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NO-dependent and NO-independent IL-1 production by a human colonic epithelial cell line under inflammatory stress

[1]Geneviève Vallette, Anne Jarry, Philippe Lemarre, Jean-Eric Branka & Christian L. Laboisse

Groupe de Recherche 'Fonctions Sécrétoires des Epithéliums Digestifs', CJF INSERM 94-04, Faculté de Médecine, 1 Rue Gaston Veil, 44035 Nantes, France

1 The present study was designed to investigate, in an *in vitro* model of the human intestinal barrier, the ability of epithelial cells to produce interleukin-1 (IL-1), the cellular mechanisms involved in IL-1 release, and the intracellular signalling pathways involved in IL-1 up-regulation during inflammatory stress.

2 This study was based on the human colonic epithelial cell line HT29-Cl.16E, maintained as polarized monolayers on filters mounted in culture chambers and treated with various proinflammatory cytokines (interferon γ (IFNγ), tumour necrosis factor α (TNFα), IL-1β) alone or in combination.

3 IL-1 production, restricted to IL-1α, was induced by the combination of IFNγ/TNFα. When IL-1β was added to IFNγ/TNFα, it led to an additional production of IL-1α. IL-1α release was associated with cell damage, as shown by the correlation between lactate dehydrogenase (LDH) release and extracellular IL-1 production, and was not accounted for by a secretory mechanism.

4 Both IFNγ/TNFα and IFNγ/TNFα/IL-1β induced inducible nitric oxide synthase (iNOS) expression as shown by quantitation of NO_2^-/NO_3^- by use of the Griess reagent, quantitation of cells scoring positive with an anti-iNOS antibody and detection of mRNAs coding for iNOS by RT–PCR. The use of N^G-monomethyl-L-arginine (L-NMMA), an inhibitor of NOS, led to the demonstration of two distinct signalling pathways in IL-1 production by HT29-Cl.16E cells, one dependent on NO (L-NMMA-sensitive) under treatment with IFNγ/TNFα/IL-1β, and the other independent of NO (L-NMMA-insensitive) under treatment with IFNγ/TNFα.

5 Moreover, we examined whether a redox-based mechanism could be responsible for the apparent discrepancy between NO production and NO implication in IL-1 production under IFNγ/TNFα and IFNγ/TNFα/IL-1β treatments. Experiments with cysteine, which acts as a powerful reductant, suggest that the nitrosonium character of NO is involved in the NO-dependent pathway in IL-1 production.

Keywords: Human colonic epithelial cell line; inflammation; interleukin-1; cytokines; inducible nitric oxide synthase; nitric oxide; N^G-monomethyl-L-arginine

Introduction

Interleukin-1 (IL-1) is a proinflammatory cytokine, mainly produced by cells of the monocyte/macrophage series, which exerts a variety of biological functions including immunological upregulation (T- and B-cell activation, induction of other cytokines, recruitment and proliferation of immune cells), and proinflammatory activity (fever, release of acute phase reactants) (for a review, see Dinarello, 1994). IL-1 exists in two active forms, IL-1α and IL-1β. IL-1 is different from most secreted proteins in that the high molecular weight precursor does not possess any signal sequence and, therefore, has no entry into the secretory pathway (Dinarello, 1994).

IL-1 is a key mediator in bowel inflammation, but there is limited knowledge about its site of production, mechanism of release, and regulation of production in the intestinal mucosa. In fact, several lines of investigation have shown that in addition to the inflammatory/immune cells, epithelial cells can be a significant contributor to IL-1 production. Interestingly, Radema *et al*. (1991) have shown an early expression of IL-1 at the mRNA level in intestinal epithelial cells, at the onset of mucosal inflammation in a rat model of experimental acute colitis. This finding led to the concept that epithelial cells under inflammatory stress may contribute to increase inflammation by producing and releasing IL-1.

Therefore, the present study was designed to investigate (1) the capacity of a human colonic epithelial cell line (HT29-Cl.16E) to produce immunoreactive IL-1 (IL-1α and/or IL-1β) in culture, (2) the mechanism of IL-1 release from epithelial cells, and (3) the intracellular signalling pathways which lead to IL-1 up-regulation during inflammation. The conditions of inflammatory stress were created by adding to the incubation medium several proinflammatory cytokines (interferon γ (IFNγ), tumour necrosis factor α (TNFα), IL-1), known to be up-regulated in inflammatory bowel diseases (Fais *et al*., 1994; Casini-Raggi *et al*., 1995; Berg *et al*., 1996).

