Available online at www.sciencedirect.com
SCIENCE DIRECT®
Immunobiology 209 (2005) 651–660
Immunobiology
www.elsevier.de/imbio

Local delivery of soluble interleukin-6 receptors to improve the outcome of alpha-toxin producing *Staphylococcus aureus* infection in mice

Tsuyoshi Onogawa*

Department of Immunology, School of Health Sciences, Kyorin University, 476 Miyashita, Hachioji-shi, Tokyo 192-8508, Japan

Received 11 March 2004; accepted 22 September 2004

Abstract

Staphylococcal alpha-toxin enhances interleukin (IL)-6 secretion in mice infected with *Staphylococcus aureus*. The role of alpha-toxin-induced IL-6 secretion in host defense has not been sufficiently clarified. In the present study, IL-6 signaling was transiently regulated using soluble IL-6 receptors (sIL-6R) to investigate the role of IL-6 in the early stage of abdominal *S. aureus* infection. In mice challenged with bacteria producing high alpha-toxin levels, the local delivery of sIL-6R was effective in improving the survival rate, the resolution of neutrophilia and the bacteria clearance. Mice that had received sIL-6R and survived showed high levels of IL-6, monocyte chemoattractant protein (MCP)-1 and tumor necrosis factor (TNF)-alpha. In contrast, mice that died in spite of the delivery of sIL-6R showed high levels of interferon (IFN)-gamma and IL-1alpha and low TNF-alpha level. When the effect of soluble gp130, a sIL-6R antagonist, was examined, the number of neutrophils increased significantly and the MCP-1 level decreased significantly, compared to the group that received sIL-6R alone; the number of viable bacteria also tended to increase as a result of the inhibition of IL-6 signaling. The cellular phosphotyrosine level in alpha-toxin-treated macrophages was reduced in cultures supplemented with recombinant IL-6 in vitro. These results suggest that IL-6 enhances bactericidal activity and reduces the number of immune cells that are activated abnormally through the regulation of inflammatory cytokines during the early stage of infection in alpha-toxin producers.

Keywords: Staphylococcus; Interleukin-6 receptor

Introduction

Staphylococcus aureus is a major cause of nosocomial infections and is a causative agent of postoperative infections and Gram-positive septic shock (Lyytikainen et al., 2002; Shanson et al., 1976; Torre-Cisneros et al., 2002). Moreover, the development of multiple antimicrobial-resistant *S. aureus* strains has become a social problem (Hiramatsu, 2001; Law et al., 1988; Sievert et al., 2002). Many studies have examined the relation between staphylococcal exotoxins as superantigens and the host immune system (i.e., the study of abnormal T lymphocyte activation) (Cameron et al., 2001; Krakauer, 1999). However, the mechanism by which staphylococcal exotoxins lead to host immunity, except for the role of superantigens, has not been sufficiently

Abbreviations: ELISA, enzyme-linked immunosorbent assay; IFN, interferon; IL, interleukin; sIL-6R, soluble IL-6 receptor; MCP, monocyte chemoattractant protein; NF-κB, nuclear factor κB; PLF, peritoneal lavage fluid; STAT, signal transducer and activator of transcription; *S. aureus*, *Staphylococcus aureus*; TNF, tumor necrosis factor

*Tel.: +81 426 91 0011; fax: +81 426 91 1094.
E-mail address: onogawa@kyorin-u.ac.jp (T. Onogawa).

0171-2985/$ - see front matter © 2005 Elsevier GmbH. All rights reserved.
doi:10.1016/j.imbio.2004.09.006

clarified; in particular, host defense mechanisms and the activation of intracellular signaling cascades have not received adequate attention.

Staphylococcal alpha-toxin is a potent pore-forming bacterial exotoxin that has been implicated as a virulence factor in human and domestic animal staphylococcal infections (Bhakdi and Tranum-Jensen, 1991; Buerke et al., 2002; Riollet et al., 2000). This toxin induces proinflammatory cytokine secretion (Bhakdi et al., 1989; Bhakdi and Tranum-Jensen, 1991), leading to various biological consequences such as the activation of nuclear factor-κB (NF-κB) in vitro (Dragneva et al., 2001). In a previous report, staphylococcal alpha-toxin was found to induce interleukin (IL)-6 secretion in macrophages, and the level of secretion was augmented by the coexistence of toxin and bacterial cells (Onogawa, 2002). Interestingly, the toxin-treated macrophages did not secrete tumor necrosis factor (TNF)-alpha, although they did secrete IL-1alpha. Bhakdi et al. (1989) showed that a high level of staphylococcal alpha-toxin induces IL-1beta secretion in monocytes, whereas a low level of toxin induces TNF-alpha secretion. Söderquist et al. (1998) demonstrated that staphylococcal alpha-toxin induced an even greater release of IL-6 in endothelial cells that had been prestimulated with IL-1beta.

