Potential Importance of IL-8: A Potent Chemokine, in Sepsis

S.J.H. van Deventer, M.A.M. von der Möhlen, and T. van der Poll

Introduction

Generalized dysfunction of the vascular endothelium, that leads to edema formation and ARDS, is in large part a result of the action of proteases and oxygen radicals that are released from activated neutrophils [1]. Bacterial products such as fMPL, complement activation products, in particular C5a, and lipid mediators including platelet activating factor (PAF), are all potent neutrophil activators. Cytokines, such as tumor necrosis factor (TNF), are also know to importantly stimulate neutrophils. In this chapter, we will discuss the potential role of a neutrophil-activating cytokine, interleukin-8 (IL-8), that is a low molecular weight protein belonging to the chemokine family (also known as small cytokine -SCY-family), in sepsis.

Interleukin-8

The small cytokine family [2] comprises a group of low molecular weight proteins (less than 10kD) that can be induced in a wide variety of cells by inflammatory stimuli, such as LPS, TNF and IL-1. Many of these proteins have leukocyte chemotactic properties, which underlies their designation as "chemokines". Indeed, IL-8 was isolated and purified to homogeneity using its neutrophil activating- and chemotactic properties as a biological marker of its presence. Many of the SCY family members, including IL-8, bind to heparin [3], and it is tempting to speculate that this may enable these cytokines to remain associated with heparan sulphates that are present on the endothelial membrane. Monocytes, endothelial cells, fibroblasts, synovial cells, keratinocytes, mesangial cells, some carcinoma cells and indeed neutrophils themselves [4], can be induced to release IL-8 after stimulation by endotoxin or/and IL-1 or/and TNF (Table 1) [3]. Serum levels of IL-8 are normally below the detection limit of currently used assay (<20 ng/l), but IL-8 may be present in normal tissue [5]. The role of this constitutively released IL-8 presently remains unknown, but we have hypothesized that it could serve as a continuously present chemotactic stimulus that would facilitate normal leukocyte recirculation through tissues.

IL-8 can activate neutrophils to degranulate, as is reflected by release of β-glucuronidase, elastase, myeloperoxidase, vitamin-12 binding protein, lacto-

J.-L. Vincent (ed.), *Yearbook of Intensive Care and Emergency Medicine 1993*

Table 1. Sources and activators of interleukin-8

leukocytes	SEA
monocytes	LPS, PMA, ConA, PHA, IL-1, TNF
lymphocytes	ConA, PHA
neutrophils	LPS, phagocytosis
promyelocytes	PMA, DMSO
fibroblasts	IL-1, TNF, virus, dsRNA
endothelial cells	LPS, IL-1, TNF
synovial cells	IL-1
keratinocytes	IL-1, Interferon-γ, TNF
epithelial cells	IL-1, TNF
mesangial cells	IL-1, TNF
chondrocytes	IL-1, TNF, dsRNA
carcinoma cells	-
osteosarcoma cells	IL-1, virus
hepatoma cells	IL-1, TNF, virus

ferrin [3]. The capacity of IL-8 to induce an oxidative burst, leading to the formation of oxygen radical species, is more controversial. It has recently been shown however, that IL-8 is a rather potent stimulator of cytochrome C type III in neutrophils that were previously primed by TNF [6]. Priming by GM-CSF has also been reported to increase the IL-8 induced oxidate burst [6], but others have not confirmed these findings [7]. Because in septic patients many cytokines are simultaneously present, neutrophils are very likely to be primed, and in these circumstances IL-8 may cause oxygen radical release *in vivo*, however [7]. Stimulation of neutrophils by IL-8 does not cause the synthesis of PAF or LTB_4 [7].

