Agents Actions, 35 (1992)

0065-4299/92/040227-05 $ 1.50+0.20/0

Interleukin-8 stimulates the formation of 15-hydroxy-eicosatetraenoic acid by human neutrophils *in vitro* *

K. Fogh, C.G. Larsen, L. Iversen and K. Kragballe

Department of Dermatology, Marselisborg Hospital, University of Aarhus, Aarhus, Denmark

Abstract

Interleukin-8 (IL-8), is a potent activator of polymorphonuclear leukocyte (PMN) functions including chemotaxis, superoxide anion production, and enzyme release and it is also chemotactic for lymphocytes. Additionally, it has recently been shown that IL-8 stimulates the formation of 5-lipoxygenase (LO) products of arachidonic acid (AA) by human PMNs. The purpose of the present study was to determine whether IL-8 also might affect the formation of 15-LO products from AA. Purified PMNs in phosphate buffered saline were preincubated with and without exogenous AA (10^{-5}–10^{-4} *M*) for 10 min. Then IL-8 was added in biologically relevant concentrations ranging from 0.1 to 100 ng/ml and incubation was carried out for 5 min at 37 °C. Lipids were then extracted from supernatants, and eicosanoids were determined by quantitative RP-HPLC. Compared with unstimulated cells, IL-8 resulted in a dose dependent increase in both LTB_4 and 15-HETE (up to 125% and 40% at 100 ng/ml, respectively). This increase in eicosanoid formation required the presence of exogenous AA. These results indicate that IL-8 is both a potent stimulator of 5-LO activity and of 15-LO activity. LTB_4 can induce both inflammation and contribute to hyperproliferation in the skin. 15-HETE in contrast has the ability to inhibit the effects induced by LTB_4. Because IL-8 is able to stimulate both LTB_4 and 15-HETE formation, the effect of IL-8 as a putative regulator of inflammatory processes may be dependent on the relative stimulation of 5-LO and 15-LO.

Introduction

A variety of proinflammatory mediators are released in cutaneous inflammation. These mediators include peptide-like substances, interleukin-1 [1, 2], complement C5a [3, 4] and bacterial F-Met peptides [5] and lipid-like mediators derived from arachidonic acid, leukotriene B_4 (LTB_4) and 12-hydroxy-eicosatetraenoic acid (12-HETE) [6, 7] and platelet activating factor (PAF) [8]. LTB_4 and 12-HETE are proinflammatory in human skin suggested by their ability to cause skin inflammation [9, 10] and epidermal proliferation [11] after topical application.

15-HETE is also a metabolite of AA formed by the 15-LO, but in contrast to LTB_4 it has no proinflammatory effect in the skin. It inhibits both the formation [12] and the *in vitro* and *in vivo* effects of LTB_4 [13, 14]. Thus 15-HETE has antiinflammatory potential. In support of this is the observed improvement of psoriasis after intralesional injections of 15-HETE [15] and the improvement of

Author for correspondence/Present address: Karsten Fogh, University of Michigan Medical Center, Department of Dermatology, Kresge I, Box 0528, Ann Arbor, Michigan 48109-0528, USA.
* Part of this work has been presented at the Annual Meeting of the European Society for Dermatological Research (E.S.D.R.), Turin, Italy, June, 1990.

carragheenan-induced experimental arthritis in dogs [16]. The regulation of eicosanoid release from human cells is poorly understood. It has been shown that the complement product C5a, FMLP and PAF have the capacity to stimulate the release of LTB_4 from neutrophils *in vitro* [17, 18]. It is unknown which factors are involved in the release of 15-HETE. However, it has been shown that substrate by itself initiates the production of 15-HETE and other 15-LO products [19, 20]. Also, it has been shown that 15-LO activity can be increased by certain non-steroidal antiinflammatory drugs/5-LO inhibitors [21, 22] and by certain hydroxy-eicosatetraenoic acids [23].

IL-8, formerly known as neutrophil-activating protein (NAP-1) [24, 25] is a newly discovered cytokine with the capacity to activate neutrophils [24, 26] and T lymphocytes *in vitro* as well as *in vivo* [24]. IL-8 can be produced by several different cell types present in the human skin including keratinocytes, fibroblasts [27] and endothelial cells [28]. Schröder et al. [3] purified considerable quantities of IL-8 from extracts of psoriatic scales suggesting a pathophysiological role of this leukocyte activating cytokine in the development of psoriasis.

