that is released basolaterally by enterocytes may participate in the recruit-
ment and subsequent activation of neutrophils. At present we have no explanation for the biological importance of apical secretion of IL 8 by enterocytes. Interestingly, it has been recently reported that IL 1 can be a growth factor for Escherichia coli, and bacteria can produce an IL 1 like activity themselves. IL 1 has been detected in mucosal cells in vivo and in non-differentiated mucosal cells in experimental colitis. Enterocytes and bacteria may therefore interact by means of cytokine secretion. Although the mucosal cytokine networks have been incompletely characterized, the ability of mucosal cells to synthesize IL 1 and IL 8 is in accordance with the hypothesis that enterocytes, in addition to endothelial cells, peripheral blood monocytes infiltrated in the mucosa, and resident lamina propria mononuclear cells play an important part in the mucosal inflammatory cascade. In conclusion, HT 29/19A cells can produce large amounts of IL 8 after stimulation by IL 1 and tumour necrosis factor, at concentrations that may occur in inflammatory bowel disease. IL 8 secretion by HT 29/19A cells is polarized according to the direction of the stimulus. Therefore, HT 29/19A cells may be used to investigate the mechanisms of polarised cytokine release in more detail.

We are indebted to Dr J A Groot (Department of Experimental Zoology, University of Amsterdam) for his help in culturing HT 29 cells. We thank Jan Wouter van der Wilt and Harry Büller for the helpful discussions. Dr S J H van Deventer is supported by a grant from the Royal Dutch Academy of Arts and Sciences. P B Bijlsma is supported by a grant from Nutricia.


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