Various truncated forms of IL-8 have been identified, that have different biological effects. Some cells are producers of longer forms, whereas other cells preferentially secrete shorter forms of IL-8. Because the currently available IL-8 ELISA's do not distinguish these different forms of IL-8, it is unknown which forms are present in the circulation of septic patients. Interestingly, plasmin can convert the longer fibroblast-derived 77 amino-acid IL-8 forms into the biologically active 72 amino-acid IL-8 by proteolytic cleavage [8], thereby enhancing its biological activity. We have previously shown that endotoxin and TNF both induce a very rapid fibrinolytic response, leading to plasmin generation in humans [9, 10], and have questioned the biological implications of this response that precedes detectable coagulation activation. In view of the proteolytic activity of plasmin on long IL-8 forms, it is possible that the early fibrinolytic response in sepsis is more related to inflammation than to coagulation or fibrinolysis. This finding is even more intriguing in view of the fact that activated blood platelets can stimulate endothelial cells to release IL-8 [11]. Hence, the coagulation and inflammatory systems may interact at multiple levels to induce and activate IL-8, and coagulation activation is thus closely linked to inflammation.

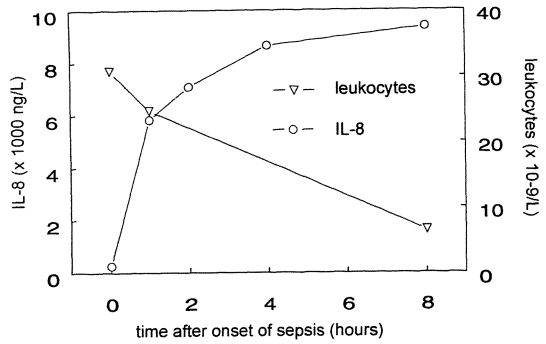

Fig. 1. Scatter diagram of IL-8 levels at the onset of gram-negative sepsis in 20 consecutive patients at the AMC, Amsterdam

IL-8 is induced in many disease states that are characterized by neutrophil infiltration (rheumatoid arthritis [12], ulcerative colitis [13], and psoriasis [14, 15]. We and others have investigated IL-8 release in experimental sepsis in subhuman primates, endotoxin- and TNF-challenged volunteers [16, 17] or cancer patients [18], and showed that both stimuli were able to induce systemic release of IL-8 with a time course that much resembled the kinetics of IL-6. Although IL-8 release was followed in time by the appearance of neutrophil activation markers in blood, including lactoferrin and elastase, the serum levels of these markers did not significantly correlate with IL-8 [16]. In these experiments in volunteers, we were not able to detect any complement activation (as measured by C3a desarg levels), but other neutrophil activators, such as TNF, were prominently induced, and therefore the extent of neutrophil activation in these experiments likely also reflected stimulation by these other factors.

High serum levels of IL-8 can be observed in experimental sepsis in subhuman primates [19], as well as in the clinical setting [20]. We have recently studied IL-8 levels at different time points in patients with gram-negative sepsis, and our results suggest that higher IL-8 levels were detected in patients that subsequently died than in survivors, and that the rise in IL-8 levels coincided with a deterioration in the clinical condition (Fig. 1 and 2).

In summary, IL-8 is induced in local inflammatory disease states, as well as in the generalized inflammatory reaction in sepsis. Neutrophils may be primed by cytokines such as TNF to react more potently to stimulation by IL-8, and priming is likely to occur in sepsis.

The Role of IL-8 in Neutrophil Recruitment in Inflammation and Sepsis

Various processes may lead to obstruction of capillaries by neutrophils in sepsis. First, it should be kept in mind that the diameter of the average pulmonary capillary is 6.5 μm, whereas the diameter of a neutrophil measures 7.5 μm. Therefore, neutrophils can only pass through the pulmonary vasculature if they are able to change shape. It is now well established that changes in the neutro-

