Some Plasma Component is Essential for IL-6 Secretion by Neutrophils

Katsutaka OISHI and Kazuhiko MACHIDA

Department of Hygiene and Public Health, School of Human Sciences Waseda University, Tokorozawa

Abstract

Recently, the neutrophil has been shown to have the capacity to produce a number of cytokines. Among these cytokines, interleukin-1 (IL-1), tumor necrosis factor- α (TNF- α) and IL-6 are typical examples of multifunctional cytokines involved in regulation of the immune response and inflammation. However, there are conflicting reports in the literature concerning the issue of whether IL-6 expression can be modulated in human neutrophils.

In this study, we showed the spontaneous secretion of IL-1 β , TNF- α and IL-6 by neutrophils. IL-6 secretion of neutrophils was effectively induced by IL-1 β . However, spontaneous or IL-1 β induced secretion of IL-6 was observed only when the cells were cultured in autologous plasma. Then we examined the spontaneous secretion of cytokines by co-cultured leukocytes (neutrophils, monocytes, and lymphocytes). Surprisingly, IL-6 was dramatically secreted by the leukocytes only when the cells were cultured in autologous plasma, although IL-1 β and TNF- α were secreted by the cells either in RPMI 1640 or in plasma. These observations suggest that the regulation of IL-6 production is qualitatively different from the production of IL-1 β or TNF- α by leukocytes.

Key words: Neutrophils, IL-6, Plasma, IL-1 β , Monocytes

Introduction

The neutrophil has long regarded as a short-lived, terminally differentiated leukocyte with limited ability to produce de novo proteins. Recently, however, it has been shown to have the capacity to produce a number of cytokines, including interleukin-1 (IL-1), tumor necrosis factor- α (TNF- α), IL-6, alpha-interferon (IFN- α), macrophage colony-stimulating factor (M-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-8¹⁾. Among these cytokines, IL-1, TNF and IL-6 are typical examples of multifunctional cytokines involved in regulation of the immune response and inflammation²⁾. Although the ability of neutrophils to synthesize these cytokines is becoming increasingly understood, little is known regarding the factors involved in the regulation of cytokine production. Moreover, there are conflicting reports in the literature concerning the issue of whether IL-6 expression can be modulated in human neu-

trophils³⁾. Several reports have indicated that IL-6 is produced by neutrophils⁴⁻⁶⁾, although other reports have not⁷⁻¹⁰⁾.

In this study, we first examined the spontaneous production of IL-1 β , TNF- α and IL-6 in the presence and absence of plasma. We demonstrated that the spontaneous production of IL-6 by neutrophils or by co-cultured leukocytes (neutrophils, monocytes, and lymphocytes) required the presence of plasma, while IL-1 β and TNF- α were produced in the absence of plasma in *in vitro* culture. Moreover, we demonstrated that the production of IL-6 by neutrophils was induced only in the presence of plasma. However, heat-treated plasma was ineffective for neutrophil production of IL-6 induced by IL-1 β . These findings showed that some plasma component was essential for IL-6 production of neutrophils, and our results explained the reason why there are conflicting reports about IL-6 production by neutrophils. Furthermore, it is suggested that the regulation of IL-6 production is qualitatively different from the production of IL-1 β or TNF- α by neutrophils.

Material and methods

Cell preparation and culture. Neutrophils were isolated from heparinized (9U/mL) venous blood of healthy volunteers by the one-step Mono-Poly Resolving Medium (Dainippon

Reprint requests to:
Kazuhiko Machida,
Department of Hygiene and Public Health, School of Human Sciences,
Waseda University, 2-579-15 Mikajima, Tokorozawa, Saitama 359,
Japan
Tel.0429-49-8111, Fax.0429-48-4314

Table 1 Spontaneous secretion of cytokines by neutrophils

Medium	IL-1 β (pg/mL)	TNF- α (pg/mL)	IL-6 (pg/mL)
RPMI 1640	1.5 $\pm$ 1.0	(ND)	(ND)
Plasma	8.2 $\pm$ 1.5	19.2 $\pm$ 1.7	442.5 $\pm$ 21.4

Neutrophils (1×10^6 cells/mL) were cultured in the presence or absence of autologous plasma. After 48 hours of culture, cell-free supernatants were collected and measured for immunoreactive cytokines. Values are expressed as means $\pm$ SD of triplicate cultures.

Abbreviation: ND, not detectable.