The results show that IL-1 is mainly produced as IL-1α by the human colonic epithelial cell line HT29-Cl.16E upon stimulation with various cytokines, that cell injury is involved in IL-1 release from these cells, and that NO-dependent and NO-independent signalling pathways are involved in IL-1 production.

Methods

Cell culture

The human colonic, mucus-secreting cell line HT29-Cl.16E (Augeron & Laboisse, 1984), a clonal derivative of the HT29 colonic carcinoma cell line (Fogh & Tremp 1975) was used as an *in vitro* model system. For the experiments, HT29-Cl.16E monolayers were formed by plating 1.2×10^6 cells on Millicell chamber inserts (0.6 cm^2; 0.45 μm porosity; Millipore). Cells were cultured in Dulbecco's modified Eagle medium (DMEM, Gibco-BRL) supplemented with 10% heat-inactivated foetal calf serum (FCS). HT29-Cl.16E cells were periodically checked

[1]Author for correspondence at: CJF INSERM 94-04, Faculté de Médecine, 1 Rue Gaston Veil, 44035 Nantes Cedex 01, France.

for mycoplasma contamination as previously described (Augeron & Laboisse, 1984), and always scored negative. HT29-Cl.16E cells, used at days 8–12 after post-seeding on chambers, formed confluent monolayers of homogenously polarized goblet cells secreting mucins at their apical surface.

Incubation of HT29-Cl.16E monolayers with cytokines

HT29-Cl.16E cells grown on chamber inserts were incubated with IFNγ (500 u ml^{-1}, Roussel-Uclaf), TNFα (500 u ml^{-1}, Boehringer Mannheim), IL-1α or IL-1β (R&D Systems), alone or in combination. These cytokines were added to the basolateral medium (DMEM/FCS 10%). Control cultures were incubated with medium alone. At various time points, incubation media (2 ml) were collected, and cell lysates (2 ml) were obtained by submitting the chamber-grown cells to three cycles of freezing and thawing. Supernatants and cell lysates were placed in aliquots and frozen at -20°C, before being assayed. In experiments aimed at assessing the action of IL-1 alone or in combination with IFNγ/TNFα on IL-1 production, either IL-1α or IL-1β was added to the incubation medium, depending on the IL-1 (β or α) assayed.

Enzyme-linked immunosorbent assay (ELISA)

IL-1α and IL-1β levels were measured in supernatants (extracellular production) and in cell lysates (intracellular production) by sensitive and specific non-cross-reacting ELISA (R&D Systems; standard limit of detection: 0.2 pg ml^{-1}). Results are expressed as pg ml^{-1} for extracellular IL-1, and as pg 10^6 cells^{-1} for intracellular IL-1. In some experiments, the IL-8 secreted in the supernatants was also measured by ELISA (R&D Systems). The results are expressed as ng ml^{-1} extracellular IL-8.

Measurement of cell viability

Cell viability was assessed by measuring lactate dehydrogenase (LDH) activity in both supernatants and cell lysates, by a standardized kinetic determination (Enzyline LDH kit, Biomérieux). The results are expressed as the percentage of LDH released, which equalled the amount of LDH released divided by the amount released plus the amount present in cell lysates.

Measurement of NO production

NO, quantified by the accumulation of nitrate (NO_2^-) and nitrite (NO_3^-) in the culture medium, was measured by use of a spectrophotometric assay (Cayman Chemicals) based on the Griess reagent (Green *et al*., 1982). The absorbance at 540 nm was measured with an Argus 400 microplate reader (Packard Instrument).

Determination of iNOS expression by immunocytochemistry

After treatment with IFNγ/TNFα or IFNγ/TNFα/IL-1 for 24 h or 48 h, HT29-Cl.16E cells were detached from their substrate by trypsin/EDTA. Cytospins were then performed, acetone-fixed, and stained with a polyclonal antibody directed to the C terminus of human iNOS (Santa Cruz Biotechnology Inc.) by use of a standard streptavidin-biotin-peroxidase technique (Dako, France). % of cells scoring positive with the anti-iNOS antibody was determined after counting the cells.

