IMMUNE RESPONSE TO INFECTION

Interleukin-8 release from human neutrophils after phagocytosis of *Listeria monocytogenes* and *Yersinia enterocolitica*

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The release of interleukin-8 (IL-8) from human neutrophils was investigated after their internalisation of the invasive bacteria *Listeria monocytogenes* and *Yersinia enterocolitica*. Three pairs of bacterial strains were used to study the influence of listeriolysin O, invasiveness and *Yersinia* virulence plasmid (pYV)-encoded proteins on neutrophil cytokine synthesis. The neutrophils secreted IL-8 into the culture supernate after exposure to all strains tested. With *L. monocytogenes*, the virulence factor listeriolysin O had no effect on IL-8 secretion, whereas a non-invasive mutant induced greater IL-8 release than the corresponding invasive strain. With *Y. enterocolitica*, expression of virulence plasmid-encoded proteins appeared to be responsible for diminished IL-8 release. Northern blot analysis showed that the induced IL-8 release was always accompanied by an enhanced level of cytoplasmic IL-8 mRNA. Cytokine priming of neutrophils with granulocyte macrophage colony-stimulating growth factor (GM-CSF), unlike granulocyte colony-stimulating factor (G-CSF), led to further enhancement of IL-8 secretion induced after internalisation of the bacteria. Again, the increased IL-8 release, from GM-CSF primed neutrophils, was accompanied by an increased level of cytoplasmic IL-8 mRNA. The results emphasise the role of IL-8 in neutrophil-mediated host defence mechanisms during the acute phase of *L. monocytogenes* and *Y. enterocolitica* infection.

Introduction

Neutrophils have been shown to play an important role during the early 'non-specific' phases of listeriosis and yersiniosis [1, 2]. *Yersinia enterocolitica*, a gram-negative enteroinvasive bacterium, causes gastroenteritis [3, 4]. All pathogenic strains carry the 70-kb *Yersinia* virulence plasmid (pYV) and express the adhesin YadA and a group of secreted proteins called *Yersinia* outer proteins (Yops) [5]. Recently, it was reported that YopH, a protein phosphotyrosine phosphatase, and YopE, which mediates distortion of actin microfilaments, are responsible for an inhibitory effect on phagocytosis [6, 7]. The inhibitory effect on human neutrophils suggests that these professional phagocytes

play an important role during host defence against this invasive bacterium [8].

Listeria monocytogenes, a food-borne pathogen, is a gram-positive, facultatively intracellular bacterium [9]. By means of specific gene products for invasion (internalin, p60), pathogenic *L. monocytogenes* strains adhere to and penetrate the intestinal epithelium [10, 11]. Once liberated into the cytoplasm of the host target cell, after dissolution of the phagosome membrane by listeriolysin O [12], *L. monocytogenes* spreads from cell to cell [13]. Neutrophils appear to be part of the innate host defences during the onset of *L. monocytogenes* infection [14, 15]. Mice depleted *in vivo* of neutrophils by use of antibody RB6-8C5 succumbed to sublethal doses of *L. monocytogenes* [1, 16], and granulocyte colony-stimulating factor (G-CSF) had a beneficial effect on the course of infection in neutrophil-depleted mice [17, 18].

Numerous studies in mice have shown that cytokines are important for resistance to *L. monocytogenes* infection [19] and cytokine expression in the spleen

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correlated with an influx of neutrophils [20]. A growing body of evidence suggests that neutrophils are not only end-stage effector cells capable of phagocytosis and inflammatory mediator release [21], but that they have the potential to secrete multiple pro-inflammatory cytokines [22]. A previous study described the release of interleukin-1 β (IL-1 β), IL-6 and tumour necrosis factor- α (TNF- α) from human neutrophils after internalisation of *L. monocytogenes* and *Y. enterocolitica* strains [23]. After cell activation, neutrophils are also able to secrete the multifunctional cytokine IL-8 [24]. IL-8 is generally reported to be a pro-inflammatory cytokine because of its chemotactic and degranulating effects on neutrophils [25] and the central role of IL-8 in many inflammatory processes is well documented [26]. Thus, neutrophils, by secreting IL-8 after internalisation of *L. monocytogenes* and *Y. enterocolitica*, might contribute to host defence during the onset of infection. The effect of listeriolysin O, invasiveness and virulence plasmid (pYV)-encoded proteins on IL-8 release from neutrophils were analysed in this study with three bacterial strain pairs. Furthermore, priming studies were performed to determine the influence of the colony-stimulating factors G-CSF and granulocyte macrophage colony-stimulating growth factor (GM-CSF) on IL-8 synthesis by neutrophils exposed to invasive bacteria.