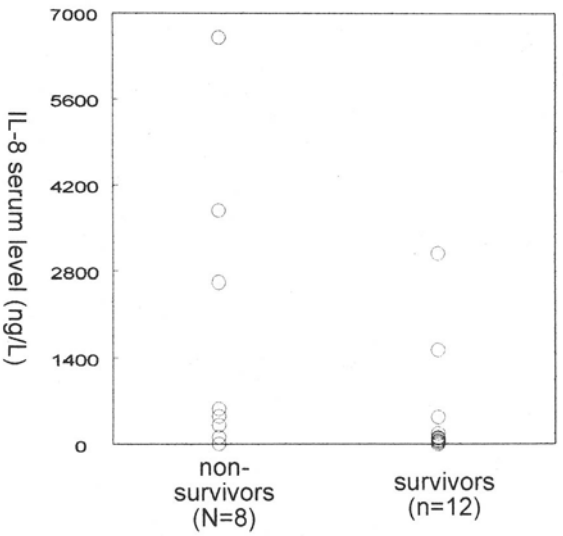

Fig. 2. Appearance of IL-8 in a 57 year old male patient with nosocomial pneumonia. The kinetics of IL-8 release differ from the transient appearance of IL-8 in endotoxin-challenged volunteers, but resemble observations in lethal gram-negative bacteremia in subhuman primates.

phil actin cytoskeleton are a prerequisite for neutrophil transmigration through small capillaries. After infusion of endotoxin in animals [21] or volunteers [9], circulating neutrophil counts rapidly decrease, and this early neutropenia is an effect of endotoxin on the neutrophil, and not on the endothelium [22]. It has been elegantly shown that endotoxin induces a stiffening of neutrophils coinciding with actin filament rearrangement [23]. This neutrophil stiffening causes retention of neutrophils in 6.5 μm pore filters, that cannot be inhibited by anti-CD18 antibodies, and therefore does not seem to be dependent on β2-integrins. Moreover, the neutrophil-stiffening effect of endotoxin is dependent on the presence of plasma [23], suggesting that this endotoxin effect is caused by activation of the LBP/CD14 pathway. These results demonstrate that endotoxin-induced neutrophils may block the microcirculation by altered cellular mechanical properties alone, and that endotoxin can induce these alterations. Thus, early neutropenia in sepsis may occur independent of the biological effects of cytokines including IL-8. On the other hand, administration of IL-8 to baboons as either a bolus injection or an 8 h infusion resulted in transient neutropenia [24], possibly related to neutrophil stiffening following IL-8 induced activation, or as a consequence of activation of neutrophil integrins (see below). The administration of IL-8 did not cause any hemodynamic alterations or induction of IL-1, TNF, or IL-6, and histopathological analysis revealed no neutrophil transmigration into the tissues. It should be kept in mind that in sepsis IL-8 is released together with IL-1 and TNF, and as will become apparent, many of the biological effects of IL-8 should be considered in view of its complex interactions with these cytokines.

Recruitment of neutrophils to inflammatory sites is a complex event, that requires specific adhesion of neutrophils to the vascular endothelium, subsequent disruption of the initial bounds, and transmigration through the endothelium. These interactions do not merely follow activation of the neutrophil, but also require active participation of the endothelial cell [25,27]. Many specific adhesion molecules that regulate these interactions have been characterized, and are now classifield as selectins (P-, L-, E-selectin), integrins, and members of the immunoglobulin superfamily (ICAM-1, ICAM-2, VCAM-1) [28]. Several adhesion molecules can be induced to alter their affinity by agonistic stimulation. Neutrophils, for example, constitutively carry integrin heterodimers on their membrane surfaces, but integrin-mediated adherence depends on a change of integrin "activation" state. There is a growing consensus that neutrophil adherence to endothelial cells follows several steps that determine the specificity of this interaction. The first change that can be seen in acute inflammation is (reversible) rolling of neutrophils over the activated endothelium, which is dependent on the presence of the neutrophil L-selectin [29], possibly by interaction with E-selectins and presumably other not yet characterized counterstructures. This rolling of neutrophils on endothelial cells slows down their passage through capillaries, and enables other neutrophil-endothelial interactions to take place. In particular, it is in this phase that endothelium-derived molecules, such as PAF and IL-8 can activate neutrophils. Activation of neutrophils by PAF causes shedding of L-selectin, and upregulation of β2-integrin heterodimers. Interleukin-8 is synthesized and secreted by endothelial cells after stimulation by endotoxin, TNF and IL-1. Some of the endothelial cell-derived IL-8 may remain cell-surface bound (possibly bound to heparan sulphate), and can activate the neutrophils that have slowed down by rolling. Stimulation of neutrophils by IL-8 causes degranulation and synthesis of oxygen radicals (in particular following "priming" by other cytokines such as TNF), and had intriguing effects on the adhesion to endothelial cells. Like PAF, IL-8 causes L-selectin shedding [30] and upregulation of β2-integrins on neutrophils [31], causing increased adhesion to the unstimulated endothelium, but it inhibits neutrophil binding to previously stimulated endothelial cells [32]. IL-8 (and PAF) also cause transendothelial migration of neutrophils, both as a consequence of its neutrophil chemotactic properties, and the fact that IL-8 is preferentially deposited subendothelially (Table 2) [33, 34].

