Lipopolysaccharide Induces CD38 Expression and Solubilization in J774 Macrophage Cells

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CD38, an ADP ribosyl cyclase, is a 45 kDa type II transmembrane protein having a short N-terminal cytoplasmic domain and a long C-terminal extracellular domain, expressed on the surface of various cells including macrophages, lymphocytes, and pancreatic β cells. It is known to be involved in cell adhesion, signal transduction and calcium signaling. In addition to its transmembrane form, CD38 is detectable in biological fluids in soluble forms. The mechanism by which CD38 is solubilized from the plasma membrane is not yet clarified. In this study, we found that lipopolysaccharide (LPS) induced CD38 upregulation and its extracellular release in J774 macrophage cells. Furthermore, it also increased CD38 expression at the mRNA level by activating the Janus kinase-signal transducers and activators of transcription (JAK-STAT) pathway. However, LPS decreased the levels of CD38 in the plasma membrane by releasing CD38 into the culture supernatant. LPS-induced CD38 release was blocked by the metalloproteinase-9 inhibitor indicating that MMP-9 solubilizes CD38. In conclusion, the present findings demonstrate a potential mechanism by which C38 is solubilized from the plasma membrane.

INTRODUCTION

CD38 (ADP-ribosyl cyclase) leukocyte antigen is a 45 kDa type II transmembrane protein having a short N-terminal cytoplasmic domain and a long C-terminal extracellular domain (De Flora et al., 2000). It is known to be expressed in lympho-monocytic lineage cells and pancreatic β cells. Furthermore, it acts as a transmembrane cell-signalling molecule as well as an ectoenzyme. The ligation of CD38 with monoclonal antibodies (mAbs) can activate some intracellular signal transduction pathways. For instance, CD38 ligation triggers activation, proliferation and mobilization of intracellular calcium (Ca²⁺) in T-lymphocytes and tyrosine phosphorylation of intracellular proteins in myeloid cells (Funaro et al., 1990; Inoue et al., 1997). In addition, it could also inhibit selectin-like adhesion of leukocytes to endothelium by blocking the interaction between CD38 and CD31, a CD38 natural ligand (Dianzani et al., 1994). CD31, a well-known member of the immunoglobulin superfamily with molecular mass of 130 kDa, is involved in the lymphocyte adhesion processes (Deaglio et al., 1996). It is expressed in monocytes, neutrophils, T-cell subsets, platelets, vascular endothelial cells and solid tumor lines (Deaglio et al., 1998).

Soluble extracellular domain of CD38 is detected in biological fluids in normal conditions and in various diseases (Funaro et al., 1996; Mallone et al., 1998). Viral infection such as acquired immunodeficiency syndrome (AIDS) increases CD38 solubilization (Funaro et al., 1996), suggesting that inflammatory signals may affect CD38 solubilization. In this study, we examined the effect of lipopolysaccharide (LPS), a bacterial cell wall component, on the expression and solubilization of CD38 in macrophages. We observed that LPS stimulates CD38 expression by activating the Janus kinase-signal transducers and activators of transcription (JAK-STAT) signaling pathway, and that the expressed CD38 was released into the extracellular domain by metalloproteinase 9.

MATERIALS AND METHODS

Reagents

IFN-β, horse radish peroxidase-linked anti-mouse antibody and horse radish peroxidase-linked anti-rabbit antibody were purchased from Abcam (USA). Purified anti-mouse IFN-β was purchased from BioLegend (USA). Anti-mouse CD38 antibody was purchased from Santa Cruz Biotechnology (USA). E. coli LPS from serotype 0111:B4, and anti-β-actin antibody were purchased from Sigma-Aldrich (USA). Inhibitors of PI3K (LY294002 and wortmannin), p38 (SB203580), JAK1 and JAK3 were purchased from Calbiochem (USA).

Cells

The J774A.1 murine macrophage cell line was obtained from the American Type Culture Collection (USA) and cultured at 37°C in Dulbecco’s Modified Eagle’s Media (DMEM, Gibco-BRL, USA) supplemented with 10% heat-inactivated and sterile-filtered fetal bovine serum (FBS), 2 mM glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin.

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penicillin, and 100 μg/ml streptomycin. The cells were passaged twice a week, and cells older than 15 passages were not used.

**Western blotting**

Cells were rinsed twice with ice-cold phosphate buffered saline and lysed in a mammalian cell lysis solution (Thermo scientific, USA) containing protease inhibitor cocktail (Roche, USA) for 40 min, and centrifugated to separate the soluble supernatants. Protein extracts (20 μg) were separated by 10% sodium dodecyl sulfate-polyacrylamide gels electrophoresis at 100 V with a running time of 120 min and transferred to polyvinylidene fluoride membranes. Membranes were blocked with 5% non-fat milk in Tris-buffered saline (pH 7.4) with 0.1% Tween 20 for 1 h at room temperature and probed overnight at 4°C with primary antibodies against CD38 and β-actin. After wash, the membranes were further incubated with species-specific horseradish phosphatase-conjugated secondary antibody for 40 min at room temperature. Immunoreactive proteins were detected by using an enhanced chemiluminescence detection system (GE Amersham life science, USA) according to the manufacturer’s recommendations.