Determination of iNOS gene expression by reverse transcriptase-polymerase chain reaction (RT–PCR)

After IFNγ/TNFα or IFNγ/TNFα/IL-1 treatment or in control cultures, total RNA was extracted with RNAzol (Bioprobe, France) according to manufacturer specifications. Total RNA (5 μg) were reverse-transcribed with 50 u murine leukemia virus-reverse transcriptase (Stratagene) and 0.5 μg of oligodT primer (Stratagene) according to standard protocols. The resulting cDNA was PCR-amplified with specific primers for human iNOS (sense: 5′-GCAGGTCGAGGACTATTTCTTTC-3′, and antisense: 5′-CTTGTTAGGAGGTCAAGTAAAGGG-3′) or for human β-actin (Clontech). Denaturation, annealing and elongation steps were 94, 60 and 72°C respectively, for 1 min each and 25 cycles.

The specificity of the PCR products obtained with iNOS primers was checked by Southern blot. The PCR products were run in agarose gel (1%), transferred to a nylon membrane and hybridized overnight at 68°C with a cDNA probe obtained from Clontech. This cDNA was previously labelled in a PCR reaction containing digoxigenin-dUTP (Boehringer Mannheim) (Lo *et al*., 1994), and the detection was performed with a digoxigenin detection kit (Boehringer Mannheim).

Statistical analysis

Data are expressed as the mean ± s.e.mean of n experiments, with three monolayers per experiment. The significance of differences was assessed by ANOVA followed by Bonferonni test or by Student's t test. In both statistical analyses, $P<0.05$ was considered significant. Spearman's test was used to evaluate the correlation degree between IL-1α and LDH production in extracellular medium.

Results

IL-1 production by HT29-Cl.16E cells treated with various cytokines

In an initial series of experiments we tested the ability of several cytokines, i.e. IFNγ, TNFα, IL-1 alone or in combination to stimulate the production of IL-1 by HT29-Cl.16E cells (Table 1). At time-point 48 h, IL-1 was measured in the supernatants and cell lysates. Exposure to IFNγ or IL-1 alone had no effect on intracellular IL-1 over 48 h incubation. A slight but significant increase in intracellular IL-1 was detected with TNFα alone ($P<0.05$, ANOVA test), without any detectable IL-1 in the supernatants. In response to treatment with the combinations of IFNγ/TNFα or IFNγ/TNFα/IL-1, IL-1 was found to be increased in both intracellular and extracellular compartments. In addition, the IL-1 level (extra- or intracellular) achieved under IFNγ/TNFα/IL-1 treatment was significantly higher ($P<0.05$, ANOVA test) than under IFNγ/TNFα treatment.

Both IL-1α and IL-1β were assayed. Only IL-1α was found to be regulated by cytokines while IL-1β levels never differed from the resting conditions whatever the treatment.

Table 1 Intra- and extracellular IL-1α levels in HT29-Cl.16E cells treated for 48 h with various cytokines

	IL-1α	
Treatment	*Cell lysates* (pg 10^6 cells)	*Cell supernatants* (pg ml^{-1})
Controls	2 ± 0.1	0§
IFNγ	0§	0§
TNFα	6.2 ± 0.16*	0§
IL-1β	1.9 ± 0.2	0§
IFNγ/TNFα	8.2 ± 1*	5.5 ± 0.7*
IFNγ/TNFα/IL-1β	16 ± 3*	16 ± 4*

§Undetectable (< 0.2 pg ml^{-1}, standard limit of detection).
*$P<0.05$ vs control as assessed by one way analysis ANOVA test.
HT-29-Cl.16E monolayers were treated for 48 h with IFNγ, TNFα (500 u ml^{-1}) or IL-1β (10^{-9} M), added to the basolateral medium alone or in combination. The IL-1α produced was measured by ELISA, both in cell lysates and in cell supernatants. The data are the mean ± s.e.mean of 6 to 15 monolayers (2–5 experiments).

Mechanisms of IL-1 release from HT29-Cl.16E cells

In order to explore the mechanism underlying the extracellular release of IL-1, we first examined the time-course of intra- and extracellular IL-1 accumulation in response to IFNγ/TNFα treatment over a 72 h period. Figure 1 shows that the kinetics of IL-1 production can be divided into 2 phases. The first phase which extends to the 24 h time-point, consists of an intracellular peak of IL-1α after 10 h of incubation, without any release of IL-1 into the extracellular medium. The second phase beginning at 24 h, consists of an accumulation of IL-1, in both intracellular and extracellular compartments.