Materials and methods

Materials

The following reagents were used: Ficoll 400 (Pharmacia, Uppsala, Sweden); macrodex (Schiwa, Glansdorf, Germany); metrizoate (Nycomed, Oslo, Norway); [α - 32 P]-dCTP (specific activity: 111 TBq/mmol) and 3 H-thymidine (specific activity: 3.18 TBq/mmol) (NEN, Dreieich, Germany); nitrocellulose sheets (Schleicher and Schuell, Dassel, Germany); gentamicin and all fine chemicals (Sigma, Deisenhofen, Germany); cell culture media and supplements (Gibco, Europe Ltd, Karlsruhe, Germany). Recombinant human IL-8 (Calbiochem, Bad Soden, Germany) was used as control standard in the IL-8 ELISA. Murine anti-human monoclonal IL-8 antibodies and goat-alkaline phosphatase-labelled anti-human IL-8 antibodies were a generous gift from Dr M. Ceska (Sandoz-Forschungsinstitut, Wien, Austria).

The IL-8 cDNA inserted into a bluescript vector (pBluescript II SK) was a generous gift from Dr D. P. Cerretti (Immunex, Seattle, Washington, USA) [27]. GM-CSF was supplied by Sandoz (Nürnberg, Germany). Listeriolysin O was purified to homogeneity by Prof. J. E. Alouf (Institut Pasteur, Paris, France).

The bacterial strains used and their characteristics are described in Table 1. The strain pair *L. monocytogenes* NCTC 7973 (Hly $^{+}$, Inv $^{+}$) and SLCC 5779 (rough variant: Hly $^{+}$, Inv $^{-}$) [11] and the isogenic strains *L. monocytogenes* Mackaness SLCC 5764 wild type (WT: Hly $^{+}$, Inv $^{+}$) and non-haemolytic Tn916 mutant (M3: Hly $^{-}$, Inv $^{+}$) [28] were kindly provided by Prof. Dr W. Goebel (Institute of Genetics and Microbiology, Würzburg, Germany). The strain pair *Y. enterocolitica* 108-C (serotype O3, virulence plasmid cured) and 108-P (serotype O3, virulence plasmid bearing) was a generous gift from Prof. Dr J. Heesemann (Institute of Medical Microbiology, Würzburg, Germany) [29].

Bacterial growth

L. monocytogenes strains were grown in brain heart infusion (BHI) broth overnight at 37°C with shaking (150 rpm). This culture was used to inoculate 10 ml of BHI medium and allowed to grow for a further 3.5 h at 37°C. *Y. enterocolitica* strains were cultured overnight at 26°C, then subcultured as above and grown for a further 4 h at 37°C. The bacteria were centrifuged (4000 g, 20 min), washed twice with PBS, and resuspended in RPMI-1640 medium to a concentration of 12.5×10^7 cells/50 μ l.

Haemolysin assay

Haemolytic activity of *L. monocytogenes* strains and of purified listeriolysin O was determined as follows. Bacteria (2.5×10^8 cells/100 μ l), the culture supernate (10–100 μ l) or purified listeriolysin O were incubated for 30 min at 37°C with 900 μ l of a suspension containing sheep erythrocytes 2% v/v, 20 mM CaCl $_2$, 10 mM Tris and 160 mM NaCl adjusted to pH 5.5 with HCl. The mixture was kept for 20 min on ice and then centrifuged. The released haemoglobin in the supernate was determined photometrically at 530 nm.