**RNA extraction and quantitative real time PCR**

Total RNA was extracted using RNaseasy mini kit (Qiagen, USA) according to the manufacturer’s instructions. Real-time PCR primer sets for mouse CD38 and GAPDH were purchased from SABiosciences (USA). Total RNA was treated with DNase I to remove residual DNA. RNA was treated with RNase-free DNase for 20 min at room temperature before reverse transcription. RNA concentrations were determined at OD_{260} absorbance. Reverse transcription was performed with 1 μg of RNA in a 20 μl reaction volume using ImProm-II™ Reverse Transcription System (Promega). Real-time quantitative PCR was then performed in a 20 μl reaction volume using SYBR premix (TaKaRa, Japan) with 10 μM of primer sets (the CD38 upstream primer, 5′-TTG TTC CGC AAG GTG CT-3′, and the CD38 downstream primer, 5′-GGG AGT TGC TGT TG-3′) and 1 μl cDNA in a 7900HT Real-time PCR machine (Applied Biosystems, USA). PCR conditions consisted of 30 s hot start at 95°C, followed by 40 cycles of 5 s at 95°C and 30 s at 60°C, with a final 15 s at 72°C. The average threshold cycle (Ct) for each gene was determined from triplicate reactions, and the levels of gene expression relative to age threshold cycle (Ct) for each gene was determined from using an enhanced chemiluminescence detection system (GE Amersham life science, USA) according to the manufacturer’s recommendations.

**Immunoprecipitation and Western blot**

For CD38 immunoprecipitation (IP), lysates were incubated overnight at 4°C with 2 μg/ml of anti-mouse CD38 antibody (eBioscience) with a 20 μl slurry of protein G plus agarose (USA) and phosphatase inhibitor cocktail. Immunoprecipitates were loaded on 7.5% polyacrylamide gels, electrophoresed and transferred. The membranes were blocked with 5% skim milk with tris-buffered saline (pH 7.4) with 0.1% Tween 20 and incubated with goat polyclonal anti-CD38 antibody (USA) for 4 h at 4°C followed by donkey anti-goat IgG-HRP (USA) for 1 h at 4°C. Proteins were developed using chemiluminescence (Amersham Pharmacia Biotech, UK).

**Statistical analysis**

Data are presented as means ± SEM. Statistical significance was determined using a two-tailed Student’s t-test and one-way ANOVA test followed by Scheffe’s post-hoc test.

**RESULTS**

**LPS increases CD38 expression at mRNA level in J774 macrophage cells**

To analyze the effect of LPS on CD38 expression in J774 cells, cells were treated with LPS (100 ng/ml) and harvested according to a time-course. The levels of CD38 mRNA were measured by quantitative real-time PCR. Real-time PCR analysis showed over 4-fold induction of CD38 mRNA expression 24 h after LPS exposure (Fig. 1).

**LPS-induced CD38 expression is mediated by the JAK-STAT signaling pathway**

To elucidate the mechanism of LPS-induced CD38 expression, the effect of several signal transduction inhibitors on LPS-induced CD38 expression was examined. J774 macrophage cells were pretreated with inhibitors of mitogen activated protein kinase (MAPK), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), phosphoinositide 3 kinase (PI3K), Janus kinase (JAK) 1 and JAK3, for 1 h and stimulated with LPS for 24 h. Wortmannin, a PI3K inhibitor, and SB203580, a p38 inhibitor, slightly decreased LPS-induced CD38 expression whereas LY294002, a PI3K inhibitor, slightly increased it (Fig. 2). Interestingly, JAK-3 inhibitor completely abolished LPS-induced CD38 expression (Fig. 2). These results suggest that LPS-induced CD38 expression is mediated by the JAK-STAT pathway.

**IFN-β increases CD38 expression at mRNA level in J774 macrophage cells**

Previous studies have shown that LPS mediates IFN-β induction and secretion through interferon regulatory factor (IRF)-3 (Sakaguchi et al., 2003). It has also been demonstrated that IFN-β activates the JAK-STAT signaling pathway (Horvath, 2004). In view of these findings, it is possible that IFN-β is involved in CD38 expression. To investigate this possibility, we treated J774 macrophage cells with IFN-β and measured CD38 mRNA expression levels by real-time PCR. IFN-β treatment