In order to determine whether the extracellular production of IL-1 which begins at 24 h could result from cellular injury caused by IFNγ/TNFα treatment, we measured the release of LDH over a 72 h incubation. As shown in Figure 2, LDH release began at 24 h, and then increased steadily up to 72 h. At this time point, cell lysis was about 40%. The levels of LDH released over time correlated with those of IL-1, as determined by Spearman's test ($P<0.001$).

In the same way as we tested the effect of IFNγ/TNFα on IL-1, a cytokine devoid of a signal peptide, we examined the effects of IFNγ/TNFα on IL-8, a signal peptide-containing cytokine whose secretion involves vesicular transport through the classical ER-Golgi pathway. As shown in Figure 3, IFNγ/TNFα treatment led to a rapid and dramatic secretion of IL-8, apparent as early as 5 h incubation. Together these findings suggest that IFNγ/TNFα-induced cytokine production from HT29-Cl.16E cells involves two distinct mechanisms, one consisting of cytokine release through cell lysis (IL-1), the other consisting of an acceleration of protein vesicular transport and secretion (IL-8).

Intracellular signalling pathways mediating the production of IL-1 in response to IFNγ/TNFα and IFNγ/TNFα/IL-1β

As iNOS is known to be induced by the combined action of inflammatory cytokines (IFNα, TNFα, IL-1β) in epithelial cells (Salzman *et al*., 1996) including the HT29 cell line (Kolios *et al*., 1995), we focused on iNOS as a potential mediator in the stimulation of IL-1 production. To detect iNOS induction, we used three complementary approaches: (1) quantitation of NO_2^-/NO_3^- levels by use of the Griess reagent, (2) quantitation of cells scoring positive with an anti-iNOS antibody, and

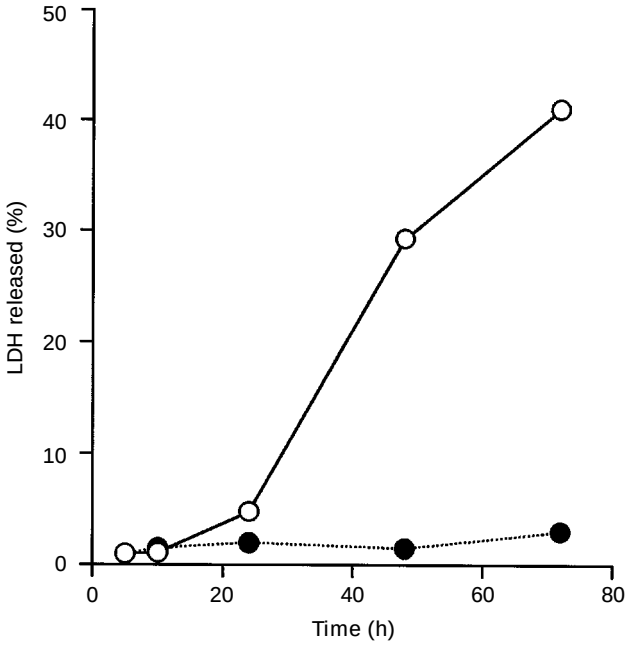

Figure 2 Time-course of LDH release in culture supernatants of IFNγ/TNFα-treated HT29-Cl.16E cells. HT29-Cl.16E monolayers were maintained in the presence (○) or absence (●) of IFNγ and TNFα (500 u ml^{-1} each). At each time point, LDH activity was assayed in both supernatants and cytosol as described in Methods. Means of 6 to 12 monolayers (2–4 experiments) are shown; vertical lines indicate s.e.mean. Data points without error bars indicate s.e.mean values less than the size of the symbol.