It has recently been shown that IL-8 can stimulate the formation of LTB_4 and its omega-oxidation products by human neutrophils [25, 29]. The purpose of the present study was to determine whether IL-8 might affect not only the formation of LTB_4 but also the formation of 15-HETE.

Materials and methods

Cell preparation

Blood was obtained from healthy human volunteers, who had not taken non-steroidal antiinflammatory drugs during the preceding 2 weeks. Neutrophils were prepared as previously described [22]. After isolation the cells were suspended at a concentration of 12×10^6/ml in phosphate buffered saline (PBS, Sigma) without calcium and magnesium, pH 7.0. Cells were kept at 4 °C until analysis.

Incubation procedure

820 µl neutrophils (12×10^6 cells/ml) were preincubated for 10 min at 37 °C with or without exogenous AA (99% pure, Sigma) at the concentrations mentioned. Then 100 µl of stock solutions (1 ng/ml-1 mg/ml in PBS) of recombinant IL-8 (Dianippon Pharmac, Japan) were added together with 40 µl $CaCl_2$ (final concentration of 3.6 m*M*) and 40 µl $MgCl_2$ (final concentration of 1.9 m*M*). In incubations with ionophore A23187 (Sigma) A23187 was added as 100 µl of a stock solution (50 µ*M*, 1% ethanol). Incubations were carried out for 5 min. at 37 °C in a shaking water bath. Control incubations without AA and without IL-8 or A23187 were carried out in the presence of ethanol. AA and A23187 were dissolved in ethanol and the final alcohol concentration never exceeded 0.2%. The incubations were terminated by cooling to 0 °C in an ice water bath.

Extraction of lipids

After centrifugation (1,500 g for 10 min at 0 °C) the supernatant was collected and 2 ml of icecold methanol was added. This mixture was kept at −20 °C for 15 min and centrifuged (1,500 g for 10 min at 0 °C) to pellet cellular debris and denatured protein and the supernatant was collected. Lipids were then extracted as previously described [22]. Briefly, the acidified supernatant (pH 3) was applied to ODS (octadecylsilyl) silica columns (C_{18}-SEP-PAK, Waters, USA), and eluted sequentially with 2 ml water, 2 ml 25% methanol, 2 ml petroleum ether and 3 ml methanol. The methanol fraction was taken to dryness under a stream of N_2 and resuspended in 70% methanol.

Reversed-phase high performance liquid chromatography (RP-HPLC)

Extracted lipids were separated by RP-HPLC as previously described. RP-HPLC was performed using a system from Waters consisting of two M-6000A pumps, a Hypersil C_{18}-column (5 µm, 100 × 4.6 mm I.D.), a model 490 programmable UV-detector, a model 730 data module and a model 721 system controller. The elution program was a modified isocratic method using methanol/water/acetic acid, 70:30:0.01 (v/v/v) as the mobile phase (solvent A). For the first 10 min the flow rate was 0.75 ml/min and thereafter the flow rate was increased to 1.25 ml/min. At 35 min the mobile phase was changed to 100% methanol (solvent B) for 10 min to elute unreacted AA. Thereafter the

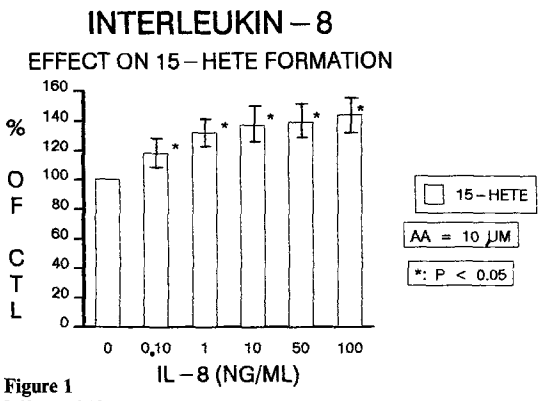

Figure 1
Effect of IL-8 on 15-HETE formation by human neutrophils. 820 µl neutrophils (12×10^6 cells/ml) were preincubated for 10 min at 37 °C in the presence of AA. Then 100 µl of stock solutions (1 ng/ml – 1 mg/l) of IL-8 were added together with 40 µl $CaCl_2$ (final concentration of 3.6 m*M*) and 40 µl $MgCl_2$ (final concentration of 1.9 m*M*). Incubations were carried out for 5 min at 37 °C in a shaking water bath. 15-HETE was determined by integrated optical density. Results are expressed as mean ± SEM of 6 experiments assayed in duplicate. *: IL-8 vs. control.

