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Role of interleukin-15 and interleukin-18 in the secretion of sIL-6R and sgp130 by human neutrophils

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Background: Available data indicate that neutrophils (PMN) produce a wide range of cytokines with the potential to modulate immune response. Recent investigation have shown that interleukin (IL)-15 and IL-18 potentiated several functions of normal neutrophils. It has been reported that IL-18-induced cytokine production may be significantly enhanced by coincident addition of IL-15.
Aims: In the present study we compared the effect of recombinant human (rh)IL-15 and rhIL-18 as well as effect of a rhIL-15 and rhIL-18 combination on the induction secretion of sIL-6Rα and sgp130 by human neutrophils.
Methods: PMN were isolated from heparinized whole blood of healthy persons. The PMN were cultured for 18 h at 37°C in a humidified incubator with 5% CO_2. rhIL-15 and/or rhIL-18 and lipopolysaccharide were tested to PMN stimulation. The culture supernatants of PMN were removed and examined for the presence of sIL-6R and sgp130 by human enzyme-linked immunosorbent assay kits. Cytoplasmic protein fractions of PMN were analysed for the presence of sIL-6R and sgp130 by western blotting using monoclonal antibodies capable of detecting these proteins. Cells were lysed and cytoplasmic proteins were electrophoresed on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The resolved proteins were transferred onto nitrocellulose and incubated with the primary monoclonal antibodies anti-sIL-6R and anti-sgp130. The membranes were incubated at room temperature with alkaline phosphatase anti-mouse immunoglobulin G. Immunoreactive protein bans were visualized by an AP Conjugate Substrate Kit.
Results and conclusion: The results of our investigation revealed that IL-15 alone, similarly to IL-18, has no significant ability for the regulation of both soluble IL-6 receptors, sIL-6R and sgp130, released by human neutrophils. It is interesting to note that the secretion of sgp130 was changed after PMN stimulation with rhIL-15 in the presence of rhIL-18. The combination of rhIL-15 and rhIL-18 was shown to induce PMN to secretion relatively higher amounts of sgp130 compared with the stimulation of PMN with rhIL-15 alone and rhIL-18 alone. The results obtained suggest that IL-15 and IL-18, belonging to the inflammatory cytokines, through the regulation of sgp130 secretion must be also considered as anti-inflammatory mediators that may influence the balance reactions mediated by the IL-6 cytokine family.

Key words: Neutrophils, Soluble interleukin-6 receptor α, Soluble gp130, Interleukin-15, Interleukin-18

Introduction

Interleukin (IL)-15 is a pleiotropic cytokine that shares the biological activities with of IL-2. IL-15 uses both β-chains and γ-chains of the IL-2 receptor for binding and signalling. The IL-15 receptor (IL-15R) complex also includes a specific α subunit (IL-15Rα), distinct from the IL-2Rα chain. IL-15R is expressed on various cells of the immune response, including T cells and B cells, NK cells and, more recently, peripheral blood neutrophils.[1–4]

IL-15 plays an important role in both innate and adaptive immunity. It stimulates antigen-driven T-cell proliferation, it has co-stimulatory activity for prolif-

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eration and immunoglobulin (Ig) production by B cells, and is an efficient activator of natural killer and lymphokine activated killer (LAK) cells.[2,4]