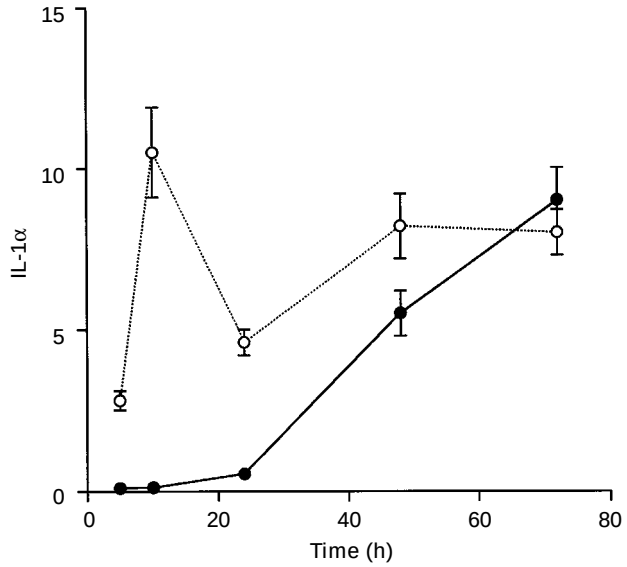

Figure 1 Intra- and extracellular IL-1α levels in IFNγ/TNFα-treated HT29-Cl.16E cells. HT29-Cl.16E monolayers were maintained in the presence of IFNγ and TNFα (500 u ml^{-1} each). At various time points, culture supernatants and cell lysates were collected as described in Methods. IL-1α concentrations were measured by ELISA and expressed as pg ml^{-1} in supernatants (●) and as pg 10^6 cells in cell lysates (○). Means of 6 to 12 monolayers (2–4 experiments) are shown; vertical lines indicate s.e.mean. Data points without error bars indicate s.e.mean values less than the size of the symbol.

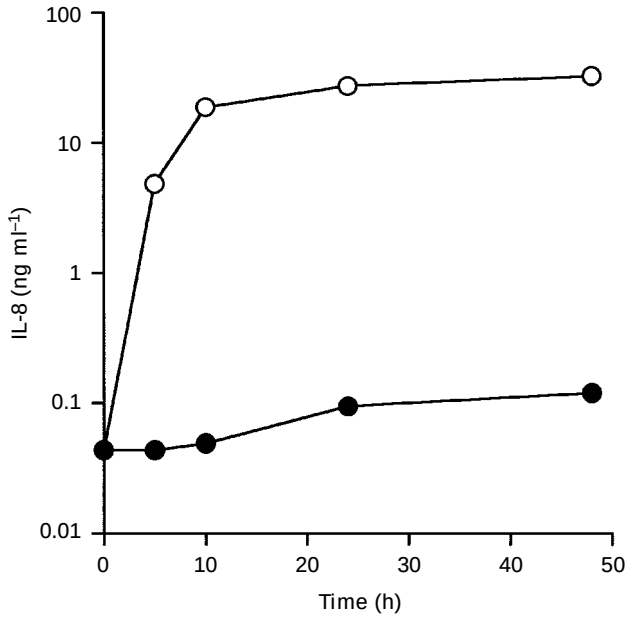

Figure 3 IL-8 secretion in culture supernatants of IFNγ/TNFα-treated HT29-Cl.16E cells. IL-8 concentrations were measured by ELISA, in culture supernatants of cells maintained in the presence (○) or absence (●) of IFNγ and TNFα (500 u ml^{-1} for each cytokine) at various time points. Means of 6 monolayers (2 experiments) are shown; s.e.mean values were less than the size of the symbol.

(3) detection of mRNAs coding for iNOS with RT–PCR. NO_2^-/NO_3^- levels were found to be significantly increased over baseline in response to treatment with IFNγ/TNFα or IFNγ/TNFα/IL-1β at 72 h (Figure 4). Table 2 shows a time-related increase in iNOS positive cells after treatment with IFNγ/TNFα and IFNγ/TNFα/IL-1β. No significant difference was observed between these two conditions at 48 h. Finally, we were able to detect mRNAs coding for iNOS in response to treatment with IFNγ/TNFα and IFNγ/TNFα/IL-1β. As shown in Figure 5a, an amplification product of 311 bp corresponding to the expected size for the iNOS fragment, was specifically obtained by RT–PCR with the RNAs from HT29-Cl.16E cells treated with IFNγ/TNFα or IFNγ/TNFα/IL-1β. This band was identified as iNOS by Southern blot analysis (Figure 5b). No band was observed with RNAs from control cells.

In order to examine whether the iNOS induction was causally related to IL-1 production, we examined the effects of N^G-monomethyl-L-arginine (L-NMMA), an inhibitor of iNOS on IL-1 production. While L-NMMA was able to block NO_2^-/NO_3^- production (Figure 4), it did not alter the IFNγ/TNFα-induced rise in IL-1 (Figure 6) whatever the dose of L-NMMA used (up to 1 mM, data not shown). Thus, IFNγ/TNFα-induced IL-1 synthesis is NO-independent. In contrast, L-NMMA blocked NO_2^-/NO_3^- production and inhibited the IL-1 rise induced by IFNγ/TNFα/IL-1β by 50% (Figure 6). This inhibition equally affected intra- and extracellular IL-1α production. D-NMMA used as a control had no inhibitory effect. L-NMMA brought the IL-1 level induced by IFNγ/TNFα/IL-1β back to that induced by IFNγ/TNFα. This finding demonstrates that the additional production of IL-1 resulting from the combined action of the 3 cytokines is NO-dependent.

