The Metalloproteinase Inhibitor GI5402 Inhibits Endotoxin-Induced Soluble CD27 and CD16 Release in Healthy Humans

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Metalloproteinases have been implicated in the cleavage of a number of cell surface immune receptors. Oral administration of the metalloproteinase inhibitor GI5402 attenuated the release of soluble CD27 and CD16 into the circulation after intravenous endotoxin injection in healthy humans.

Metalloproteinases (MPs) are involved in the shedding of multiple cell surface molecules (7). The capacity of MPs to cleave cell-associated tumor necrosis factor alpha (TNF-α) has received much attention. MP inhibitors prevent TNF-α release by mononuclear cells in vitro and in mice in vivo and protect mice from a lethal dose of lipopolysaccharide (LPS) (5, 13, 15).

We have previously shown that oral administration of the MP inhibitor GI5402 markedly reduced the release of soluble TNF-α in the circulation after a bolus intravenous injection of LPS into healthy humans (4). GI5402 did not influence the LPS-induced decreases in monocyte and granulocyte TNF-α receptor expression but did attenuate the rise in the concentrations of soluble TNF-α receptors in plasma. MPs are also involved in the shedding of other surface molecules, including other members of the TNF-α receptor family and the immunoglobulin G (IgG) Fcγ receptor type III (FcγRIII) (7, 14).

CD27 is a lymphocyte-specific member of the TNF-α receptor family (1, 11). Like other members of this family, CD27 is expressed as a transmembrane protein that consists of two identical subunits. After activation of lymphocytes, a soluble form of CD27 is released into the extracellular environment, most likely via proteolytic cleavage (6, 12). CD16 exists in two forms, each encoded by a different gene (2). Only neutrophils constitutively express FcγRIIIb, which is the major source for circulating soluble CD16 (8). FcγRIIIb is shed from the surface of activated neutrophils in vitro by MP-mediated cleavage (14).

Little is known about the regulation of CD27 and CD16 expression during infection in vivo. In the present study we sought to determine whether the cellular expression of these surface molecules and the concentrations in plasma of their soluble forms change after injection of LPS into normal subjects and to establish the role of MPs herein.

The present study was performed simultaneously with an investigation on the effect of GI5402 on TNF-α and TNF-α receptors, the results of which have been published (4). The study was approved by the Research and Ethics committees of the Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands, and written informed consent was obtained from all volunteers prior to study entry. The study was designed as a double-blind, randomized, cross-over, placebo-controlled investigation in which seven male volunteers (mean age, 22 years; range, 20 to 25 years) were treated with LPS on two occasions, with an interval of 6 weeks between. On one study occasion, fasting subjects were given an oral dose of GI5402 as a 100-mg tablet, which was followed 20 min later by an intravenous injection of LPS. On the other occasion, matching placebo preceded the LPS injection. Escherichia coli LPS, lot G (UPS, Rockville, Md.), was administered over 1 min into an antecubital vein at a dose of 4 ng/kg of body weight. Blood was obtained from a cannulated forearm vein 0.5 h before LPS injection (i.e., directly prior to administration of GI5402 or placebo), directly before LPS administration (i.e., time zero), and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, and 24 h thereafter. Blood for FACSscan analyses was collected in heparin-containing Vacutainer tubes; all other samples were drawn in potassium-EDTA-containing tubes. Leukocyte counts and differentials were assessed with a Stekker analyzer (Counter STKS; Coulter, Bedfordshire, United Kingdom). All blood samples (except samples for flow cytometry) were centrifuged at 2,000 × g for 20 min at 4°C, and plasma was stored at −20°C until assays were performed. Soluble CD27 was measured by enzyme-linked immunosorbent assay (ELISA) precisely according to the instructions of the manufacturer (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service [CLB], Amsterdam, The Netherlands; detection limit, 5 U/ml). Soluble CD16 was determined by ELISA as described previously (10). The lower limit of detection of this assay was 15.6 pg/ml. Blood obtained for FACSscan analysis was processed exactly as described previously (4). Cell surface CD27 was measured on lymphocytes with a mouse anti-human CD27 monoclonal antibody (clone 9F4; CLB); membrane-bound CD16 expression was determined on granulocytes with a mouse anti-human CD16 monoclonal antibody (clone CLBFcRgran1; CLB). All fluorescence-activated cell sorting (FACS) reagents were used at the concentrations recommended by the manufacturers, and all analyses were also conducted with the appropriate control antibodies (murine IgG1 or IgG2 [Becton Dickinson & Co., Rutherford, N.J.]). For each test, 10⁵ cells were counted. The mean cell fluorescence (MCF) at >570 nm of forward and side angle scatter-gated lymphocytes and granulocytes was assessed. Data are presented as the difference (linear units) in MCF intensities of specifically and nonspecifically stained cells. Values are given as means ± standard errors (SE). Differences between GI5402 and placebo treatment pe-
periods were tested by analysis of variance (ANOVA) for repeated measures. Changes of variables over time were analyzed using one-way ANOVA. A two-tailed \( P \) value of <0.05 was considered significant.

