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Invited Review

Neutrophils as a source of cytokines in inflammation

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Summary. The recruitment of neutrophils into inflammatory foci is a fundamental process observed in inflammation. The function of neutrophils has long been regarded only as an effector cell that kills the invading pathogens. Recent evidence has demonstrated that neutrophils are capable of producing inflammatory cytokines. The findings are, however, mainly based on the findings obtained *in vitro*. It has not been fully elucidated if neutrophils could synthesize and secrete cytokines *in vivo*. Animal models of inflammation are essential to address the issue and provide insight into the involvement of neutrophils in producing cytokines.

Key words: Neutrophil, Cytokine, Acute inflammation

Introduction

Inflammation is a host response when noxious stimuli, such as pathogens, invade into tissues. Neutrophil recruitment into the foci is one of the characteristic features of inflammation, and neutrophils play a central role in eliminating the pathogens. The function of neutrophils has long been thought to be the phagocytosis and the killing of invading pathogens through the release of enzymes stored in granules and the generation of reactive oxygen intermediates (Klebanoff and Clark, 1978; Bainton, 1988). It has been believed that neutrophils are terminally differentiated cells and incapable of protein synthesis because of the relative scarcity of ribosome and endoplastic reticulum (Cline, 1961). The function of neutrophils, such as phagocytosis and the release of lysosomal contents, remains when mRNA and protein synthesis are blocked (Cline, 1961). In parallel with the development of cytokine research, neutrophils were also considered as one of the sources of cytokines, but this notion was not definitely proved until the usage of highly sensitive techniques, such as molecular biology and immunoassays, in the 1980's. In this review, we describe the production of cytokines by neutrophils both *in vitro* and *in vivo*, and verify which cytokine is produced by infiltrating neutrophils and influences the inflammatory responses *in vivo*.

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Cytokine production by neutrophil

We for the first time suggested the production of a lymphocyte proliferation potentiating factor by infiltrating neutrophils (Yoshinaga et al., 1975, 1980; Goto et al., 1984a,b), proved it to be interleukin (IL)-1ß (Goto et al., 1988), and immunohistochemically demonstrated the production of IL-1ß by infiltrating neutrophils (Goto et al., 1989). No IL-1ß was detected in peripheral neutrophils (Goto et al., 1984a,b) and IL-1ß production in neutrophils that were stimulated *in vitro* was completely inhibited when synthesis of mRNA and protein was inhibited (Yoshinaga et al., 1987), suggesting *de novo* synthesis of IL-1ß in infiltrating neutrophils. In those days, macrophages were believed to be the main source of IL-1ß at inflammatory sites. However, we proved that the majority of the cells producing IL-1ß were infiltrating neutrophils (Goto et al., 1989). *In vitro*, Granelli-Piperno et al. (1977) first demonstrated that neutrophils were capable of producing a protein; plasminogen activator. Then, evidence in this decade has led to a consensus that neutrophils have the capability to produce a variety of proteins including cytokines (Lloyd and Oppenheim, 1992; Cassatella, 1995a). These cytokines include tumor necrosis factor (TNF) α, IL-1α, IL-1ß, IL-3, IL-6, IL-8, growth-related protein (GRO) a, macrophage inflammatory protein (MIP)-1α, MIP-1ß, monocyte chemoattractant protein (MCP)-1, interferon-gamma-inducible protein (IP)-10, IL-10, IL-12, IL-1 receptor antagonist (IL-1Ra), granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte CSF (G-CSF), macrophage CSF (M-CSF), transforming growth factor (TGF) ß, interferon (IFN) α and platelet activating factor (PAF) (Table 1). In agreement with the *in vitro* observations, recent *in vivo* studies showed that neutrophils are the cells producing TNFα, IL-1α, IL-1ß, IL-6, IL-8, GROα, MCP-1 and IL-1Ra (Table 2), all of which are considered to play some roles in inflammation. Because of the potential to produce various kinds of cytokines, it is assumed that neutrophils may contribute to the inflammatory

Table 1. Cytokines produced by neutrophils *in vitro*.