Recent investigation have shown that IL-15 potentiated several functions of normal neutrophils (PMNs) involved in the innate immune response against invading pathogens. IL-15 was observed to enhance phagocytosis, nuclear factor-κB activation and IL-8 production, and to delay apoptosis of these cells.[4–6] Available data indicate that PMN produce a wide range of cytokines with potential to modulation of immune response. The induction of PMN can lead to the synthesis of cytokines such as IL-1, IL-3, IL-6, IL-8, IL-12, transforming growth factor-β1 interferon-α, granulocyte-colony stimulating factor, granulocyte–macrophage-colony stimulating factor (GM-CSF), tumor necrosis factor (TNF)-α, growth-related oncogene (Gro)α, macrophage inflammatory protein (MIP)-1α and MIP-1β.[4,7] It was also found that PMN, similar to the other immune cells, are able to produce naturally occuring proteins that regulate the activity of certain cytokines in biological fluids or in tissue culture supernatants.[7] PMNs have the ability to simultaneous release IL-6 and its regulators: soluble receptors, sIL-6Rα and sgp130, that control both local and systemic IL-6-mediated responses.[7,8] Because various stimuli are able to induce the cellular release of cytokines and their soluble receptors, it is of interest to examine the role of other inflammatory cytokines in the regulation of IL-6 regulatory protein production. Previous studies in our laboratory have established that IL-18 is a promising candidate for the enhanced secretion of IL-6 by human neutrophils but not for both soluble receptors of IL-6.[8] It is known that IL-18 does not act alone, but in combination with other cytokines, such as IL-12 and/or IL-15. Different combinations of IL-18, IL-12 and IL-15 induce distinct effects. For example, McInnes *et al*. reported that IL-18-induced cytokine production may be significantly enhanced by coincident addition of IL-15.[9]

In the present study we compared the effect of recombinant human (rh)IL-15 and rhIL-18 as well as synergistic effect of rhIL-15 with rhIL-18 on the induction secretion of sIL-6Rα and sgp130 by human neutrophils. Studies of the interrelations between these mediators may provide new data on the inflammatory network cytokine controlled by these cells.

Materials and methods

Neutrophils and peripheral blood mononuclear cells (PBMC) were isolated from heparinized (10 U/ml) whole blood of 15 healthy persons by Gradisol G gradient (1.115 g/ml). This method enables simultaneous separation of two highly purified leukocyte fractions: mononuclear cells containing 95% lymphocytes, and polymorphonuclear cells containing 94% PMN. The purity of isolated PMN and PBMC was determined by May–Grunewald–Giemsa staining.

Cells were suspended in the culture medium (RPMI-1640, autologous serum, penicillin and streptomycin) to provide 5×10^6 cells/cm^3. The PMN were cultured in 96-well plates (Falcon) for 18 h at 37°C in a humidified incubator with 5% CO_2 (Nuaire™). RhIL-15 (50 ng/ml; R&D Systems, Minneapolis, USA) and/or rhIL-18 (50 ng/ml; R&D Systems) and LPS (50 ng/ml; Sigma) were tested to stimulate secretion by PMN. After 18 h incubation, the culture supernatants of PMN were removed and examined for the presence of sIL-6R and sgp130 by human ELISA kits (R&D Systems) obtained from BIOKOM.

Cytoplasmic protein fractions of PMN and PBMC were analysed for the presence of sIL-6R and sgp130 by western blotting using monoclonal antibodies capable of detecting these proteins, anti-sIL-6R and anti-sgp130, respectively (R&D Systems, from BIOKOM). Cells were lysed directly by sonication, and cytoplasmic proteins were electrophoresed on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The resolved proteins were transferred onto nitrocellulose and incubated with the primary monoclonal antibodies anti-sIL-6R and anti-sgp130. The membranes were incubated at room temperature with alkaline phosphatase anti-mouse IgG. Immunoreactive protein bans were visualized by the AP Conjugate Substrate Kit.

Results

SIL-6Rα and sgp130 protein expression in human PMN and PBMC detected by western blot

We used a specific monoclonal antibody directed against the intracellular forms of sIL-6R and sgp130 for western blot in PMN and for comparison in PBMC. As shown in Fig. 1, the antibody in human unstimulated and LPS-stimulated PMN and PBMC identified bands of 110 and 55 kDa, respectively, from each donor.