Intravenous injection of LPS modestly but significantly decreased lymphocyte CD27 expression, which reached a nadir after 6 h (MCF decreased from 77 ± 13 to 52 ± 10) (Fig. 1, upper panel) \((P < 0.05 \text{ over time})\). This effect was associated with an increase in the plasma concentrations of soluble CD27, peaking after 1.5 h (from 191.3 ± 17.5 to 251.4 ± 18.7 U/ml) (Fig. 1, lower panel) \((P < 0.05 \text{ over time})\). Administration of GI5402 did not influence the changes in lymphocyte CD27 but significantly reduced the LPS-induced increase in the amount of soluble CD27 in the circulation \((P < 0.001 \text{ for the difference between treatment groups})\).

Unlike other members of the TNF receptor family, CD27 expression is restricted to lymphocytes (1, 11). Activation of T cells by stimuli that mimic antigen-specific stimulation elicits a strong upregulation of CD27 surface expression (3, 18). Other mitogenic stimuli such as phorbol esters reduce CD27 expression (3, 18). Activation of lymphocytes also results in the shedding of soluble CD27, most likely by proteolytic cleavage of the transmembrane receptor (6, 12). The plasma concentrations of soluble CD27 have been found to increase in various inflammatory conditions, including psoriasis, renal transplant, and filariasis (11). In addition, circulating soluble CD27 levels are elevated in patients with B-cell malignancies, in whom they strongly correlate with the tumor load (19). We here report that experimental endotoxemia in healthy humans is associated

![FIG. 1. Lymphocyte CD27 (top panel) and plasma soluble CD27 (lower panel) after intravenous injection of LPS in subjects receiving either GI5402 (○) or placebo (●) given 20 min prior to LPS challenge (lot G, 4 ng/kg). FACS results are expressed as the difference between specific MCF and nonspecific MCF. All data are means ± SE. P value indicates the difference between treatment groups. NS, nonsignificant.](http://iai.asm.org/).
with a decrease in lymphocyte CD27 expression and in soluble CD27 levels in plasma. In addition, our results suggest that MPs are involved in the cleavage of soluble CD27 from the cell surface.

We confirmed the role of MPs in the shedding of CD16 in vivo. Activation of neutrophils is associated with the release of CD16 from the cell surface (9, 14), and soluble CD16 in plasma is mainly derived from neutrophils (8). In the present study, LPS given at a dose that does result in detectable activation of granulocytes (e.g., degranulation, upregulation of CD11b, and downmodulation of TNF receptors) (16, 17), did not significantly influence granulocyte CD16 expression. It should be noted, however, that FACS analysis in this model can only be conducted on cells found in the circulation. Therefore, it is possible that granulocytes that become attached to the vascular endothelium after LPS injection do shed their CD16. This may also explain the observed rise in soluble CD16 concentrations in plasma, a finding that is in line with earlier reports of elevated soluble CD16 levels at sites of inflammation (2).

REFERENCES


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