CYOKYNE	ASSAY(S)	REFERENCE
TNFα	Northern blot	Lindemann et al., 1989
IL-1α	Northern blot, Bioassay	Lindemann et al., 1988
IL-1ß	Partial sequence, Bioassay Northern blot, Bioassay	Goto et al., 1988 Lindemann et al., 1988
IL-3	Bioassay, Immunoassay	Kita et al., 1991
IL-6	Northern blot, Bioassay	Cicco et al., 1990
IL-8	Northern blot, Bioassay, Immunoassay	Bazzoni et al., 1991
GRO	Northern blot, Immunoassay	Gasperini et al., 1995
MIP-1α	Northern blot, Bioassay, Immunoassay	Kasama et al., 1993
MIP-1ß	Northern blot	Kasama et al., 1994
MCP-1	Northern blot, RT-PCR, Immunoassay	Burn et al., 1994
IP-10	Northern blot, Immunoassay	Cassatella et al., 1997
IL-10	RT-PCR, Immunoassay	Romani et al., 1997
IL-12	Northern blot, Bioassay, Immunoassay	Cassatella et al., 1995b
IL-1Ra	RT-PCR, Immunoassay	Haskill et al., 1991
GM-CSF	Bioassay, Immunoassay	Kita et al., 1991
C-CSF	Northern blot, Bioassay, Immunoassay	Lindemann et al., 1989
M-CSF	Northern blot, Bioassay, Immunoassay	Lindemann et al., 1989
TGFß	Northern blot, Immunoassay	Grotendorrst et al., 1989
IFNα	Northern blot, Immunoassay	Shirafuji et al., 1990
PAF	Bioassay	Sisson et al., 1987

Note: There are many other reports describing the production of cytokines by neutrophils in vitro. Representative reports are shown in this table. Abbreviations: TNFα, tumor necrosis factor α; IL, interleukin; GRO, growth-related protein; MIP, macrophage inflammatory protein; MCP-1, monocyte chemoattractant protein-1; IP-10, interferon-gamma-inducible protein-10; IL-1RA, IL-1 receptor antagonist; GM-CSF, granulocyte-macrophage colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; M-CSF, macrophage colony-stimulating factor; TGFß, trasforming growth factor ß; IFNα, interferon α; PAF, platelet activating factor.

responses through the secretion of these cytokines. Animal models of inflammation are essential to confirm this assumption.

Production of cytokines at inflammatory sites

We investigated the production of inflammatory cytokines such as TNFα, IL-1ß, IL-8, MCP-1 and IL-1Ra in a rabbit arthritis model induced with LPS. Injection of LPS into rabbit knee joints resulted in the production of these cytokines in the joint fluids. Maximum levels of TNFα, IL-1ß, IL-8, MCP-1 and IL-1Ra in joint fluids were detected at 2, 6, 2, 4 and 9 h after LPS-injection, respectively (Matsukawa et al., 1993, 1997, 1998a). Intraarticular injection of LPS also induced a massive leukocyte infiltration in the joint cavity and majority of the cells (>90%) were neutrophils for up to 12 h after the injection. When the amounts of cytokines in the infiltrating leukocytes were measured, the infiltrating leukocytes, mainly neutrophils, contained immunoreactive IL-1ß, IL-8, MCP-1 and IL-1Ra. Immunohistochemically, these cytokines were positively stained in the infiltrating neutrophils (Matsukawa et al., 1993, 1997, 1998a). Thus, it is possible that infiltrating neutrophils are the sources of IL-1ß, IL-8, MCP-1 and IL-1Ra at the site of inflammation. In contrast, no TNFα was detected in the infiltrating leukocytes. Also, TNFα was not detected in the infiltrating neutrophils in other types of rabbit inflammation, such as monosodium urate crystal-induced arthritis (Matsukawa et al., 1998b), LPS-induced pleurisy (Edamitsu et al., 1995) and casein-induced peritonitis (Mori et al., 1994). Although TNFα was detected in neutrophils in models of acute lung injury (Feiken et al., 1995; Imamura et al., 1997), infiltrating neutrophils generally do not appear to be the cell source of TNFα in acute inflammation occurred in cavities.