Table 1. Strains used in this study and their characteristics

Strain	Serovar	Listeriolysin phenotype	Invasion phenotype
<i>L. monocytogenes</i>			
NCTC 7973	1/2a	Hly $^{+}$	Inv $^{+}$ p60 $^{+}$
SLCC 5779	1/2a	Hly $^{+}$	Inv $^{-}$ p60 $^{-}$
SLCC 5764 (Mackaness) WT	1/2a	Hly $^{+}$	Inv $^{+}$ p60 $^{+}$
SLCC 5764 (Tn916 mutant) M3	1/2a	Hly $^{-}$	Inv $^{+}$ p60 $^{+}$
<i>Y. enterocolitica</i>			
108-P (virulence plasmid-bearing)	O3		Inv $^{+}$
108-C (virulence plasmid-free)	O3		Inv $^{+}$

Preparation of neutrophils

Human neutrophils were prepared from heparinised venous blood of healthy donors on a Ficoll-metrizoate gradient followed by dextran sedimentation, as described previously [30]. The remaining erythrocytes were lysed by exposing the cells to hypotonic conditions. After preparation, neutrophils were washed twice with PBS (modified Dulbecco phosphate-buffered saline consisting of 137 mM NaCl, 8 mM Na₂HPO₄, 2.7 mM KH₂PO₄, and 2.7 mM KCl, pH 7.4) and a third time with RPMI-1640 medium. This method gave a cell preparation containing >97% pure neutrophils (1–2% eosinophils and <2% mononuclear cells) as shown by morphological examination of Wright-stained smears and non-specific esterase staining.

Phagocytosis of bacteria

Bacteria were labelled by the addition of [³H]-thymidine (55.5 kBq) to a 10-ml volume of bacterial culture after incubation for 1 h. The culture was incubated at 37°C for a further 2.5 h, then the bacteria were washed twice in PBS and resuspended in RPMI-1640 medium at 12.5×10^7 cells/50 μ l. The bacteria incorporated 40–60% of the added radioactivity [31]. For stimulation experiments, radioactively labelled neutrophils (2×10^7 /ml) in 500 μ l of PBS were mixed with 50 μ l of the bacterial suspension in the absence of serum and incubated at 37°C for 5 or 30 min. The neutrophils were then separated from the bacteria by centrifugation at 300 g for 15 min. Adherent *Y. enterocolitica* were removed from the neutrophils by incubation for 30 min on ice with 500 μ l of PBS-EDTA (40 mM)-lysozyme (100 μ g/ml); adherent *L. monocytogenes* were removed by treatment with PBS-EDTA-lysozyme-lysostaphin (1 μ g/ml). Neutrophils were lysed in distilled water to determine the percentage of ingested bacteria, determined by liquid scintillation counting (Rack beta 1209, LKB, Turku, Finland). The escape of listeriolysin O-producing *L. monocytogenes* strains into the cytoplasm of neutrophils was verified by microscopy.

Stimulation experiments

Neutrophil viability was determined by trypan blue exclusion and only cell preparations with a viability >95% were used. Neutrophils (5×10^6) were exposed to 12.5×10^7 bacteria in a volume of 1 ml (multiplicity of infection = 25:1). After culture for 1 h in RPMI-1640 medium in a water-saturated atmosphere containing CO₂ 5% at 37°C, gentamicin (50 μ g/ml) was added to prevent overgrowth of the neutrophils. For cytokine priming, GM-CSF 10 ng or G-CSF 10 ng, diluted in RPMI-1640 medium, or the medium alone (as control), were added to neutrophils in a volume of 600 μ l. After 15 min, bacteria or medium were added. Unprimed neutrophils were incubated for 4 h to obtain maximal IL-8 release after exposure to *L. monocytogenes* and *Y. enterocolitica*.

With GM-CSF-priming, a culture time of 3 h was optimal. After incubation, cell-conditioned media were harvested by centrifugation and stored at –70°C until used for IL-8 determination.

Cytokine immunoassay

Interleukin-8 was analysed by enzyme-linked immunosorbent assay (ELISA) as described previously [32]. This ELISA method detected IL-8 at concentrations ≥ 30 pg/ml.

RNA extraction and Northern blot analysis

Total cellular RNA was extracted according to the protocol of Chomczynski and Sacchi [33]. For Northern blot analysis, 20 μ g of total RNA were electrophoresed under denaturing conditions on an agarose 1.2% w/v-formaldehyde gel. The RNA was blotted on to GeneScreen Plus membranes (NEN, Dupont) by capillary transfer and hybridised under stringent conditions to a ³²P-labelled nick-translated IL-8 cDNA probe (a 300-bp *KpnI/EcoRI* cDNA fragment) [34]. Standardisation was performed with respect to the ethidium bromide-stained 28S and 18S rRNA of the blotted total RNA [27]. Equal RNA loading and transfer were checked by ultraviolet examination of the gels and blots. Blots were quantified by laser densitometry.