Western blot analysis showed that the samples of unstimulated PMN and PBMC contained a 110 kDa protein that was stained by an anti-sgp130 monoclonal antibody. These samples also contained a 55 kDa protein stained by an anti-sIL-6R monoclonal antibody. The LPS-stimulated PMN and PBMC expressed a little increased sIL-6R protein in comparison with the unstimulated cell lines. In contrast, sgp130 expression in unstimulated and LPS-stimulated cells was on the same level. Comparison of sIL-6R and sgp130 bands indicated that the expression of sgp130 is more intensive than expression of sIL-6R.

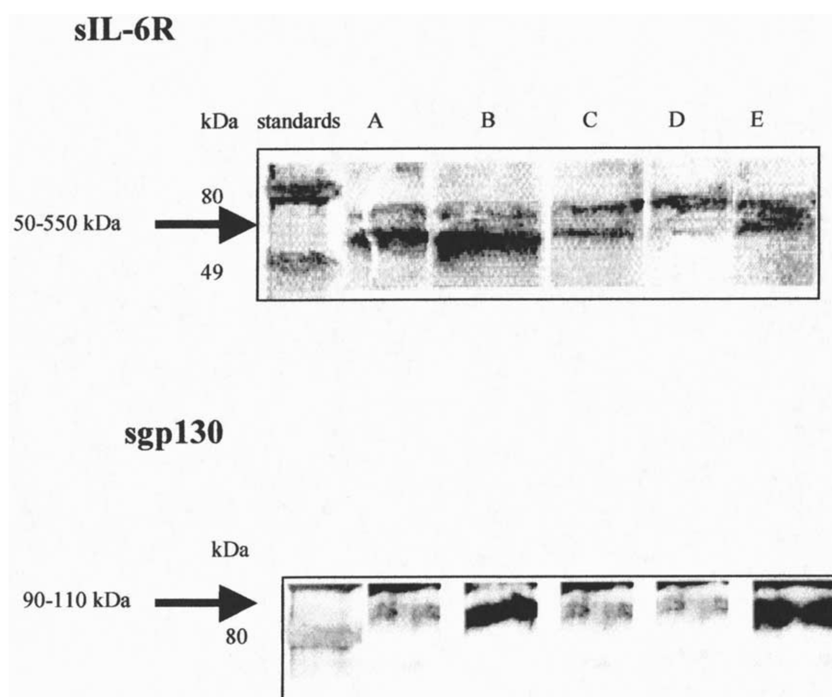

FIG. 1. Western blot analysis of sIL-6R and sgp130 proteins expression in human PMN and PBMC cells. We showed detection of sIL-6R and sgp130 proteins from human unstimulated (line A) and LPS-stimulated (line B), rhIL-15-stimulated (line C), rhIL-18-stimulated (line D), and rhIL-15+rhIL-18-stimulated (line E) PMNs. Whole cell lysates were obtained from fresh PMN of healthy donors. One hundred micrograms of total proteins from PMN were loaded onto a 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and transferred to a nitrocellulose filter. The filter was incubated with 1:1000 dilution of respective antibody. Sizes of protein standards are given in kilodaltons. One representative western blot of a few independent experiments is shown.

SIL-6Rα and sgp130 concentrations in the culture supernatants of PMN detected by enzyme-linked immunosorbent assay

In the culture supernatants of unstimulated PMN we observed about 10 times more sgp130 than sIL-6Rα (23.8 ng/ml and 2.27 ng/ml, respectively).

Similar to rhIL-18, rhIL-15 did not have a significant effect on the release of sIL-6Rα and sgp130. However, concentrations of both soluble molecules in the culture supernatants of rhIL-15-stimulated PMN were non-significantly higher than those in the culture supernatants of rhIL-18-stimulated PMN (Fig. 2).

LPS stimulation led to a significant increase in the sIL-6R secretion by PMN in comparison with unstimulated PMN. We did not observe that kind of relation in the case of sgp130 secretion by PMN (Fig. 2).

Release of sIL-6R by PMN stimulated with rhIL-15 and rhIL-18 together were in the levels of sIL-6R in the culture of PMN stimulated with rhIL-15 only.