Are cytokines secreted from neutrophils?

The above described findings proved the production of cytokines by neutrophils *in vivo*. However, they did not address the degree to which endogenous cytokine was secreted from infiltrating neutrophils. We therefore measured the production of these cytokines in neutrophil-depleted rabbits, in which LPS-induced neutrophil infiltration was scanty whereas the influx of mononuclear cells was maintained. The peak levels of TNFα, IL-8 and MCP-1 in neutrophil-depleted rabbits were similar to the findings observed in normal rabbits. In contrast, no IL-1ß was detected in neutrophil-depleted rabbits, and the level of IL-1Ra in neutrophil-depleted rabbits was far lower than that in normal rabbits (Matsukawa et al., 1997, 1998a), indicating that the cells secreting TNFα, IL-8 and MCP-1 are resident cells in the joint cavity whereas the cells secreting IL-1ß and IL-1Ra are infiltrating neutrophils. As expected, synovial lining cells were positively stained for TNFα, IL-8 and MCP-1 (Matsukawa et al., 1997, 1998a)

However, antigenic IL-8 and MCP-1, and its mRNA were consistently detected in the infiltrating neutrophils (Matsukawa et al., 1997, 1998a). The peak levels of IL-8 and MCP-1 in the joint fluids, which were detected at 2 and 4 h after LPS-injection, respectively, were observed a few hours before the number of infiltrating neutrophils reached the peak (9 h post-injection). Despite the production of IL-8 and MCP-1 by infiltrating neutrophils, the IL-8 and MCP-1 appear to be retained inside the cells. Although it is suggested that IL-8 and MCP-1 produced by neutrophils might promote further recruitment of neutrophils and monocytes, this notion does not seem to apply at the site of LPS-induced arthritis. *In vitro* studies showed that IL-8 and MCP-1 produced by neutrophils were secreted into the culture supernatant (Bazzoni et al., 1991; Cassatella et al., 1992; Strieter et al., 1992;). It remains unclear why neutrophils

Table 2. Cytokines produced by neutrophils*in vivo*.