Does IL-8 promote or inhibit neutrophil recruitment in sepsis? In general, a chemotactic factor causes migration of the target cell along its concentration gradient, and in a condition were high IL-8 levels circulate, one would expect inhibition of neutrophil transmigration. In view of the following facts, the effects of IL-8 on neutrophil recruitment in sepsis are more complex however. First, IL-8 tissue levels in sepsis are presently not known, and in view of the capacity of several tissue cells to produce large amounts of IL-8, could be very high. Second, different forms of IL-8 have different effects on neutrophil recruitment, and it is not known whether in sepsis longer or shorter IL-8 forms circulate. Third, as has been discussed, the neutrophil-activating effects of IL-8 are importantly modulated by priming of neutrophils.

Table 2. Biological activities of IL-8

***In vitro* Biological Activities of IL-8**	
Neutrophils	– direct migration
	– degranulation
	– respiratory burst (ox. radicals) in primed cells
	– activation of arachidonate-5-lipoxygenase in presence of exogenic arachidonic acid
	– growth inhibitory activity on *Candida albicans* (independent of superoxide production)
	– inhibition of adhesion to cytokine activated endothelial monolayers
	– stimulation of adhesion to unactivated endothelial cells
T-cells	– chemotaxis
***In vivo* Biological Activities of IL-8**	
– neutrophil attraction and migration	
– skin reactions (plasma leakage, neutrophil accumulation)	
– in presence of PGE_2 (vasodilatator) -- neutrophil infiltration, plasma protein extravasation	
– intradermal -- accumulation lymphocytes and neutrophils connective tissue	
– in lymphatic tissue (in high endothelial venules of drained lymph nodes) -- accelerated emigration of lymphocytes	
– intravenous IL-8 -- local immediate and profound neutrophilia, systemic reaction more delayed	
– oxidative burst	
– no induction of TNF / IL-1 / IL-6	

Blocking IL-8

Various drugs have been shown to block transcription of the IL-8 gene, or interfere with release of IL-8 protein. Cyclosporin A, for example, blocks IL-8 transcription in PMA and PHA-stimulated T-cells [35], but this finding probably has minor relevance for sepsis. More importantly, corticosteroids [36] and IL-1 receptor antagonist (IL-1ra) [37, 38] are able to importantly reduce the release of IL-8 after LPS. Not only do these data suggest that IL-1 is involved in the induction of IL-8, but these findings may in part explain the efficacy of IL-1ra in sepsis.

Conclusion

IL-8 is a small cytokine that is prominantly induced in local inflammatory disease states, and high levels of IL-8 are detected in septic patients. IL-8 has neutrophil chemotactic- and activating properties, that are of importance for neutrophil recruitment to local sites of inflammation. In sepsis, IL-8 may play an important role in early host response to serious gram-negative infection, in particular in neutrophil recruitment and activation. Its biological functions should be considered in conjunction with the effects of other cytokines, as well as the coagulation system.

