



Short Communication

Effect of IL-18 on IL-1 β and sIL-1RII Production by Human Neutrophils

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Abstract. In the present study we investigated the effect of interleukin 18 (IL-18) on the production of IL-1 β and soluble IL-1 receptor II (sIL-1RII) by human neutrophils. The results obtained indicate that recombinant human IL-18 (rhIL-18) induces IL-1 β and, to a lesser extent, sIL-1RII production by human neutrophil isolated from peripheral blood. However, this effect was less important than lipopolysaccharide (LPS) stimulation. Additionally, our observations suggest that IL-18 can induce priming of neutrophils for IL-1 β and, to a lesser extent, sIL-1RII production by LPS-stimulated cells. The ability of IL-18 to serve as an effective modulator for IL-1 β and its regulatory protein may have significance in the inflammatory and immune reactions mediated by IL-1 β .

Key words: neutrophil; interleukin 18; interleukin 1 β ; soluble interleukin 1 receptor II.

Introduction

Neutrophils, or polymorphonuclear leukocytes (PMN), the main cells of the innate immune response, are capable of simultaneous release of interleukin 1 β (IL-1 β) and its regulatory protein, soluble IL-1 receptor type II (sIL-1RII)^{3, 4}. Since sIL-1RII has been shown to inhibit or antagonize the effects of IL-1 β , its production by large numbers of tissue-invading neutrophils may suggest a mechanism by which the effects of IL-1 β are regulated. The sIL-1RII retain their affinity for the ligand IL-1 but do not induce signal transduction and, thus, appear to function as natural inhibitors of IL-1 β ⁷. IL-1 β and sIL-1RII belong to the large IL-1 family, also involving IL-18¹.

IL-18, originally described as an interferon γ (IFN- γ)-inducing factor, plays an important role in immunologic and inflammatory reactions^{1, 2}. IL-18 is structu-

rally similar to the IL-1 family and functionally similar to IL-12^{1, 6}. IL-18 has direct proinflammatory properties. Puren et al.⁶ reported that, in human unstimulated peripheral blood mononuclear cells (PBMC), IL-18 induces synthesis of IL-1 β , tumor necrosis factor α (TNF- α) and IL-8.

In the present study we investigated the effects of recombinant human IL-18 (rhIL-18) on the induction of IL-1 β and sIL-1RII production by peripheral blood neutrophils. Since the binding of lipopolysaccharide (LPS) to receptors on leukocytes triggers the production of highly active mediators, such as IL-1 β , we compared the effect of rhIL-18 stimulation with that of LPS stimulation of PMN. In addition, we estimated the priming effect of rhIL-18 on the secretion of IL-1 β and its regulators by LPS-stimulated cells.

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Materials and Methods

PMN were isolated from heparinized (10 U/ml) whole blood of healthy donors by Gradisol G gradient, washed and resuspended at 5×10^6 cells/ml in RPMI 1640 containing 100 U/ml penicillin, 100 µg/ml streptomycin and 10% autologous serum. The purity of the PMN was determined by May-Grunewald-Giemsa staining (94%). The cells were cultured in flat-bottomed 96-well plates (Falcon) with rhIL-18 (50 ng/ml, R&D System) in the presence or absence of anti-IL-18 mAbs (20 µg/ml, R&D System) or LPS (100 g/ml, Sigma-Chemical Co) 18 h at 37°C in a humidified incubator with 5% CO₂.

In the culture supernatants of the PMN, the concentrations of IL-1β and sIL-1RII were measured with ELISA kits (R&D System).

The results are expressed as mean ± standard deviation ($\bar{x} \pm SD$). Data were analyzed according to variance and Student's *t*-test. A *p*-value of less than 0.05 was considered to represent a statistically significant difference.

Results

Supernatant IL-1β levels were significantly higher in the cultures of PMN stimulated with LPS and rhIL-18 compared with unstimulated control cells. Although the increase of IL-1β in the culture supernatants of rhIL-18-stimulated PMN was statistically significant (*p*<0.05), the amounts of IL-1β was 2-fold lower than by LPS stimulation. When PMN were pre-incubated with rhIL-18, the production of IL-1β was enhanced compared with LPS stimulation alone (Fig. 1).

Recombinant human IL-18 stimulation led to a slight increase of sIL-1RII secretion by PMN. Recombinant human IL-18 pre-incubation of PMN did not have a significant influence on the release of sIL-1RII by LPS-stimulated cells in comparison with LPS stimulation alone (Fig. 2).

The ratios between sIL-1RII and IL-1β in the supernatants of rhIL-18-stimulated cells were higher than those in supernatants of LPS-stimulated cells (7.36 and 4.85, respectively).