Analysis of data

All experiments were carried out at least three times. The data were calculated as mean and SEM. The significance was evaluated by the two-tailed Student's *t* test for independent means; *p* < 0.05 was considered significant.

Results

Phagocytosis of bacteria

The uptake of the three bacterial strain pairs by neutrophils after an incubation time of 30 min is depicted in Table 2. Similar phagocytosis data were obtained after an incubation time of 5 min (data not shown). The data are expressed as the percentage of

Table 2. Extent of bacterial phagocytosis by neutrophils

Strain	Mean percentage uptake (SEM)
<i>L. monocytogenes</i>	
NCTC 7973	3.7 (0.6)
SLCC 5779	17.3 (2.1)
SLCC 5764 WT	2.8 (0.5)
SLCC 5764 M3	3.1 (0.4)
<i>Y. enterocolitica</i>	
108-P	20.3 (3.5)
108-C	28.4 (2.9)

radioactively labelled bacteria taken up by the neutrophils. All the bacterial strains were phagocytosed. The uptake of the two invasive *Y. enterocolitica* strains (> 20%) differed significantly from that of the three invasive *L. monocytogenes* strains (< 5%). Only the non-invasive *L. monocytogenes* strain SLCC 5779, which forms long chains of cells, was phagocytosed extensively (17.3%). The isogenic *L. monocytogenes* strain pair WT and M3 were internalised to the same degree, indicating that the virulence factor listeriolysin O did not influence the phagocytosis of *L. monocytogenes* by neutrophils. The virulence plasmid-cured *Y. enterocolitica* strain 108-C was readily phagocytosed (28.4%), whereas uptake of virulence plasmid-bearing strain 108-P expressing the Yops and YadA was only 20.3%, verifying the anti-phagocytic activity of the pYV-encoded virulence factors.

IL-8 release after internalisation of bacteria

Human neutrophils were exposed to the bacterial strain pairs of *L. monocytogenes* and *Y. enterocolitica* for 4 h. The amount of IL-8 measured in the culture supernates was maximal after this incubation time and is shown in Fig. 1. As a positive control, the IL-8 release from neutrophils stimulated with Ca-ionophore A23187 is depicted. The isogenic *L. monocytogenes* strain pair WT and M3 induced similar IL-8 secretion from neutrophils (0.76 SEM 0.04 and 0.83 SEM 0.06 ng/ml, respectively). Therefore, the virulence factor listeriolysin O did not appear to influence IL-8 release from human neutrophils. This result was verified by incubation of neutrophils with purified listeriolysin O (0.1–10 U/ml). No IL-8 synthesis was induced (data not shown). After incubation of neutrophils with the *L. monocytogenes* strain pair NCTC 7973 and SLCC

Fig. 1. IL-8 release from human neutrophils (5×10^6) cultured for 4 h in the presence of medium, *L. monocytogenes* WT, *L. monocytogenes* M3, *L. monocytogenes* NCTC 7973, *L. monocytogenes* SLCC 5779, *Y. enterocolitica* 108-C, *Y. enterocolitica* 108-P, and Ca-ionophore A23187 (6 μ M), respectively; columns represent mean values (ng/ml) and vertical bars represent the SEM; $n = 4$; * $p < 0.05$ versus supernates from non-infected neutrophils (medium).

5779, larger amounts of IL-8 were secreted into the culture supernate than with strain pair WT and M3. However, the invasive strain NCTC 7973 induced significantly lower amounts of IL-8 (1.88 SEM 0.22 ng/ml) compared to the non-invasive *L. monocytogenes* strain SLCC 5779 (3.14 SEM 0.45 ng/ml). Thus, the observed greater phagocytosis of the non-invasive strain correlated with an enhanced IL-8 secretion from neutrophils.