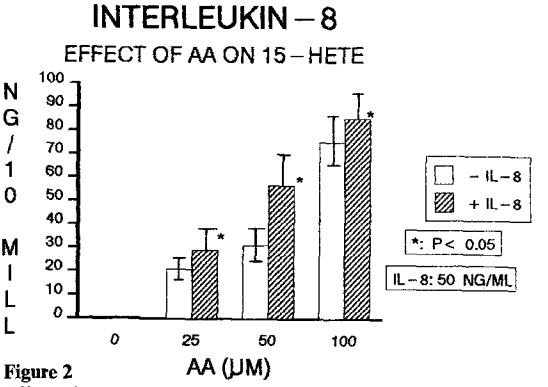

Figure 2
Effect of exogenous AA on IL-8 stimulated 15-HETE formation by human neutrophils. 820 µl neutrophils (12×10^6 cells/ml) were preincubated for 10 min at 37 °C in the presence of AA (0–100 µ*M*). Then 100 µl of a stock solution (0.5 mg/l) of IL-8 were added together with 40 µl $CaCl_2$ (final concentration of 3.6 m*M*) and 40 µl $MgCl_2$ (final concentration of 1.9 m*M*). Incubations were carried out for 5 min at 37 °C in a shaking water bath. 15-HETE was determined by integrated optical density. Results are expressed as mean ± SEM of 6 experiments assayed in duplicate. *: IL-8 vs. control.

column was re-equilibrated with solvent A before the next analysis. The UV-detector was programmed to 270 nm from 0–15 min and to 235 nm from 15–35 min. The eluent was monitored by the UV-detector producing the UV-absorption as a function of wavelength for each chromatographic peak. Quantification of fatty acid metabolites was made by comparison of integrated areas of unknown peaks with those of authentic standards. All peaks in the chromatograms were identified and no unknown peaks were seen [22]. Recovery of ^3H-labeled eicosanoids as internal standards was determined by scintillation counting in a Tricarb scintillation counter as previously described [22]. Tritiated standards were added before extraction and purification. The elution times were standardized daily. The eluent was collected by a fraction collector programmed to collect 1 min fractions.

Statistical Analysis

Results are expressed as the mean ± standard error of the mean (SEM). Statistical significance was assessed by Wilcoxon's ranksum test. A p value below 0.05 was considered significant.

Results

Fig. 1 shows the effect of IL-8 on the formation of 15-HETE, by human neutrophils *in vitro* in the presence of 10 µ*M* AA. IL-8 lead to a dose-dependent increase in 15-HETE formation in the concentration range 0.1–100 ng/ml. At 100 ng/ml there was a 40% increase in 15-HETE formation. For comparison also LTB_4 formation was determined and there was a 125% increase in LTB_4 at 100 ng/ml (data not shown). Incubations with 10 µ*M* AA, but without IL-8 resulted in the formation of 4.7 ± 0.9 ng $LTB_4/10^7$ cells and of 6.7 ± 0.8 ng 15-HETE/10^7 cells.

The increase in 15-HETE formation was dependent on the presence of exogenous AA (Fig. 2) Increased concentrations of AA lead to an increase in 15-HETE formation. IL-8 had an additive effect on AA-induced eicosanoid formation. Also the formation of LTB_4 formation was dependent on the presence of exogenous AA (data not shown). To assess the potency of IL-8 on eicosanoid formation, the effect of the calcium ionophore A23187, a potent activator of LTB_4 formation [30] was determined. 15-HETE release was increased 40% by IL-8 (100 ng/ml), whereas A23187 did not cause any significant increase in the formation of 15-HETE (Fig. 3). In contrast, A23187 (5 µ*M*) induced a 550% increase in LTB_4 formation,