Discussion

In the present study we found an interesting relationship between IL-1β and its regulatory protein simultaneously secreted by unstimulated human neutro-

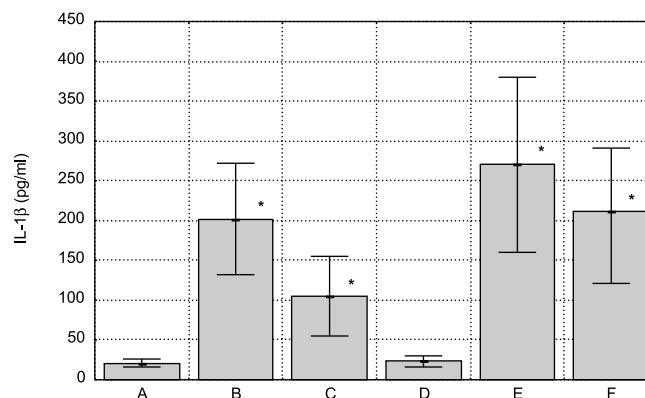


Fig. 1. IL-1β concentrations in the culture supernatants of unstimulated, IL-18- and LPS-stimulated human neutrophils as mean value ± SD, **p*<0.05 compared with unstimulated cells. A – unstimulated, B – LPS, C – IL-18, D – IL-18 + anti-IL-18, E – IL-18 + LPS, F – IL-18 + anti-IL-18 + LPS

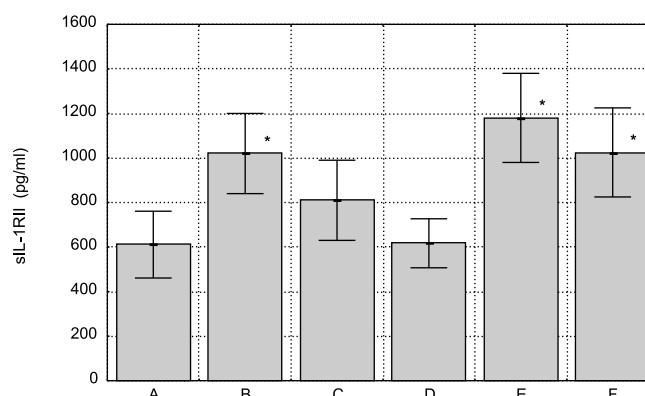


Fig. 2. Soluble IL-1RII concentrations in the culture supernatants of unstimulated, IL-18- and LPS-stimulated human neutrophils as mean value ± SD, **p*<0.05 compared with unstimulated cells. Explanations see Fig. 1

phils. These cells produced a small amount of IL-1β and a relatively large amount of sIL-1RII. This interrelation was changed in the cultures with rhIL-18. We demonstrated a significant effect of rhIL-18 on IL-1β production by PMN but not on sIL-1RII production. However, this effect is less important in comparison with LPS stimulation. Observations including the LPS effect are in agreement with the data of ROUX-LOMBARD⁷ and PENTON-ROLL et al.⁵, who demonstrated that LPS is a powerful inducer of IL-1β and sIL-1RII in monocytes.

The results obtained indicate that rhIL-18 also has the ability to prime neutrophils for IL-1β and, to a lesser extent, sIL-1RII secretion. The data presented in this study and earlier demonstrate the ability of IL-18

to enhance IL-1 β production by PBMC and appear to indicate an essential role of IL-18 in IL-1 β -mediated reactions. Additionally, IL-18 together with IL-12 are capable of inducing IL-6 and TNF- α production by mononuclear cells, which might lead to an increase of sIL-1RII secretion by neutrophils. It has been reported that treatment of PMN with TNF- α results in a massive, rapid, and protein synthesis-independent release of a soluble form of IL-1RII from the PMN surface⁴.

In the present study we also observed that rhIL-18 stimulation led to an increase of the sIL-1RII/IL-1 β ratio in comparison with LPS stimulation. This may have different implications in states associated with a higher concentration of IL-18. Soluble IL-1RII has a high affinity for IL-1 β , so a relationship between this regulator and its ligand may have a significant influence on IL-1 β -mediated reactions. This might lead to a limiting of the inflammatory process. Thus, sIL-1RII secreted by neutrophils is thought to be a part of a paracrine and autocrine regulatory system modulating IL-1 β functions in the immune response.

In conclusion, our data suggest that the capacity of IL-18 to serve as an effective modulator for the interrelation between IL-1 β and sIL-1RII production by human neutrophils may have significance in the inflammatory and other reactions mediated by IL-1 β . Understanding the complex inflammatory networks involving IL-18 and IL-1 β as well as its regulatory protein may provide novel strategies for augmenting or diminishing the early innate immune response.

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