References

1. Miller EJ, Cohen AB, Nagao S, et al. (1992) Elevated levels of NAP-1/Interleukin-8 are present in the airspaces of patients with adult respiratory distress syndrome and are associated with increased mortality. Am Rev Respir Dis 146:427–432
2. Van Deventer SJH, Cerami AC (1992) Novel endotoxin-induced cytokines. In: Ryan JC, Morrison DC (eds) Bacterial endotoxic lipopolysaccharides. Vol II. Immunopharmacology and pathophysiology. CRC press Boca Ruton, pp 197–212
3. Van Damme J (1991) Interleukin-8 and related molecules. In: Thomson AW (ed) The cytokine handbook. Academic Press Ltd. London, pp 201–214
4. Cassatella MA, Bazzoni F, Ceska M, Ferro I, Baggiolini M, Beron G (1992) IL-8 production by human polymorphonuclear leukocytes. The chemoattractant formyl-methionyl-leucyl-phenylalanine induces the gene expression and release of IL-8 through a pertussis toxin-sensitive pathway. J Immunol 148:3216–3220
5. Hommes D, Jansen J, Smit F, et al. (1993) Increased mucosal IL-8 production in ulcerative colitis (Submitted)
6. You A, Kitagawa S, Kasahara T, Matsushima K, Saito M, Takaku F (1991) Stimulation and priming of human neutrophils by interleukin-8: Cooperation with tumor necrosis factor and colony-stimulating factors. Blood 78:2708–2714
7. Wirthmueller U, Baggioloni M, de Weck AL, Dahinden C (1991) Receptor-operated activation of polymorphonuclear leukocytes: Different effects of NAP-1/IL-8 and fMET-LEU-PHE or C5a. Biochem Biophys Res Comm 176:972–978
8. Nakagawa H, Hatakeyama S, Ikesue A, Miyai H (1991) Generation of interleukin-8 by plasmin from AVLPR-interleukin 8, the human fibroblast-derived neutrophil chemotactic factor. FEBS (Lett) 282:412–414
9. Van Deventer SJH, Büller HR, ten Cate JW, Aarden L, Hack E, Sturk A (1990) Experimental endotoxemia in humans: Analysis of cytokine release and coagulation, fibrinolytic, and complement pathways. Blood 76:2520–2526
10. Van der Poll T, Levi M, Büller HR, et al. (1991) Fibrinolytic response to tumor necrosis factor in healthy subjects. J Exp Med 174:729–732
11. Van Deventer SJH, Jansen J. (Unpublished results)
12. Peichl P, Ceska M, Broell H, Effenberger F, Lindley IJ (1992) Human neutrophil activating peptide/interleukin-8 acts as an autoantigen in rheumatoid arthritis. Ann Rheum Dis 51:19–22
13. Mahida YR, Ceska M, Effenberger F, Kurlak L, Lindley I, Hawkey CJ (1992) Enhanced synthesis of neutrophil activating peptide-1/interleukin-8 in active ulcerative colits. Clin Sci 82:273–275
14. Anttila HS, Reitamo S, Erkko P, Ceska P, Moser B, Baggiolini M (1992) Interleukin-8 immunoreactivity in the skin of healthy subjects and patients with palmoplantar pustulosis and psoriasis. J Invest Dermatol 98:96–101
15. Schröder JM (1992) Generation of NAP-1 and related peptides in psoriasis and other inflammatory skin diseases. Cytokines 4:54–76
16. Van Deventer SJH, Hart M, van der Poll T, Hack EC, Aarden LA (1993) Endotoxin and tumor necrosis factor alpha induced IL-8 release in humans. J Infect Dis (in press)
17. Martich GD, Danner RL, Ceska M, Suffredini AF (1991) Detection of interleukin-8 and tumor necrosis factor in normal humans after intravenous endotoxin: The effect of antiinflammatory agents. J Exp Med 173:1021–1024
18. Van Meir E, Ceska M, Effenberger F, et al. (1992) Interleukin-8 is produced in neoplastic and infectious diseases of the human central nervous system. Cancer Research 52:4297–4305
19. Redl H, Schlag G, Bahrami S, Schade U, Ceska M, Stutz P (1991) Plasma neutrophil-activating peptide-1/interleukin-8 and neutrophil elastase in a primate bacteria model. J Inf Dis 164:383–388
20. Hack CE, Hart M, Strack van Schijndel RJM, et al. (1992) Interleukin-8 in sepsis: Relation to shock and inflammatory mediators. Infect Immun 60:2835–2842
21. Meyrick B, Brigham KL (1983) Acute effects of Escherichia coli endotoxin on the pulmonary microcirculation of anaesthesized sheep. Structure function relationships. Lab Invest 48:458–470
22. Haslett C, Worthen GS, Giclas PC, Morrison DC, Henson JE, Henson PM (1987) The pulmonary vascular sequestration of neutrophils in endotoxemia is initiated by an effect of endotoxin on the neutrophil in the rabbit. Am Rev Respir Dis 136:9–18

