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A Novel Pathway Regulating Lipopolysaccharide-Induced Shock by ST2/T1 Via Inhibition of Toll-Like Receptor 4 Expression

Matthew J. Sweet,* Bernard P. Leung,* Daiwu Kang,† Morten Sogaard,† Kerstin Schulz,* Vladimir Trajkovic,2* Carol C. Campbell,* Damo Xu,3* and Foo Y. Liew 3*

ST2/ST2L, a member of the IL-1R gene family, is expressed by fibroblasts, mast cells, and Th2, but not Th1, cells. It exists in both membrane-bound (ST2L) and soluble forms (ST2). Although ST2L has immunoregulatory properties, its ligand, cellular targets, and mode of action remain unclear. Using a soluble ST2-human IgG fusion protein, we demonstrated that ST2 bound to primary bone marrow-derived macrophages (BMM) and that this binding was enhanced by treatment with LPS. The sST2 treatment of BMMs inhibited production of the LPS-induced proinflammatory cytokines IL-6, IL-12, and TNF-α but did not alter IL-10 or NO production. Treatment of BMMs with sST2 down-regulated expression of Toll-like receptors-4 and -1 but induced nuclear translocation of NF-κB. Administration of sST2 in vivo after LPS challenge significantly reduced LPS-mediated mortality and serum levels of IL-6, IL-12, and TNF-α. Conversely, blockade of endogenous ST2 through administration of anti-ST2 Ab exacerbated the toxic effects of LPS. Thus, ST2 has anti-inflammatory properties that act directly on macrophages. We demonstrate here a novel regulatory pathway for LPS-induced shock via the ST2-Toll-like receptor 4 route. This may be of considerable therapeutic potential for reducing the severity and pathology of inflammatory diseases. The Journal of Immunology, 2001, 166: 6633–6639.

Previously, ST2/T1 was independently identified as an oncogene and serum-responsive gene expressed in fibroblasts (1–3). It was subsequently found to be expressed by mast cells (4) and by Th2, but not Th1, cells (5–7). Although ST2/T1 is a member of the IL-1R family, it does not bind IL-1α, IL-1β, or IL-1R antagonist (8, 9). Functional ligands for ST2 have not been demonstrated, although ST2 binding proteins have been reported (8, 9). In the mouse, differential mRNA processing within the ST2 gene allows for the production of both a membrane-bound form of ST2, which is expressed primarily by hemopoietic cells (ST2L), and a soluble (s) form of ST2, which is predominantly expressed by fibroblasts (10). Hence, ST2 is identical with the extracellular region of ST2L except for an additional nine amino acids, which are present at the C terminus of ST2. More recently, another alternatively spliced ST2 transcript has been described in humans, the functional consequences of which are unclear (11).

Transcription of ST2/ST2L is controlled by two distinct promoters: an upstream promoter directs transcription in hemopoietic cells such as mast cells, while a promoter 10.5 kb downstream directs fibroblast-specific expression (10). Regulation of ST2/ST2L expression between mice and humans appears to be conserved, because two promoters also control ST2/ST2L expression in human cells (12). Studies using either anti-ST2 Abs or a recombinant s form of ST2 have demonstrated important roles for this molecule in regulating Th1/Th2-associated immune responses in vitro and in experimental disease models in vivo (6, 13). Such data are supported by studies using ST2-deficient mice (14).

Macrophages respond to bacterial products such as LPS, bacterial or CpG-containing DNA, peptidoglycan, and muramyl dipeptide by producing a cascade of proinflammatory cytokines including IL-1α/β, IL-6, IL-12, IL-18, and TNF-α, adhesion molecules, and inflammatory mediators that mediate innate immunity and prime the acquired immune response. Systemic bacterial infections trigger dysregulated production of these molecules, ultimately leading to disseminated intravascular coagulation, multiple organ failure, and mortality. The toxic effects of LPS in vivo are primarily conferred by macrophages (15). LPS-mediated activation of macrophages involves presentation of LPS by CD14 to members of the Toll-like receptor (TLR) family, particularly TLR4 (16–18). Signaling through TLR members involves recruitment of the IL-1R-associated kinase signaling complex, leading to activation of NF-κB and transcription of responsive genes (19). It is clear that a multitude of other signaling pathways including mitogen-activated protein kinase, erk-1/2, p38, c-Jun N-terminal kinase, and ceramide-activated protein kinase are also involved in inflammatory responses. ST2 expression was detected in human monocytes after LPS stimulation in vitro and in...
the muscle and spleen of mice after LPS injection (21). Proinflammationatory stimuli including TNF-α, IL-1α, and IL-1β induced ST2 expression in 3T3 cells and, exposure to UV light triggered ST2 expression in vivo (22). We have investigated the role of ST2 in regulating the LPS response using a sST2-human (h)IgG-Fc fusion protein (sST2). We report here that macrophages expressed a sST2-binding activity that was up-regulated by LPS. Treatment of macrophages with sST2 down-regulated expression of TLR4 and TLR1 but induced nuclear translocation of NF-κB. Furthermore, sST2 suppressed inflammatory responses induced by LPS both in vitro and in vivo. Thus, sST2 represents a novel pathway for regulating endotoxic shock and may have considerable therapeutic potential as an anti-inflammatory agent through its ability to deactivate macrophages.