In contrast to sIL-6R, secretion of sgp130 by PMN after stimulation with rhIL-15 and rhIL-18 together was higher than sgp130 secretion by PMN after stimulation with rhIL-15 or rhIL-18 alone.

Discussion

Results of our investigation revealed that IL-15, similarly to IL-18, has no important ability in the regulation of both soluble IL-6 receptors, sIL-6R and sgp130, release by human neutrophils.

The lack of effect of these cytokines on the sIL-6R secretion appears to confirm data demonstrated by other authors who did not find a significant influence of most inflammatory cytokines on sIL-6Rα production. They demonstrated that IL-1β, IL-6, IL-4, IL-10 or TNF-α did not affect the sIL-6R production.[10] We have found that LPS alone stimulated significantly greater amounts of sIL-6Rα than medium controls, rhIL-15 alone, rhIL-18 alone as well as the combination of rhIL-15 and rhIL-18.

Similarly, the secretion of sgp130 was independent of the presence of LPS, rhIL-15 or rhIL-18. It is interesting to note that the secretion of sgp130 was changed after PMN stimulation with rhIL-15 in the presence of rhIL-18. The combination of rhIL-15 and rhIL-18 was shown to induce PMN to secretion relatively higher amounts of sgp130 compared with the stimulation of PMN with rhIL-15 alone and rhIL-18 alone. Our results indicate that rhIL-15 alone and rhIL-18 alone, in the used concentrations, do not