23. Erzurum SC, Downey GP, Dohert DE, Schwab B III, Elson E, Worthen GS (1992) Mechanism of lipopolysaccharide-induced neutrophil retention. Relative contributions of adhesive and cellular mechanical properties. J Immunol 149:154–162
24. Van Zee KJ, Fischer E, Hawes AS, et al. (1992) Effects of intravenous IL-8 administration in nonhuman primates. J Immunol 148:1746–1752
25. Springer TA (1990) Adhesion receptors of the immune system. Nature 346:425–433
26. Butcher EC (1991) Leukocyte-endothelial cell recognition: Three (or more) steps to specificity and diversity. Cell 67:1033–1036
27. Zimmerman GA, Prescott SM, McIntyre TM (1992) Endothelial cell interaction with granulocytes: Tethering and signaling molecules. Immunol Today 13:93–100
28. Rot A (1992) Endothelial cell binding of NAP-1/IL-8: Role in neutrophil emigration. Immunol Today 13:291–294
29. Von Adrian UH, Chambers JD, McEnvoy L, Bargatze RF, Arfors K-E, Butcher EC (1991) Two-step model of leukocyte-endothelial cell interaction in inflammation: Distinct roles for LECAM-1 and the leukocyte beta2 integrins in vivo. Proc Nat Acad Sci USA 88:7538–7542
30. Smith CW, Kishimoto TK, Abbass O, et al. (1991) Chemotactic factors regulate lectin adhesion molecule 1 (LECAM-1)-dependant neutrophil adhesion to cytokine-stimulated endothelial cells in vitro. J Clin Invest 87:609–618
31. Detmers P, Lo SK, Olson-Egbert E, Walz A, Baggiolini M, Cohn ZA (1990) Neutrophil-activating protein-1/interleukin-8 stimulates the binding activity of the leukocyte adhesion receptor CD11b/CD18 on human neutrophils. J Expl Med 171:1155–1162
32. Hechtman DH, Cybulsky MI, Fuchs HJ, Baker JB, Gimbrone MA Jr (1991) Intravascular IL-8. Inhibitor of polymorphonuclear leukocyte accumulation at sites of acute inflammation. J Immunol 147:883–892
33. Huber AR, Kunkel SL, Todd RF III, Weiss SJ (1991) Regulation of transendothelial neutrophil migration by endogenous interleukin-8. Science 254:99–102
34. Kuypers TW, Hakkert BC, Roos D (1992) Neutrophil migration across monolayers of cytokine-prestimulated endothelial cells: A role for cell-associated platelet-activating factor in migration and modulation of surface antigen expression. J Cell Biol 117:565–572
35. Zipfel PF, Bialonski A, Skerka C (1991) Induction of the IL-8/NAP-1 gene family in human T Lymphocytes is suppressed by cyclosporin A. Biochem Biophys Res Comm 181:179–183
36. Tobler A, Meier R, Seitz M, Dewald B, Baggiolini M, Fey MF (1992) Glucocorticoids downregulate gene expression of GM-GSF, NAP-1/IL-8, and IL-6, but not in human fibroblasts. Blood 79:45–51
37. Fischer E, Marano MA, van Zee KJ, et al. (1992) Interleukin-1 receptor blockade improves survival and hemodynamic performance in Escherichia coli septic shock, but fails to alter host responses to sublethal endotoxemia. J Clin Invest 89:1551–1557
38. DeForge LE, Tracey DE, Kenney JS, Remick DG (1992) Interleukin-1 receptor antagonist protein inhibits interleukin-8 expression in lipopolysaccharide-stimulated human whole blood. Am J Pathol 140:1045–1054