Materials and Methods

Cell lines and reagents

RMPI 1640 medium (Life Technologies, Paisley, U.K.), containing 10% FCS, penicillin/streptomycin, and glutamine (complete medium) was used for culture of primary cells and cell lines. Marine bone marrow-derived macrophages (BMMs) were derived from cells of the femurs of adult BALB/c mice (Harlan Olac, Bicester, U.K.). Briefly, femurs were flushed with complete medium, and cells were plated in complete medium containing 10 U/ml rhCSF-1 (a gift from Chiron, Emeryville, CA, provided by D. Hume, Brisbane, Australia) on 10-cm bacteriological plastic plates (Bibby Sterilin, Staffordshire, U.K.) for 7 days in a 37°C incubator containing 5% CO₂. In some experiments, 10% L929 cell-conditioned medium was used as a source of CSF-1 instead of rhCSF-1. The marine macrophage-like cell line RAW264 was obtained from American Type Culture Collection (Manassas, VA) and was cultured in complete medium on 10-cm bacteriological plastic plates. For in vitro experiments, LPS from Salmonella minnesota (Sigma, Poole, U.K.) was used at a concentration of 100 ng/ml in all cell culture experiments. For in vivo experiments, LPS from S. enteritidis (Sigma) was used at a dose of 18 mg/kg body weight. The sST2-IgG fusion protein was prepared as previously described (13). Briefly, a mammalian expression plasmid containing the sST2 cDNA was described previously (25). Double-stranded oligonucleotides (Sigma-Geno- sys, Poole, U.K.) used were NF-κB (26) corresponding to an element from the TNF-α promoter that contains an NF-κB binding site (5'-CAAA CAGGGGGCTTTCCCTCCTC-3') and GATA corresponding to an element from the ST2 promoter that contains a GATA-1 binding site (5'- CCGTTCTAAGCTGTGAAGGAGG-3'). Both strands of the NF-κB oligonucleotide were end-labeled with polynucleotide kinase and [γ-32P]ATP, annealed, separated on a NAP-5 column (Sigma), and used for gel shift assays. Cold competition analysis was performed by incubating binding reactions with a 10-fold molar excess of either self (NF-κB) or an unrelated oligonucleotide (GATA). Supershift/Ab blocking experiments were performed by addition of 1 μl of 100 μg/ml of either anti-p50, anti-p52, anti-p65, or anti-c-rel Abs (BD PharMingen).