In this context we reasoned that NO was (1) unable *per se* to induce IL-1 synthesis and (2) that an additional induction by IL-1 of a 'NO-responsive target' was needed to mediate the effects of IFNγ/TNFα/IL-1. As our recent findings showed that a NO donor with strong nitrosonium character

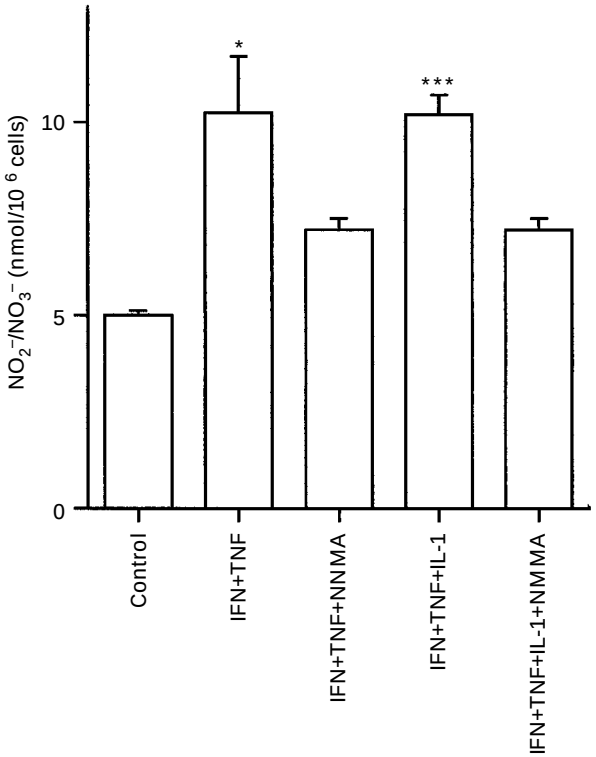

Figure 4 NO_2^-/NO_3^- levels in HT29-Cl.16E cells treated with IFNγ/TNFα or IFNγ/TNFα/IL-1β in the presence and absence of L-NMMA. Chamber-cultured cells were treated for 72 h with IFNγ/TNFα (500 u ml^{-1} each) or IFNγ/TNFα/IL-1β (10^{-9} M IL-1β) in the presence and absence of the iNOS inhibitor L-NMMA (500 μM). NO_2^-/NO_3^- levels were measured in the supernatants by use of Griess reagent (see Methods). Results are expressed as nmol NO_2^-/NO_3^- per 10^6 cells. Mean$\pm$s.e.mean of 6 monolayers (2 experiments). *$P<0.05$, ***$P<0.001$ vs control value as assessed by Student's t test.

Table 2 Immunocytochemical demonstration of inducible NO synthase (iNOS) expression in HT29-Cl.16E cells treated with IFNγ/TNFα or IFNγ/TNFα/IL-1β

	Time of incubation	
	24 h	*48 h*
Controls	0.4$\pm$0.2%	0.3$\pm$0.1%
[IFNγ/TNFα]-treated cells	2$\pm$0.8%	60.5$\pm$12.5%
[IFNγ/TNFα/IL-1β]-treated cells	2.2$\pm$0.2%	70$\pm$9%

HT29-Cl.16E monolayers were treated or not (control) with IFNγ/TNFα or IFNγ/TNFα/IL-1β (IFNγ and TNFα: 500 u ml^{-1}; IL-1β: 10^{-9} M) for 24 h or 48 h. Acetone-fixed cytospins were stained with an immunoperoxidase technique, by a polyclonal antibody directed to inducible NO synthase (iNOS), as mentioned in Methods. Results are expressed as the % of cells scoring positive with the iNOS antibody. Mean$\pm$s.e.mean of 3 separate experiments.