CYTOKINE	MODEL/DISEASE	METHOD(S)	REFERENCE
TNFα	LPS-induced lung injury (rat)	Northern blot, In situ hybridization, Immunohistochemistry	Xing et al., 1993
	Crohn's disease (human)	Immunohistochemistry	Beil et al., 1995
	Wound healing (mouse)	In situ hibridization, Immunohistochemistry	Feiken et al., 1995
	LPS-induced lung injury (rabbit)	Immunohistochemistry	Imamura et al., 1997
IL-1α	LPS-induced pneumonitis (rat)	Northern blot	Ulich et al., 1992
IL-1ß	Casein-induced peritonitis (rabbit)	Northern blot, Immunohistochemistry	Goto et al., 1989
	LPS-induced pneumonitis (rat)	Northern blot	Ulich et al., 1992
	LPS-induced arthritis (rabbit)	ELISA, Immunohistochemistry	Matsukawa et al., 1993
	Chronic sinusitis (human)	In situ hybridization	Tokushige et al., 1994
	Rheumatoid arthritis (human)	Northern blot, ELISA	Beaulieu and McColl, 1994
	Rheumatoid arthritis (human)	Northern blot	Quayle et al., 1995
	LPS-induced pleurisy (rabbit)	ELISA	Edamitsu et al., 1995
	Caerulein-induced pancreatitis (mouse)	RT-PCR, Immunohistochemistry	Fink and Norman, 1996
	LPS-induced lung injury (rabbit)	Immunohistochemistry	Imamura et al., 1997
	Crystal-induced arthritis (rabbit)	ELISA, Immunohistochemistry	Matsukawa et al., 1998b
	LPS-induced uveitis (rabbit)	ELISA, Immunohistochemistry	Mo et al., 1998
	LPS-induced lung injury (mouse)	Western blot, Immunohistochemistry	Parsey et al., 1998
IL-6	Septic shock (mouse)	Bioassay, immunohistochemistry	Terebuh et al., 1992
IL-8	Rheumatoid arthritis (human)	Northern blot, ELISA	Beaulieu and McColl, 1994
	Psoriasis (human)	In situ hybridization, Immunohistochemistry	Gillitzer et al., 1996
	Inflammatory bowel disease (human)	In situ hybridization, Immunohistochemistry	Grimm et al., 1996
	Casein-induced peritonitis (rabbit)	Northern blot	Mori et al., 1994
	LPS-induced lung injury (rabbit)	ELISA, Immunohistochemistry	Matsukawa et al., 1997
	LPS-induced arthritis (rabbit)	Immunohistochemistry	Imamamura et al., 1997
	Crystal-induced arthritis (rabbit)	ELISA, Immunohistochemistry	Matsukawa et al., 1998b
Groα	Rheumatoid arthritis (human)	ELISA	Koch et al., 1995
MCP-1	Bleomycin-induced lung injury (rat)	Immunohistochemistry	Sakanashi et al., 1994
	Cutaneous delayed hypersensitivity (rat)	Immunohistochemistry	Rand et al., 1996
	Collagen-induced arthritis (rat)	Immunohistochemistry	Ogata et al., 1997
	LPS-induced arthritis (rabbit)	RT-PCR, Fluoroimmunoassay, Immunohistochemistry	Matsukawa et al., 1998a
	Crystal-induced arthritis (rabbit)	RT-PCR, Fluoroimmunoassay, Immunohistochemistry	Matsukawa et al., 1998a
IL-1Ra	LPS-induced pneumonitis (rat)	Northern blot	Ulich et al., 1992
	Casein-induced peritonitis (rabbit)	Bioassay, Cloning	Goto et al., 1992
	LPS-induced arthritis (rabbit)	ELISA, Immunohistochemistry	Matsukawa et al., 1993
	Rheumatoid arthritis (human)	Northern blot, ELISA	Beaulieu and McColl, 1994
	LPS-induced pleurisy (rabbit)	ELISA	Edamitsu et al., 1995
	LPS-induced lung injury (rabbit)	Immunohistochemistry	Imamura et al., 1997
	LPS-induced uveitis (rabbit)	ELISA, Immunohistochemistry	Mo et al., 1998
	Crystal-induced arthritis (rabbit)	ELISA, Immunohistochemistry	Matsukawa et al., 1998b

do not secrete IL-8 or MCP-1 in LPS-induced arthritis. What is the function of the IL-8 and MCP-1? The other questions which remain to be answered are; although leukocytes can produce TNFα and synovial cells can produce IL-1ß and IL-1Ra *in vitro*, why do the leukocytes not produce TNFα *in vivo*? Why do synovial cells not produce IL-1ß and IL-1Ra *in vivo*? The inflammatory response is not a static event, but a dynamic event (Hayashi, 1975). The production and secretion of cytokines may depend on the sequence of events during inflammation. Further study is required to completely understand the mechanisms underlying these phenomena.

The regulation of cytokines secreted by infiltrating neutrophils and their involvement in inflammation

We here focused on IL-1ß and IL-1Ra because IL-1ß and IL-1Ra are the cytokines secreted from infiltrating neutrophils in LPS-induced arthritis.

The production of IL-1ß in the joint fluids was inhibited by anti-TNFα mAb, rabbit recombinant (rr)IL-1Ra and anti-IL-8 IgG (Matsukawa et al., 1997), but not by anti-MCP-1 IgG (Matsukawa et al., 1998a). Intra-articular injection of rrTNFα or human IL-1ß into rabbit knee joints induced the production of IL-1ß (Miyamoto et al., 1997; Matsukawa et al., 1998b). We also showed that injection of rrIL-8 into rabbit knee joints induced the production of IL-1ß (Matsukawa et al., 1995), although IL-8 did not induce the production of IL-1ß in circulation when it was injected intravenously (Van et al., 1992). As described, the cells secreting TNFα and IL-8 were synovial lining cells whereas infiltrating neutrophils were responsible for the production of IL-1ß. Administration of rrIL-1Ra inhibited the leukocyte infiltration and cartilage degradation induced with LPS