Quantification of mRNA

Total RNA was prepared using RNAzol B (Biogenesis, Poole, U.K.) according to the manufacturer’s instructions. RNA was treated with DNase 1 (Ambion, Austin, TX) and reverse transcribed to cDNA using Superscript reverse transcriptase (Life Technologies). Negative control samples (no first-strand synthesis) were prepared by performing reverse transcription reactions in the absence of reverse transcriptase. The cDNA levels of TLR4, TLR1, IL-12 p40, IL-12 p35, and hypoxanthine phosphoribosyltransferase (HPRT) were quantitated by real-time PCR using an ABI prism 7700 sequence detector according to the manufacturer’s instructions (Applied Biosystems, Foster City, CA). The cDNA levels during the linear phase of amplification were normalized against HPRT. Determinations were made in triplicate and expressed as mean ± SD. Primers and probes used were as follows: for TLR4, sense, 5’-AGGAATTCTTCTGGACAAAT-3’, antisense: 5’-CAAAAATTTTGTCCACACCCACCA-3’; for TLR1, sense: 5’-TGTTGTCGCCGTACACAATA-3’, antisense: 5’-AGAGGACCCCCTGTTCTCA-3’; probe: 5’-CACACTCGTATTTGATAGACGTTCAATCCGCTTGATAC-3’; for IL-12 p40, sense: 5’-GGATAATGTTCTGCACTCTGAT-3’, antisense: 5’-CAAGCTGACCTCATCGAATTAAAAT-3’, probe: 5’-CACTGGATACCCCTGGGATTACAT-3’, probe: 5’-AACAGAACCTTCTCGAGAGATACCACAT-3’; for IL-12 p35, sense: 5’-AGGGACCCATCATCACACGACCAAA-3’, antisense: 5’-AGGGACAACCTGCCTGTCTCTTCCGTA-3’, probe: 5’-CAAGCTGACCTCATCGAATTAAAAT-3’. For HPRT, sense: 5’-GAGATCGAGACCCGCTCTCA-3’, antisense: 5’-GCAATCGACGCGACCAAA-3’, antisense: 5’-CAGGGCTTTCCCTCCTCCTC-3’, and for HPRT, sense: 5’-GCCATACGACCCGCTCTCA-3’, antisense: 5’-GCAATCGACGCGACCAAA-3’, antisense: 5’-CAAGCTGAGATTACCCCTGGGATTACAT-3’. In additional experiments, the Student’s t test was used for cytokine determinations. For experiments using anti-ST2 Ab, age- and sex-matched BALB/c mice (8-wk-old male mice; n = 12/group) were injected i.p. with 18 mg/kg LPS then 1 h later were injected i.p. with 100 μg/mouse hIgG control or 100 μg/mouse sST2. Serum was collected from mice 2 h after LPS injection for determination of cytokines. Statistical analysis

Statistical analysis was performed using Minitab software (Minitab, State College, PA). For in vivo trials, the two-tailed log-rank test was used, and the Student’s t test was used for cytokine determinations.

Results

Soluble ST2 binds to the surface of BMMs

Using a sST2-hIgG fusion protein, we analyzed the ability of BMMs to bind sST2. BMMs were initially preincubated with Fe block (anti-CD16/anti-CD32) and then incubated with either sST2 or hIgG. Cells were then analyzed by flow cytometry using an anti-hIgG mAb. Cells incubated with normal hIgG had a staining pattern identical with the isotype control. In contrast, cells incubated with sST2 were markedly stained with anti-hIgG (Fig. 1A). Hence, BMMs express a cell surface binding activity for sST2. Importantly, this binding activity was markedly enhanced by overnight exposure of BMMs to LPS (Fig. 1B). In additional experiments, BMMs were incubated with human IgG and then reacted
expression of ST2L mRNA in BMMs by RT-PCR, we have been
differentiation (13).

The production of IL-6, IL-12, and TNF-α were analyzed by ELISA. Treatment with sST2 markedly inhibited
harvested at regular intervals, and concentrations of cytokines
were obtained in two separate experiments.

with anti-ST2 Ab. Cells incubated with hlgG showed no difference in binding between anti-ST2 Ab and the isotype control, thus, indicating that macrophages do not express detectable levels of cell surface ST2L (data not shown).

Treatment with sST2 inhibits LPS-induced proinflammatory cytokine production from macrophages
Because binding of sST2 to BMMs was up-regulated by LPS treatment, we investigated the reciprocal involvement of sST2 in regulating the LPS response. BMMs were pretreated for 1 h with sST2 or hlgG (both at 50 μg/ml) or medium alone and then stimulated with LPS (100 ng/ml) for up to 48 h. Culture supernatants were harvested at regular intervals, and concentrations of cytokines were analyzed by ELISA. Treatment with sST2 markedly inhibited the production of IL-6, IL-12, and TNF-α (Fig. 2). This was apparent at all time points examined, indicating that inhibition by sST2 was not overcome by persistent exposure to LPS. In contrast, NO and IL-10 (data not shown) production in response to LPS was not inhibited by treatment with sST2. Production of IL-6 and TNF-α, but not IL-12, in response to LPS in the murine macrophage-like cell line RAW264.7 was also suppressed by sST2 treatment (data not shown). Hence, the inhibition mediated by sST2 appears to be selective.