Table 2 Spontaneous secretion of cytokines by co-cultured leukocytes

Medium	IL-1 β (pg/mL)	TNF- α (pg/mL)	IL-6 (pg/mL)
RPMI 1640	5.8 $\pm$ 0.2	3.3 $\pm$ 1.3	(ND)
Plasma	11.3 $\pm$ 0.6	30.2 $\pm$ 3.9	531.7 $\pm$ 18.0

Leukocytes (neutrophils, monocytes and lymphocytes) were cocultured in the presence or absence of autologous plasma. After 48 hours of culture, cell-free supernatants were collected and measured for immunoreactive cytokines. Values are expressed as means $\pm$ SD of triplicate cultures.

Abbreviation: ND, not detectable.

Pharmaceutical Co., Osaka, Japan) separation procedure^{11, 12}. The remaining neutrophils were washed twice and cultured in RPMI 1640 medium (Dainippon Pharmaceutical Co., Osaka, Japan) or in autologous plasma, pH 7.4, containing 10 U/mL penicillin and 100 μ g/mL streptomycin. Neutrophils were more than 95% pure. All steps of cell preparation were normally performed at room temperature ($25 \pm 2^\circ\text{C}$) to avoid changes of receptor expression¹³. Cell suspensions (1×10^6 cells/mL) were incubated at 37°C in a 5% CO_2 atmosphere.

Co-culture system. Neutrophils and the other kinds of leukocytes were obtained by one-step centrifugation as described above (*Cell preparation and culture*). Neutrophils (1×10^6 cells/mL) and the other leukocytes (5×10^5 cells/mL, including 83% lymphocytes and 16% monocytes) were cocultured for 48 hours. Autologous plasma or RPMI 1640 was used for culture media.

ELISA assay for cytokines. Immunoreactive IL-1 β , TNF- α , and IL-6 in culture supernatants were measured with commercially available assay kits. Assays for IL-1 β and IL-6 (R & D Systems, Inc., Minneapolis, MN) had a sensitivity of 0.3 pg/mL and 0.7 pg/mL, respectively. The TNF- α assay (Endogen, Inc., Cambridge, MA) had a sensitivity of 2 pg/mL. These assays do not cross-react significantly with other known cytokines.

Results

Influence of autologous plasma on spontaneous secretion of cytokines by neutrophils. Neutrophils were cultured in RPMI 1640 or in autologous plasma. Autologous plasma used in this study contained 1 pg/mL of IL-6, whereas both IL-1 β and TNF- α were undetectable. Cytokine release by neutrophils was negligible in RPMI 1640 (Table 1). However, exposure of neutrophils to autologous plasma resulted in IL-6 secretion in culture supernatants. On the other hand, levels of IL-1 β and TNF- α in the culture supernatants were not so dramatic in comparison to the level of IL-6.

Influence of autologous plasma on spontaneous secretion of cytokines by co-cultured leukocytes. Neutrophils, lymphocytes and monocytes were co-cultured in RPMI 1640 or in autologous plasma (Table 2). Co-cultured leukocytes secreted low levels of IL-1 β and TNF- α in RPMI 1640. However, IL-6 was not secreted at all in RPMI 1640. On the other hand, IL-1 β , TNF- α and IL-6 were secreted by leukocytes in autologous plasma. It was surprising that IL-6 secretion by leukocytes was dramatically induced in the plasma culture, Although levels of IL-1 β and TNF- α were not so increased compared with the incubation with RPMI 1640.

Fig. 1 Released IL-6 immunoreactivity from neutrophils stimulated with 20ng/mL IL-1 β . Neutrophils (1×10^6 cells/mL) were cultured in RPMI 1640 only, in RPMI 1640 with IL-1 β (20ng/mL), in autologous plasma only, and in the plasma with IL-1 β for 48 hours. Immunoreactive IL-6 in the supernatants was determined by ELISA. Values are expressed as means $\pm$ SD of triplicate cultures.

Fig. 2 Influence of untreated and heat-treated plasma on IL-6 secretion by neutrophils stimulated with IL-1 β . Neutrophils (1×10^6 cells/mL) were cultured with 20ng/mL IL-1 β in untreated plasma and in heat-treated plasma. The plasma was heated at 56°C for 20 minutes. Immunoreactive IL-6 in the supernatants was determined by ELISA. Values are expressed as means $\pm$ SD of triplicate cultures.

Requirement of plasma component for IL-6 induction by IL-1 β .