The internalisation of the two invasive *Y. enterocolitica* strains led to differences in IL-8 release from neutrophils. Both strains induced a significantly enhanced IL-8 synthesis compared to the constitutive IL-8 secretion of 98 pg/ml (Fig. 1, medium). This latter might be the result of an activation process due to the plastic surfaces of the incubation vials used. Strain 108-P induced a significantly lower IL-8 release than its isogenic, plasmid-free strain 108-C (1.70 SEM 0.14 versus 2.36 SEM 0.31 ng/ml). Thus, the lower phagocytosis of the plasmid-bearing strain 108-P, expressing the virulence factor YadA and the phagocytosis-inhibitory factors YopH and YopE, correlated with a diminished IL-8 release from neutrophils.

IL-8 mRNA accumulation by neutrophils

Northern blot analysis was performed to elucidate the molecular background of the observed IL-8 secretion pattern from human neutrophils after internalisation of the bacteria. Fig. 2 shows a representative IL-8 Northern blot from these experiments. At the bottom, the total RNA blotted on nitrocellulose and stained with ethidium bromide is presented, verifying that

Fig. 2. Northern blots showing cytoplasmic IL-8 mRNA steady state levels in human neutrophils cultured for 2 h. Neutrophils were incubated with (1) medium; (2) *L. monocytogenes* WT; (3) *L. monocytogenes* M3; (4) *L. monocytogenes* NCTC 7973; (5) *L. monocytogenes* SLCC 5779; (6) *Y. enterocolitica* 108-C; (7) *Y. enterocolitica* 108-P or (8) Ca-ionophore A23187 (6 μ M), respectively; bottom: ethidium bromide-stained total RNA after blotting on to the filter.

equal amounts of RNA were analysed in each lane of the Northern blot shown. Compared to the medium control (lane 1) neutrophils accumulated IL-8 mRNA after incubation with each bacterial strain (lanes 2–7). As shown in lane 8, neutrophil stimulation with the Ca-ionophore A23187 served as a positive control. The amounts of cytoplasmic IL-8 mRNA after phagocytosis of *Y. enterocolitica* 108-C and 108-P (lanes 6 and 7) were similar. Therefore, in comparison to strain 108-C the diminished IL-8 release observed after phagocytosis of strain 108-P was not due to a lower amount of IL-8 mRNA. In contrast, the isogenic *L. monocytogenes* strain pair WT and M3 (lanes 2 and 3) induced a lower cytoplasmic IL-8 mRNA level than the strain pair NCTC 7973 and SLCC 5779 (lanes 4 and 5). Therefore, the observed differences in the IL-8 secretion pattern induced by these *L. monocytogenes* strain pairs correlated with the amounts of IL-8 mRNA in neutrophils measured after phagocytosis.

IL-8 release from neutrophils after GM-CSF and G-CSF priming

In the mouse model of *L. monocytogenes* infection, exogenously added colony-stimulating factor enhanced resistance of neutrophil-depleted mice as determined by increased survival and reduced bacterial growth. To examine the basis of this protective effect, IL-8 secretion by G-CSF- as well as by GM-CSF-primed neutrophils was determined. Fig. 3 shows the IL-8 release data from GM-CSF-primed neutrophils after incubation for 3 h, the time of maximal IL-8 release. It is evident that GM-CSF priming by itself induced an enhanced IL-8 release from neutrophils (0.13 SEM 0.1 versus 0.72 SEM 0.2 ng/ml). Furthermore, IL-8

secretion due to internalisation of all bacterial strains under investigation was significantly enhanced by GM-CSF priming. In contrast, G-CSF priming did not alter IL-8 release from neutrophils either exposed to *L. monocytogenes* and *Y. enterocolitica* strains or to medium alone (data not shown).

IL-8 mRNA levels in GM-CSF- and G-CSF-primed neutrophils

To determine whether the increased IL-8 secretion of GM-CSF-primed neutrophils might be accompanied by an enhanced cytoplasmic IL-8 mRNA level, Northern blot experiments were performed. Fig. 4 shows the amount of IL-8 mRNA after exposure of neutrophils to the pathogenic *L. monocytogenes* strain NCTC 7973. As can be seen, GM-CSF-primed neutrophils accumulated IL-8 mRNA (lane 2, 83% of maximum) compared to the medium control (lane 1, 61% of maximum). Neutrophils primed with G-CSF (lane 3, 58% of maximum) did not accumulate IL-8 mRNA. The IL-8 accumulation induced after uptake of *L. monocytogenes* NCTC 7973 (lane 4, 85% of maximum) was further enhanced in GM-CSF-primed neutrophils (lane 5, 100% = maximum). In contrast, priming with