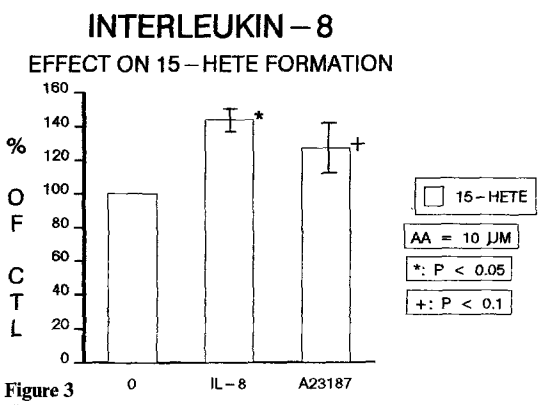

Figure 3
Comparison of IL-8 and A23187 on 15-HETE formation by human neutrophils. 820 µl neutrophils (12×10^6 cells/ml) were preincubated for 10 min at 37 °C in the presence of AA (0–100 µ*M*). Then 100 µl of a stock solution of IL-8 (1.0 mg/l, final concentration of 100 ng/ml) or A23187 (50 µ*M*, final concentration of 5 µ*M*) were added together with 40 µl $CaCl_2$ (final concentration of 3.6 m*M*) and 40 µl $MgCl_2$ (final concentration of 1.9 m*M*). Incubations were carried out for 5 min at 37 °C in a shaking water bath. 15-HETE was determined by integrated optical density. Results are expressed as mean ± SEM of 6 experiments assayed in duplicate. *: IL-8 vs. control, +: A23187 vs control.

whereas the IL-8 induced LTB_4 release was increased by 125% at 100 ng/ml (data not shown).

Discussion

We have shown that IL-8 in a dose-dependent way increases the formation of LTB_4 and 15-HETE by human neutrophils *in vitro*. This stimulation of both LTB_4 and 15-HETE formation is dependent on the presence of exogenous arachidonic acid and therefore reflects increased activity of 5-LO and 15-LO.

IL-8 was formerly known as neutrophil-activating protein (NAP-1), but we have recently suggested that this newly discovered cytokine should be termed interleukin-8 [24]. IL-8 has the capacity to stimulate a number of neutrophil functions [25, 26]. Furthermore, IL-8 is chemotactic for T lymphocytes [24]. It has recently been shown that IL-8 has the capacity to increase the formation of LTB_4 from exogenous AA [25, 29]. In the present study we have confirmed this finding, and shown that in addition IL-8 also has the capacity to increase the formation of 15-HETE.

The requirement for exogenous AA for LTB_4 formation depends on the stimulus. The calcium ionophore A23187 leads to the formation of large amounts of LTB_4 without the addition of AA by a mechanism that involves a rise in intracellular calcium and an activation of phospholipase [31]. As with IL-8, the formation of LTB_4 induced by FMLP or C5a requires the presence of exogenous AA [17]. These findings indicate that these agents stimulate LTB_4 formation without activating phospholipase A_2. Thus, substrate availability seems to be critical for the stimulation of LTB_4 formation by IL-8.

Activation of 15-LO is poorly understood. There is evidence that the formation of 15-HETE only requires substrate for product formation [19, 20] and we have previously shown that increase in 15-HETE formation by neutrophils is associated with an inhibition of LTB_4 formation [22]. In this study we have found that AA leads to the formation of relatively large amounts of 15-HETE and that IL-8 is a potent stimulus for 15-HETE formation in contrast to A23187 that leads to no significant increase in 15-HETE formation (Fig. 3). However, the activation of 15-LO still remains to be clarified. The present results indicate that the activity of IL-8 may partly be mediated through an activation of the 5- and 15-LO pathways of arachidonic acid (i.e. of LTB_4 and 15-HETE formation). Because LTB_4 and 15-HETE have opposite effects on inflammatory processes (pro-inflammatory and anti-inflammatory, respectively), activation of their formation may be of importance in the regulation of inflammatory processes. Therefore, it remains to be determined whether IL-8 *in vivo* leads to an activation of LTB_4 and 15-HETE formation.

Received 29 May 1991; accepted by I. Ahnfeld-Rønne, 19 September 1991.

References

[1] R. D. R. Camp, N. J. Fincham, F. M. Cunningham, M. W. Greaves, J. Morris and A. Chu, *Psoriatic skin lesions contain biologically active amounts of an interleukin 1-like compound.* J. Immunol. *137*, 3469–3471 (1986).