IL-6 is a multifunctional cytokine produced during infection and inflammation and is induced by IL-1 and TNF-alpha (Kishimoto et al., 1992; Taga and Kishimoto, 1997). IL-6 also acts as an anti-inflammatory agent in inflammation-related diseases (Cuzzocrea et al., 2002; Schindler et al., 1990) and improves the mortality rate of *Escherichia coli*-infected mice but does not affect the rate of LPS-induced mortality (Dalrymple et al., 1996). Regarding the role of IL-6 in inflammation induced by a bacterial cell-free supernatant, the complex of IL-6 and its soluble receptor (sIL-6R) has been reported to induce monocyte chemoattractant protein (MCP)-1, aiding in the resolution of neutrophilia and the initiation of the immune response by the transition from neutrophils to monocytes during inflammation (Hurst et al., 2001; Marin et al., 2001). Thus, the effect of alpha-toxin-induced IL-6 secretion in animals with *S. aureus* infections was considered a topic of interest.

The present study utilized the delivery of sIL-6R to investigate the role of IL-6 signaling on the survival rate, leukocyte count, and cytokine responses in staphylococcal alpha-toxin producers.

Materials and methods

Bacteria

S. aureus KU-01-06-37 and KU-01-12-44 were isolated from clinical specimens at Kyorin University Hospital, Tokyo, Japan. These strains have been shown to produce staphylococcal enterotoxin C, toxic shock syndrome toxin-1, and alpha-toxin and have been classified as coagulase type II. The alpha-toxin levels in the supernatants of the overnight cultures of KU-01-06-37 and KU-01-12-44 were found to be 1566.7 ng/ml [corresponding to 45 hemolytic units (HU)] and 291.4 ng/ml (corresponding to 9 HU), respectively, using an enzyme-linked immunosorbent assay (ELISA) (unpublished data). The KU-01-06-37 strain, but not the KU-01-12-44 strain, is lethal in mice.

Mice

Male ddY mice (25–27 g, 5 weeks old) were obtained from Nippon SLC (Shizuoka, Japan). The mice were reared under specific pathogen-free conditions at $23 \pm 2\,^{\circ}C$ and $45 \pm 10\%$ humidity and were fed a commercial diet and water ad libitum. All animal care and husbandry techniques were in accordance with the Guidelines for Animal Experimentation of the Japanese Association for Laboratory Animal Science.

S. aureus administration and analysis

Two groups of mice were challenged intraperitoneally (i.p.) with *S. aureus*. One group was challenged with a sub-lethal dose of *S. aureus* (1×10^8 cells/mouse) to examine the kinetics of cytokines. In a second group of mice, the endogenous IL-6 level was transiently regulated using the administration (i.p.) of recombinant human sIL-6R (100 ng/mouse, Genzyme-Techne, MN, USA) 1 h after bacterial challenge (5×10^8 cells/mouse) to examine the effect of IL-6 at the site of infection. A control group was treated with saline containing 0.1% bovine serum albumin.

Peritoneal lavage fluid (PLF) was obtained by lavage with saline at 1, 2, 3, 6, 9, 12, 18 or 24 h after bacterial challenge in the first group of mice and at 3, 6 or 9 h after bacterial challenge in the second group of mice that received sIL-6R. Upon death in the second group, the peritoneal cavity was washed immediately at 6 or 9 h after the bacterial challenge. The PLFs obtained from each group were spun at $4\,^{\circ}C$ for $160g$ for 5 min, and the supernatants were collected and stored at $-80\,^{\circ}C$ until use in the cytokine assay. The cell pellets were resuspended in saline for the hematological tests.

Cumulative survival

In the sIL-6R delivery experiment, the condition of the sIL-6R-treated mice was checked every hour, and mortality during the 24-h period following the bacterial challenge was recorded. The survival rates were calculated using the Kaplan–Meier method and analyzed for