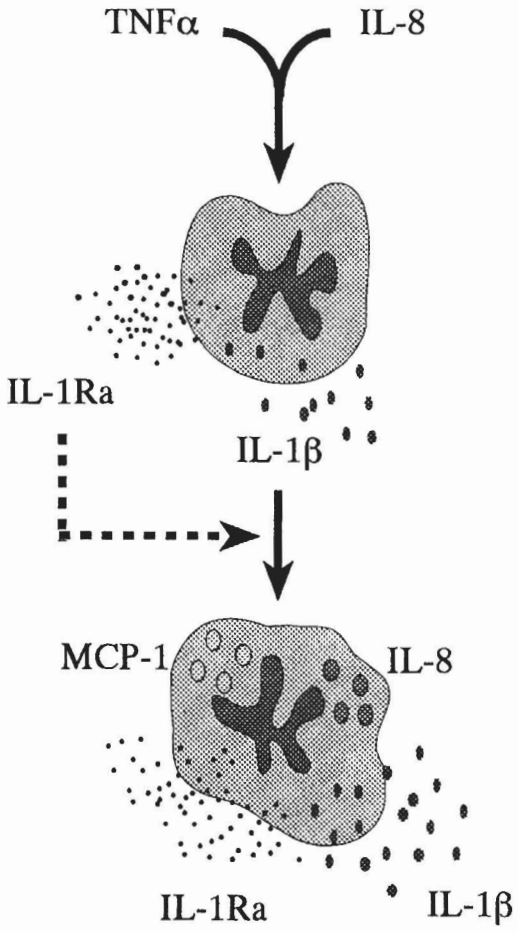

Fig. 1. Possible cytokine network regulating IL-1ß/IL-1Ra release by neutrophils in LPS-induced rabbit arthritis. TNFα and IL-8, produced by synovial cells, induce the production of IL-1ß and IL-1Ra by neutrophils. IL-1ß induces further production of IL-1ß and IL-1Ra. Endogenous IL-1Ra down-regulates the production of IL-1ß. IL-8 and MCP-1 produced by neutrophils are retained inside the cells.

(Matsukawa et al., 1993). These findings suggest that TNFα and IL-8, produced by synovial lining cells, induce the subsequent production of IL-1ß by neutrophils. IL-1ß has an autocrine/paracrine effect and regulates its own synthesis. IL-1ß appears to amplify the inflammatory responses (Fig. 1).

IL-1Rα, a naturally occurring receptor antagonist against IL-1 has no agonist activity (Eisenberg et al., 1990; Goto et al., 1992; Arend, 1993). The production of endogenous IL-1Ra in joint fluids after LPS-injection was delayed compared with that of TNFα, IL-1ß, IL-8 and MCP-1, and the level of endogenous IL-1Ra was inhibited by blocking the activity of either TNFα or IL-8 (Matsukawa et al., 1997). Anti-MCP-1 IgG did not inhibit the production of endogenous IL-1Ra (Matsukawa et al., 1998a). Intraarticular injection of rrTNFα, rrIL-1ß, or rrIL-8 resulted in the production of endogenous IL-1Ra in the joint fluids (Miyamoto et al., 1997; Matsukawa et al., 1998a). Administration of neutralizing anti-IL-1Ra mAb enhanced LPS-induced production of IL-1ß and leukocyte infiltration (Fukumoto et al., 1996). The cells secreting endogenous IL-1Ra were infiltrating neutrophils. All these findings taken together suggest that endogenous IL-1Ra, produced by infiltrating neutrophils, is regulated by the stimulation with TNFα, IL-1ß and IL-8, and down-regulates the inflammatory responses (Fig. 1).

Concluding remarks

We have here described the production of TNFα, IL-1ß, IL-8, MCP-1 and IL-1Ra in a rabbit arthritis model induced with LPS, and elucidated which cytokine was produced and secreted from infiltrating neutrophils. However, the cytokine profile appears to be different among the types of inflammation and the places where inflammatory responses are evident. It remains unclear if cytokines other than TNFα, IL-1ß, IL-8, MCP-1 and IL-1Ra could be released from neutrophils at the site of inflammation. Animal models of inflammation will pave the way to address the complicated issues.

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