[2] P. M. Dowd, R. D. R. Camp and M. W. Greaves, *In vivo effects of human recombinant interleukin 1 in normal human skin.* J. Invest. Dermatol. *88*, 250 (1987).

[3] J.-M. Schröder and E. Christophers, *Identification of $C5a_{des\,arg}$ and an anionic neutrophil-activating peptide (ANAP) in psoriatic scales.* J. Invest. Dermatol. *87*, 53–58 (1986).

[4] T. Ternowitz, P. Bjerring, P. H. Andersen, J.-M. Schröder and K. Kragballe, *Methotrexate inhibits the human C5a induced skin response in psoriatic patients.* J. Invest. Dermatol. *89*, 192–196 (1987).

[5] H. J. Showell, R. J. Freer, S. H. Zigmond, E. Schiffmann, S. Aswanikumar, B. Corcoran and E. L. Becker, *The structure-activity relations of synthetic peptides as chemotactic factors and inducers of lysosomal enzyme secretion for neutrophils.* J. Exp. Med. *143*, 1154–1169 (1976).

[6] K. Fogh, T. Herlin and K. Kragballe, *Eicosanoids in acute and chronic psoriatic lesions. Leukotriene B_4, but not 12-hydroxy-eicosatetraenoic acid is present in biologically active concentrations in acute guttate lesions.* J. Invest. Dermatol. *92*, 837–841 (1989).

[7] E. A. Duell, C. N. Ellis and J. J. Voorhees, *Determination of 5,12, and 15-lipoxygenase products in keratomed biopsies of normal and psoriatic skin.* J. Invest. Dermatol. *91*, 446–450 (1988).

[8] A. I. Mallet and F. M. Cunningham, *Structural identification of platelet activating factor in psoriatic scale.* Biochem. Biophys. Res. Commun. *126*, 192–198 (1985).

[9] R. D. R. Camp, R. R. Jones, S. Brain, P. Woollard and M. W. Greaves, *Production of intraepidermal microabscesses by topical application of Leukotriene B_4.* J. Invest. Dermatol. *82*, 202–204 (1984).

[10] P. M. Dowd, A. K. Black, P. M. Woollard, R. D. R. Camp and M. W. Greaves, *Cutaneous responses to 12-hydroxy-eicosatetraenoic acid (12-HETE).* J. Invest. Dermatol. *84*, 537–541 (1985).

[11] F. W. Bauer, P. C. M. van de Kerkhof and R. M. Maassen-De Grood, *Epidermal hyperproliferation following the induction of microabscesses by leukotriene B_4.* Br. J. Dermatol. *114*, 409–412 (1986).

[12] J. Y. Vanderhoek, R. W. Bryant and J. M. Bailey, *Inhibition of leukotriene biosynthesis by the leukocyte product 15-hydroxy-5,8,11,13-eicosatetraenoic acid.* J. Biol. Chem. *255*, 10064–10066 (1980).

[13] T. Ternowitz, K. Fogh and K. Kragballe, *15-Hydroxy-eicosatetraenoic acid (15-HETE) specifically inhibits LTB_4-induced chemotaxis of human neutrophils.* Skin Pharmacol. *1*, 93–99 (1988).

[14] T. Ternowitz, P. H. Andersen, P. Bjerring, K. Fogh, J.-M. Schröder and K. Kragballe, *15-Hydroxy-eicosatetraenoic acid specifically inhibits the LTB_4-induced skin response.* Arch. Dermatol. Res. *281*, 401–405 (1989).

[15] K. Fogh, H. Søgaard, T. Herlin and K. Kragballe, *Improvement of psoriasis vulgaris after intralesional injections of 15-hydroxy-eicosatetraenoic acid (15-HETE).* J. Am. Acad. Dermatol. *18*, 279–285 (1988).

[16] K. Fogh, E. S. Hansen, T. Herlin, V. Knudsen, T. B. Henriksen, H. Ewald, C. Bünger and K. Kragballe, *15-Hydroxy-eicosatetraenoic acid (15-HETE) inhibits carragheenan-induced experimental arthritis and reduces synovial fluid Leukotriene B_4 (LTB_4).* Prostaglandins *37*, 213–228 (1989).