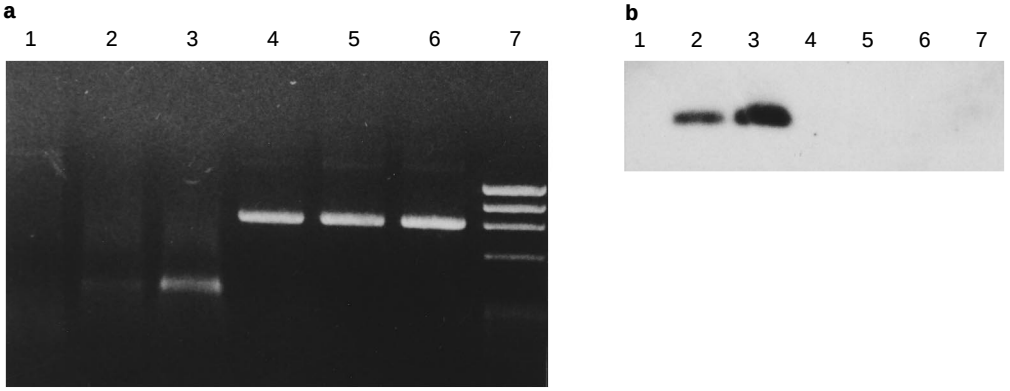

Figure 5 iNOS gene expression in control and IFNγ/TNFα- or IFNγ/TNFα/IL-1β-treated HT29Cl.16E cells. RT–PCR analysis of iNOS (lanes 1 to 3) and β-actin (lanes 4 to 6) expression in HT29-Cl.16E monolayers treated with IFNγ/TNFα, IFNγ/TNFα/IL-1β for 30 h. The PCR products were (a) run in ethidium bromide-stained agarose gel (1%) and (b) then transferred to a nylon membrane for Southern analysis with a digoxigenin-labelled iNOS probe. Lanes 1 and 4: control cells (no treatment); lanes 2 and 5: cells treated with IFNγ/TNFα; lanes 3 and 6: cells treated with IFNγ/TNFα/IL-1β; lane 7: molecular weight marker (ϕX174/HaeIII).

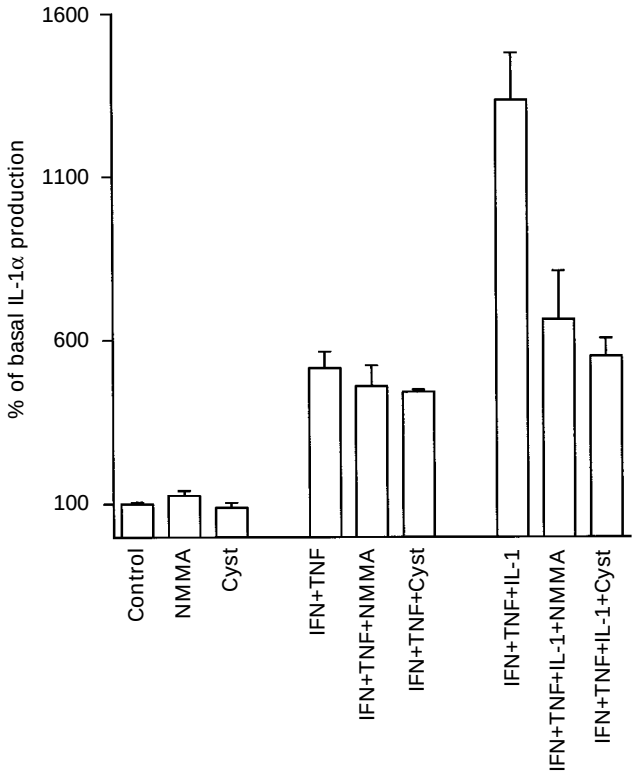

Figure 6 IL-1α production by IFNγ/TNFα- or IFNγ/TNFα/IL-1β-treated HT29-Cl.16E cells in the presence and absence of L-NMMA or cysteine. Chamber-cultured cells were treated for 72 h with IFNγ/TNFα (500 u ml^{-1} each) or IFNγ/TNFα/IL-1β (10^{-9} M IL-1β) in the presence and absence of the iNOS inhibitor L-NMMA (500 μM) or cysteine (cyst., 1.2 mM). Results are expressed as percentage of the basal total IL-1 production (intracellular + extracellular). Mean $\pm$ s.e. of 6 monolayers (2 experiments). Range of baseline IL-1α levels: 1.5–4.5 pg; range of maximal IL-1α levels under IFNγ/TNFα/IL-1β stimulation: 25–42 pg.

was able to induce IL-1 synthesis (Vallette *et al*., 1996), we elected to test whether this 'NO-responsive target' could behave as a NO^+ carrier. To this end, HT29-Cl.16E cells were incubated with IFNγ/TNFα/IL-1 in the presence of 1.2 mM cysteine. As shown in Figure 6, cysteine inhibited IL-1α induction in response to IFNγ/TNFα/IL-1β, the magnitude of this inhibitory effect being similar to that obtained with L-NMMA.