Fig. 3. IL-8 release from non-primed (□) and GM-CSF-primed (10 ng/ml) (■) human neutrophils (5×10^6) cultured for 3 h. The cells were exposed to medium, *L. monocytogenes* WT, *L. monocytogenes* M3, *L. monocytogenes* NCTC 7973, *L. monocytogenes* SLCC 5779, *Y. enterocolitica* 108-C or *Y. enterocolitica* 108-P, respectively. Columns represent the mean values (ng/ml) and vertical bars represent the SEM of four experiments performed in triplicate; # $p < 0.05$ versus supernatants from non-primed, non-infected cells; * $p < 0.05$ versus supernatants from GM-CSF-primed, non-infected neutrophils.

Fig. 4. (A) Expression of IL-8 mRNA in human neutrophils primed with GM-CSF or G-CSF and exposed to *L. monocytogenes* NCTC 7973. The cells were cultured for 2 h with (1) medium, (2) GM-CSF (10 ng/ml), (3) G-CSF (10 ng/ml), (4) *L. monocytogenes* NCTC 7973, (5) GM-CSF + *L. monocytogenes* NCTC 7973; (6) G-CSF + *L. monocytogenes* NCTC 7973; a representative Northern blot is presented. (B) Densitometry of the representative northern blot presented above; * $p < 0.05$ versus medium control ($n = 3$).

G-CSF was without any influence on the cytoplasmic IL-8 mRNA level induced by internalisation of *L. monocytogenes* NCTC 7973 (lane 6, 86% of maximum).

Discussion

This study was performed to analyse the release of IL-8 from human neutrophils after internalisation of the enteroinvasive bacteria *L. monocytogenes* and *Y. enterocolitica*. The data show that neutrophils secreted IL-8 after phagocytosis of both *L. monocytogenes* and *Y. enterocolitica* and there was a correlation between the amount of phagocytosed bacteria and IL-8 secretion. The non-invasive *L. monocytogenes* strain SLCC 5779, growing in long cell filaments [11], was phagocytosed most readily and induced the highest IL-8 release. Therefore, the property of invasiveness for epithelial cells plays no role with regard to phagocytosis and IL-8 induction by neutrophils. In contrast, epithelial cells secreted IL-8 only after their infection by invasive bacteria [35]. Although the phagocytic process is finished as early as 5 min after exposure to the bacteria, it seems to be the stimulus for enhanced IL-8 gene expression up to 4 h afterwards [36]. On further incubation, decreased cell viability and perhaps IL-8 degradative processes due to aminopeptidase N might be responsible for the reduced IL-8 levels found in the culture supernates (data not shown) [37].

IL-8 release and IL-8 mRNA levels after uptake of the isogenic *L. monocytogenes* strain pair WT and M3 were similar. These data suggest that one of the most important virulence factors of *L. monocytogenes*, listeriolysin O, does not excite neutrophils for enhanced IL-8 synthesis. This result was further substantiated by experiments with purified listeriolysin O. In contrast, the positive effect of listeriolysin O on IL-1 and TNF- α synthesis by murine macrophages has been reported [38, 39]. We have also observed an increased IL-8 release from human peripheral blood mononuclear cells (c. 20% monocytes, 80% lymphocytes) incubated with purified listeriolysin O (unpublished observations). Therefore, it seems that the virulence factor listeriolysin O induces IL-8 gene expression in a cell type specific manner. It remains to be determined whether murine granulocytes are able to express IL-8 after exposure to listeriolysin O.

L. monocytogenes strains WT and NCTC 7973 were similarly phagocytosed by neutrophils, but the strain NCTC 7973 induced the release of twice as much IL-8 compared to strain WT. Recently, Vasquez and co-workers reported that listeriolysin O by itself was not primarily responsible for TNF- α release from murine macrophages [38]. They suggested that listeriolysin O, by disruption of the phagolysosome, allows other bacterial molecules liberated into the cytoplasm to induce TNF- α release. It may be that strains WT and

NCTC 7973 differ in the production of other molecules which are responsible for the observed differences in IL-8 release from neutrophils.