[17] R. M. Clancy, C. A. Dahinden and T. E. Hugli, *Arachidonate metabolism by human polymorphonuclear leukocytes stimulated by N-Formyl-Met-Leu-Phe or complement component C5a is independent on phospholipase activation.* Proc. Natl. Acad. Sci. U.S.A. *80*, 7200–7204 (1983).

[18] L. M. Ingraham, T. D. Coates, J. M. Allen, C. P. Higgings, R. L. Baehner and L. A. Boxer, *Metabolic, membrane, and functional responses of human polymorphonuclear leukocytes to platelet-activating factor.* Blood *59*, 1259–1266 (1982).

[19] E. Morita, J.-M. Schröder and E. Christophers, *Production of 15-hydroxyeicosatetraenoic acid by purified human eosinophils and neutrophils.* Scand. J. Immunol *32*, 497–502 (1990).

[20] J. Turk, R. L. Maas, A. R. Brash, L. J. Roberts and J. A. Oates, *Arachidonic acid 15-lipoxygenase products from human eosinophils.* J. Biol. Chem. *257*, 7068–7076 (1982).

[21] J. Y. Vanderhoek, S. L. Ekborg and J. M. Baily, *Nonsteroidal anti-inflammatory drugs stimulate 15-lipoxygenase/leukotriene pathway in human polymorphonuclear leukocytes.* J. Allergy. Clin. Immunol. *74*, 412–417 (1984).

[22] K. Fogh, T. Herlin and K. Kragballe, *In vitro inhibition of leukotriene B_4 formation by exogeneous 5-lipoxygenase inhibitors is associated with enhanced generation of 15-hydroxy-eicosatetraenoic acid (15-HETE) by human neutrophils.* Arch. Dermatol. Res. *280*, 430–436 (1988).

[23] J. Y. Vanderhoek, H. T. Karmin and S. L. Ekborg, *Endogenous hydroxyeicosatetraenoic acids stimulate the human polymorphonuclear leukocyte 15-lipoxygenase pathway.* J. Biol. Chem. *260*, 14482–14487 (1985).

[24] C. G. Larsen, A. O. Anderson, E. Appella, J. J. Oppenheim and K. Matsushima, *The neutrophil-activating protein (NAP-1) is also chemotactic for T lymphocytes.* Science *243*, 1464–1466 (1989).

[25] J.-M. Schröder, *The monocyte-derived neutrophil activating peptide (NAP/interleukin 8) stimulates human neutrophil arachidonate-5-lipoxygenase, but not the release of cellular arachidonate.* J. Exp. Med. *170*, 847–863 (1989).

[26] T. Yoshimura, K. Matsushima, S. Tanaka, E. A. Robinson, E. Appella, J. J. Oppenheim and E. J. Leonard, *Purification of a human monocyte-derived neutrophil chemotactic factor that has peptide sequence similarity to other host defense cytokines.* Proc. Natl. Acad. Sci. U.S.A. *84*, 9233–9237 (1987).

[27] C. G. Larsen, A. O. Anderson, J. J. Oppenheim and K. Matshusima, *Production of interleukin-8 by human dermal fibroblasts and keratinocytes in response to interleukin-1 or tumor necrosis factor.* Immunology *68*, 31–36 (1989).

[28] A. Sica, K. Matshusima, J. M. Wang, N. Polentarutti, E. Dejana, F. Colotta and A. Mantovani, *IL-1 transcriptionally activates the monocyte-derived neutrophil chemotactic factor/IL-8 gene in endothelial cells.* Immunology *69*, 548–553 (1989).

[29] U. Wirtmueller, M. Baggiolini, A. L. de Weck and C. A. Dahinden, *Receptor-operated activation of polymorphonuclear leukocytes: different effects of NAP-1/IL-8 and fMET-LEU-PHE or C5a.* Biochem. Biophys. Res. Commun. *176*, 972–978, (1991).

[30] P. Borgeat and B. Samuelsson, *Metabolism of arachidonic acid in polymorphonuclear leukocytes. Effect of calcium ionophore A 23187.* Proc. Natl. Acad. Sci. U.S.A. *76*, 2148–2152 (1979).

[31] C. A. Rouzer and B. Samuelsson, *Reversible, calciumdependent membrane association of human leukocyte 5-lipoxygenase.* Proc. Natl. Acad. Sci. U.S.A. *84*, 7393–7397 (1987).