To examine whether some plasma component is essential for neutrophil secretion of IL-6, neutrophils were stimulated by IL-1 β with or without plasma (Fig. 1). IL-1 is a potent inducer of IL-6, because the IL-6 promoter is known to be rapidly and transiently activated by IL-1¹⁴. As shown in Fig. 1, IL-6 secretion from neutrophils was effectively increased by IL-1 β only when the cells were cultured in autologous plasma. However, IL-1 β was ineffective in IL-6 secretion from neutrophils when the cells were cultured in RPMI 1640. It should be noted that neutrophils cultured in the plasma could secrete IL-6 without IL-1 β , although the neutrophils cultured in RPMI 1640 could not secrete IL-6 even when the cells were exposed to IL-1 β . Moreover, heat-treated plasma had a decreased effect on the IL-6 induction by IL-1 β in comparison to the untreated plasma (Fig. 2). These results suggested that some heat-sensitive component in the plasma was essential for IL-6 secretion by neutrophils.

Discussion

While there have been many reports indicating that neutrophils can express and secrete several cytokines after treatment *in vitro*, it is uncertain if these cells can express these cytokines *in vivo*. In this study, we demonstrated that neutrophils can spontaneously secrete substantial levels of IL-6 in autologous plasma, although the cells failed to secrete IL-6 in RPMI 1640. Moreover, neutrophils secreted IL-6 after 24 hours of incubation in plasma. This observation suggested that neutrophils could not only transiently but continuously produce IL-6. However, IL-6 production in neutrophils may be negatively regulated *in vivo*, since IL-6 has been suggested to be involved in the pathogenesis of a variety of diseases, plasma cell neoplasms and glomerulonephritis¹⁴. Actually, the production of IL-6 in various cells is positively or negatively regulated by a variety of signals¹⁵.

In this study, we showed that neutrophils could spontaneously secrete IL-6 in autologous plasma, although the cells failed to secrete IL-6 without plasma. Moreover, IL-6 secretion from neutrophils was dramatically increased by IL-1 β when the cells were cultured in autologous plasma. It should be noted that heat-treated plasma had less effect on IL-6 induction by IL-1 β . These results suggested that some plasma component was essential for IL-6 production in neutrophils. Our suggestion may be supported by many other reports. On the one hand, Takeichi et al.¹⁰ reported that neutrophils stimulated with concanavalin A, lipopolysaccharide (LPS), or zymosan produced IL-1 α and IL-1

β , but that IL-6 could not be produced. However, in their study, no fetal bovine or human serum was added to the media. Wang et al.⁷ have indicated that neutrophils stimulated by LPS produce IL-8, IL-1 α , IL-1 β and TNF- α , but IL-6 production could not be detected in heat-inactivated fetal calf serum (FCS). On the other hand, Cicco et al.⁶ have reported that IL-6 production in neutrophils is induced by TNF- α or GM-CSF, but we think the FCS used in their study had an essential role in IL-6 production. Melani et al.⁴ have reported that neutrophils express IL-6 after being stimulated with GM-CSF in 10%FCS. They postulated that the IL-6 expression was downregulated by an unknown starvation factor or handling procedures, since differences were found between neutrophils prepared from heparinized peripheral blood immediately after withdrawal and cells purified from buffy coats of blood bank donors. Moreover, it is known that monocytes secrete IL-6 without any stimulus in *in vitro* culture¹⁶. However, our data obviously indicated that IL-6 secretion in monocytes was not induced without plasma (Table 2). These reports and our results suggest that some plasma component is essential for IL-6 production not only in neutrophils but in other leukocytes. Recently, it is known that the cooperative interactions between nuclear factor (NF) -IL6 and NF- κ B play an important role in the expression of IL-6¹⁷. NF-IL6 and NF- κ B are not normally expressed in any tissues but can be activated after stimulation with LPS, IL-1, IL-6 or TNF^{2,14}. However, in this study IL-6 secretion was not induced by IL-1 β alone.

IL-6 is a multifunctional cytokine that is produced by a range of cells and plays a central role in host defense mechanisms¹⁴. However, the question of whether human neutrophils produce IL-6 is still a matter of discussion¹⁸. In this study, we demonstrated that neutrophils can secrete substantial levels of IL-6 in the presence of plasma. This result strongly suggests that one must take into consideration that neutrophils can be a major source of IL-6 production. Actually, elevated levels of IL-6 can be detected in the synovial fluid from affected joints of patients with active rheumatoid arthritis¹⁹, and neutrophils may be one of the sources of IL-6 production.

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