Discussion

Three main findings were obtained from this study: (1) IL-1α production by a human epithelial intestinal cell line was regulated by several cytokines acting in combination, (2) the extracellular release of IL-1 was dependent on cell damage as shown by the correlation with LDH release, and (3) there were two signalling pathways regulating cytokine-induced IL-1 production, i.e. a NO-independent and a NO-dependent pathway.

Several lines of investigation have shown that proinflammatory cytokines are able to affect several biological functions of intestinal epithelial cells including cell viability (Jarry *et al*., 1992), HLA-DR expression (Colgan *et al*., 1994; Kvale & Brantzaeg, 1995), macromolecular and ionic secretion (Jarry *et al*., 1994; Colgan *et al*., 1994; Besançon *et al*., 1994), NO production (Kolios *et al*., 1995), and IL-8 secretion (Lammers *et al*., 1994; Gross *et al*., 1995).

As for IL-1 production by intestinal epithelial cells, the regulation of production of this cytokine, as well as its mechanism of release, have remained poorly understood up to now. This situation is mainly due to the paucity of *in vitro* studies. For example, Panja *et al*. (1994) failed to demonstrate IL-1β expression in human isolated intestinal epithelial cells at the mRNA level. IL-1α but not IL-1β transcripts were found to be constitutively expressed in the rat epithelial cell line IEC6, and IL-1 bioactivity was found to be restricted to the intracellular compartment (Stadnyk *et al*., 1995). These authors failed to demonstrate any quantitative change in IL-1α production by cytokines in this model. However, various studies based on human keratinocytes in culture support the concept that IL-1 production by epithelial cells can be regulated by cytokines (Ansel *et al*., 1990; Phillips *et al*., 1995). This concept is further strengthened by our study demonstrating an upregulation of IL-1α by the combined action of several cytokines (IFNγ, TNFα, IL-1β) in an *in vitro* model of human intestinal epithelial cells.

In addition, our study provides clues to the mechanisms by which an epithelial cell can control the extracellular release of 2 cytokines, i.e. IL-8 and IL-1α, which are simultaneously upregulated by the same stimulus. IL-8 is directly channelled to the extracellular surface through the classical secretory pathway, while IL-1 accumulates in the cytosol and needs an additional event, i.e. cellular damage, to be released. The dependence of IL-1 release on cellular injury leading to cell necrosis or apoptotic cell death has been already noted in mice peritoneal macrophages by Hogquist *et al*. (1991). The biological relevance of this temporal hierarchy in the release of the two cytokines, i.e. IL-1 and IL-8 may reside in the fact that the main function of IL-8 is to recruit inflammatory cells (Baggiolini *et al*., 1989), while the main role of IL-1 is to activate the recruited immune cells at the inflammatory site and to extend their life (Martin & Resch, 1988; Dinarello, 1994).

Finally, our work shows that both NO-independent and NO-dependent signalling pathways are involved in up-regulating IL-1α production in this culture model of human intestinal epithelial cells. Data from the recent literature together with our experimental results provide a framework to rationalize the differential effect of L-NMMA on IL-1 production in two situations characterized by similar inductions of iNOS. In particular our finding of an inhibitory effect of cysteine on IL-1α induction by IFNγ/TNFα/IL-1β is best rationalized in terms of S-nitrosylation of cysteine, which would limit the interaction of NO^+ equivalents with thiol groups at redox modulatory sites of proteins involved in IL-1 synthesis, i.e. transcription factors. Thus, our explanation assumes that NO^+ equivalents are formed under IFNγ/TNFα/IL-1β treatment. The intracellular formation of NO^+ equivalents represented by iron-nitrosyl complexes (Stamler *et al*., 1992) has been described upon treatment of various cell types including macrophages (Lancaster & Hibbs, 1990) and islets of Langerhans with inflammatory cytokines (Corbett *et al*., 1992). NO^+ equivalents are able to induce IL-1 synthesis as shown by our recent demonstration that nitroprusside, a compound with a strong NO^+ character, induces IL-1 in HT29-Cl.16E cells (Vallette *et al*., 1996).

Together these findings suggest that IL-1 added to IFNγ/TNFα could induce the formation of NO^+ equivalents destined to activate transcription factors by acting at their redox modulatory sites (Arnelle & Stamler, 1995).

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