The amount of IL-8 released upon phagocytosis of *Y. enterocolitica* strain 108-P was reduced in comparison with that released upon uptake of strain 108-C. *Yersinia* outer proteins (Yops) and YadA encoded by the virulence plasmid play an important role in inhibiting the ingestion of *Y. enterocolitica* by neutrophils [7, 8]. Therefore, one reason for the diminished IL-8 release by neutrophils after uptake of strain 108-P might be the reduced phagocytosis of this virulence plasmid carrying strain. Recently, Schulte and co-workers [40] reported that epithelial cells infected with a virulence plasmid-bearing (pYV⁺) *Y. enterocolitica* strain released lower amounts of IL-8 than epithelial cells infected with an isogenic non-virulent, plasmid-cured (pYV⁻) strain. Neither the lower infection rate of the virulent pYV⁺ strain nor the cytotoxic potential of YopE and YopH – known to inhibit phagocytosis by neutrophils – was responsible for the reduced IL-8 secretion pattern. However, YopB and YopD were involved in the suppression of IL-8 release from epithelial cells as well as in the TNF- α release from murine macrophages [40, 41], but the mechanism of this inhibitory effect is unknown.

IL-8 secretion by the neutrophils correlated with enhanced cytoplasmic IL-8 mRNA levels, suggesting an increased rate of IL-8 gene transcription or an altered IL-8 mRNA stability. No differences were observed in the amount of IL-8 mRNA induced by *L. monocytogenes* NCTC 7973 and SLCC 5779 or by *Y. enterocolitica* 108-C and 108-P, respectively (Fig. 2). It is possible that the observed differences in IL-8 release might have been due to enhanced secretion of the cytoplasmic IL-8 pool. A recent study has shown that the phagocytosis of *L. monocytogenes* and *Y. enterocolitica* diminished the respiratory burst activity and altered the leukotriene metabolism of human neutrophils [31]. In contrast, the release of the pro-inflammatory cytokines TNF- α , IL-1 β and IL-6 from human neutrophils was induced after exposure to these invasive bacteria [23]. Also, murine macrophages exposed to *L. monocytogenes* showed a suppressed respiratory burst but an increased IL-1 synthesis [39]. The protective role of IL-1, IL-6 and TNF- α against a *L. monocytogenes* infection of mice was described recently [38, 42, 43]. As IL-8 activates neutrophils for chemotaxis, exocytosis, receptor expression and respiratory burst [25], the data from the present study suggest that neutrophils, by secreting the pre-inflammatory cytokines IL-1 β , IL-6, TNF- α and IL-8, might overcome the dysfunctions in mediator release and phagocytic activity induced by these bacteria [23, 31].

The observed IL-8 release from epithelial cells subsequent to infection with either *L. monocytogenes* or *Y. enterocolitica* [35], emphasises the role of this

cytokine during the early phase of infection by these bacteria. Therefore, neutrophils chemotactically recruited and activated by IL-8 in a paracrine or autocrine manner, or both, should be well armed for phagocytosis of *Y. enterocolitica* [7, 8] and of cytotoxicity of *L. monocytogenes*-infected cells [14, 15, 44].

Epithelial cells are also able to synthesise GM-CSF [45]. The present study clearly revealed that human neutrophils primed with GM-CSF accumulated more IL-8 mRNA and secreted more IL-8 into the culture supernate than unprimed cells. In contrast, G-CSF priming showed no influence on either IL-8 protein or IL-8 mRNA levels. The data suggest that epithelial cells of mucosal surfaces might prime recruited neutrophils for an enhanced IL-8 release and effector cell functions in *L. monocytogenes* and *Y. enterocolitica* infections [46].

An essential contribution of neutrophils in the course of cell-mediated immunity during primary and secondary infections was recently observed [47, 48]. There is some evidence that IL-8 is also chemotactic for T-lymphocytes and suppresses IL-4 production by CD4⁺ T cells [49]. One may speculate that neutrophils, by secreting IL-8, are involved in the generation of protective Th1-mediated cellular immune responses.

In agreement with our previous study on cytokine release from human neutrophils exposed to *L. monocytogenes* and *Y. enterocolitica* [23], the present data on IL-8 suggest that neutrophils modulate the cytokine network in infected tissue. During the course of *L. monocytogenes* or *Y. enterocolitica* infection, the neutrophil might be involved in protective inflammatory as well as immune responses.

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