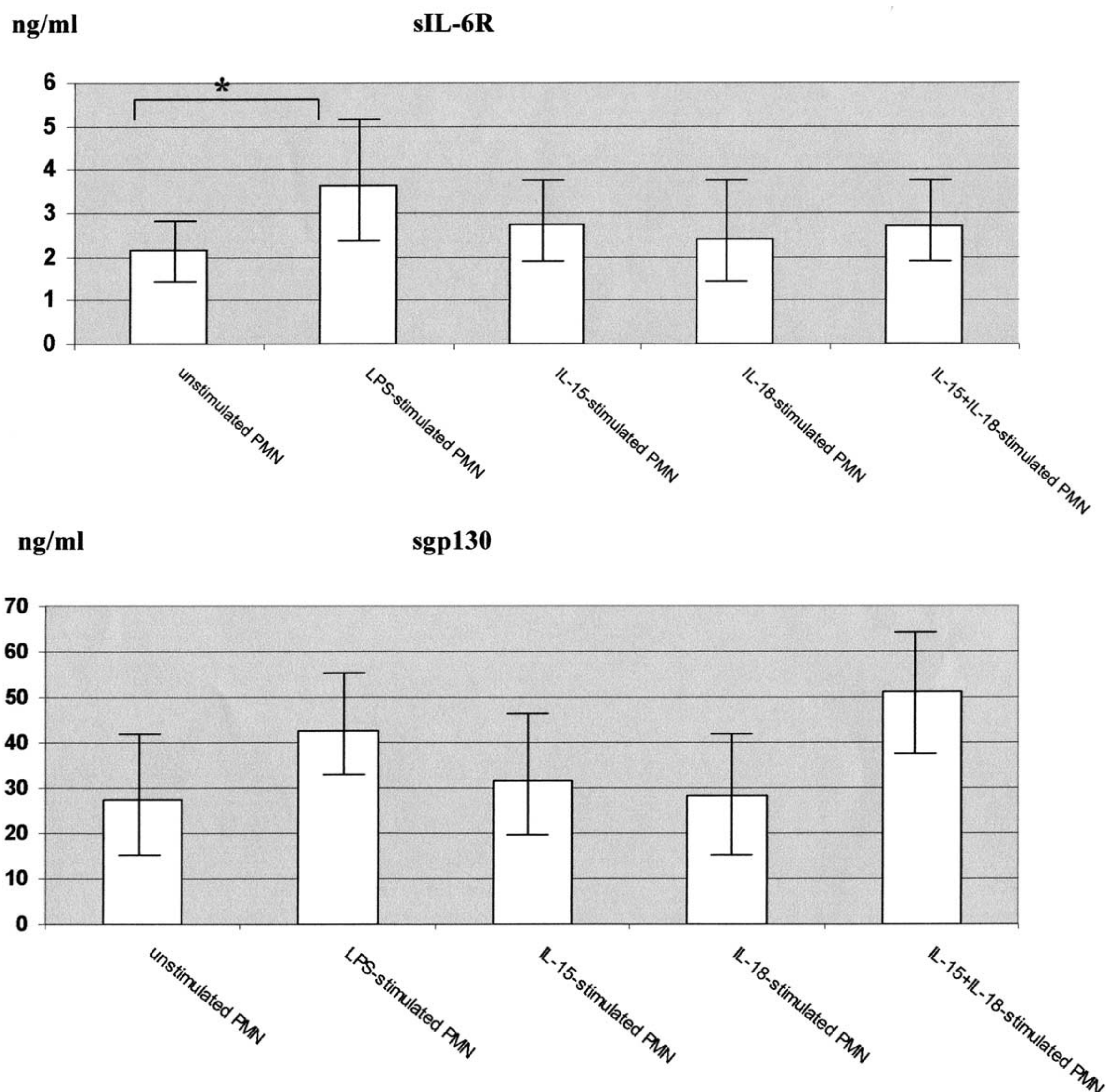

FIG. 2. The concentrations of sIL-6R and sgp130 in the culture supernatants of LPS-stimulated, rhIL-15-stimulated and rhIL-18-stimulated PMN. * Statistical difference with unstimulated cells ($p < 0.05$).

provide signals sufficient to induce soluble IL-6 receptor release by human neutrophils.

However, there are available data suggesting a significant role of IL-15 and IL-18 in regulatory function of PMN.[5,6] An important role for IL-18 in the stimulation of PMN activity was recently documented. It has been found that the capacity of PMN to release IL-1β, IL-6, IL-8 and TNF-α was enhanced by the presence of IL-18.[11–13] In our previous study we indicated that IL-18 is also a promising candidate for the enhanced secretion of IL-6 by human neutrophils. However, we have not found a significant effect of IL-18 on the soluble IL-6 receptors.[12] Additionally, we investigated an effect of IL-15 on the simultaneous secretion of IL-1β and its soluble receptor type II (sIL-1RII) by human neutrophils from normal and tumour-bearing hosts. Our observations indicated that IL-15 induces IL-1β but not sIL-1RII release by these cells.[13]

The observation indicating various actions of cytokine alone and of cytokine in combination with others are in agreement with reports of different authors. For instance, Fehniger *et al*. demonstrated that the optimal co-stimulatory monokine combination for GM-CSF production by human natural killer cells was IL-18 and IL-15.[14]

A probable explanation for the synergistic effect of IL-18 with IL-15 on the secretion of sgp130 by PMN may be an influence of these cytokines on their specific membrane-bound receptor expression. A recent report characterizing IL-15R and IL-18R expression on PMN revealed that these cells constitutively express both IL-15R and IL-18R mRNA and proteins.[5,11] It is possible that IL-15 and IL-18 synergize in inducing sgp130 production by PMN via induction of their membrane-bound receptor expression on PMN, IL-18R can be up-regulated by IL-15 and, conversely, IL-15R may be regulated by the

presence of IL-18. Further examinations involving estimation of membrane-bound receptor expression are needed to confirm of these suggestions.

In conclusion, the results obtained revealed that IL-15 and IL-18 belonging to the inflammatory cytokines, through the regulation of sgp130 secretion, should also be considered anti-inflammatory mediators that may influence the balance reactions mediated by the IL-6 cytokine family, involving also IL-11, leukemia inhibiting factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor (CNTF) or cardiotrophin (CT)-1. However, the clinical studies involving inflammation and other pathological conditions may be helpful to explain an importance of these observations.

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