We then conducted dose-response studies. BMMs were preincubated with increasing concentrations (0.1–50 μg/ml) of sST2 or hlgG for 1 h and then cultured with LPS for 8 h before supernatants were assayed for IL-6 by ELISA. Treatment with sST2 caused a dose-dependent decrease in LPS-induced IL-6 production compared with the hlgG control, with significant effect from 0.1 μg/ml and a 6-fold reduction at 50 μg/ml (data not shown). This effect was not due to toxicity induced by sST2 because cell viability (assessed by MTT assay) was not altered by treatment with sST2 (data not shown). The dose of sST2 used for additional experiments (50 μg/ml) was similar to or lower than the concentration of sST2 used by others in an earlier report on Th1/Th2 cell differentiation (13).

Although we have sometimes detected very low levels of expression of ST2L mRNA in BMMs by RT-PCR, we have been unable to detect membrane-bound ST2L by flow cytometry (data not shown). To confirm that the effect of sST2 on macrophages did not involve competition between sST2 and presumptive low levels of ST2L for an LPS-inducible protein, we pretreated BMMs for 2 h with sST2, washed the cells thoroughly to remove sST2 in culture medium, and then stimulated cells with LPS for 8 h. We found that sST2 inhibited LPS-induced IL-6 production irrespective of whether sST2 had been removed before stimulation with LPS (data not shown). Hence, sST2 appears to trigger anti-inflammatory signals in macrophages directly.

NF-κB is activated in BMMs by sST2
One mechanism by which sST2 could suppress LPS-induced proinflammatory cytokine production is by blocking LPS-initiated nuclear translocation of NF-κB or specifically mobilizing NF-κB p50 homodimers, which can have inhibitory effects on gene expression (27). To test this possibility, we assessed levels of NF-κB in BMMs pretreated with 50 μg/ml of hlgG or sST2 for 30 min before stimulation with LPS for 45 min. Unexpectedly, sST2 was itself able to activate NF-κB in BMMs to the same extent as LPS, and no additional activation or repression by LPS was observed (Fig. 3A). Cold competition analysis indicated that the induced band was indeed NF-κB, because unlabeled self oligonucleotide, but not an unlabeled oligonucleotide containing a GATA-1 site, could compete for binding (Fig. 3A). NF-κB induced by sST2 was primarily composed of p65, c-rel, and p50 as assessed by supershift analysis (Fig. 3B). The inducible complex did not contain the rel family member p52, because treatment with an anti-p52 Ab did not alter binding. On the basis of supershift analysis, LPS-induced NF-κB could not be distinguished from sST2-induced NF-κB (data not shown). Hence, LPS and sST2, which clearly have opposing effects on macrophage cytokine production, both induced p65/c-rel/p50-containing NF-κB.

TLR4 and TLR1 levels in BMMs are suppressed by sST2
Because members of the TLR family, particularly TLR4, are essential for macrophage responses to LPS, we examined the effect

FIGURE 1. BMMs express an ST2-binding activity. A, BMMs were harvested, preincubated at 4°C in complete medium in the presence of Fc block, and then incubated with 50 μg/ml of either sST2 or hlgG. Cells were then stained with an anti-hlgG Ab and analyzed by flow cytometry. The dotted line indicates isotype control, the thin line represents hlgG-treated cells, and the thick line depicts sST2-treated cells stained with anti-hlgG Ab. B, BMMs were cultured for 20 h in the presence of medium or LPS (100 ng/ml) and then analyzed as described in A. The dotted line indicates isotype control, the thin line represents BMM control cells treated with sST2 and stained with anti-hlgG Ab, and the thick line depicts LPS-treated BMMs treated with sST2 and stained with anti-hlgG Ab. Similar results were obtained in two separate experiments.

FIGURE 2. Time course of inhibition of LPS responses by sST2. BMMs were pretreated with either medium, 50 μg/ml hlgG, or 50 μg/ml sST2 for 1 h and then activated with LPS (100 ng/ml) for up to 48 h. Levels of IL-6, IL-12, and TNF-α in supernatants were determined by ELISA. NO production was assessed by the Griess reaction. Data are presented as the mean of triplicates ± SD. IL-10 levels were not affected (data not shown).

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of sST2 on TLR mRNA levels in BMMs using quantitative real-time PCR. Fig. 4 shows that treatment of BMMs with either sST2 or LPS down-regulated expression of TLR4 mRNA 3-fold, and the combination of these stimuli suppressed TLR4 mRNA levels further. Interestingly, TLR1, which may also be involved in the response to LPS (28), was differentially regulated by LPS and sST2; sST2 repressed TLR1 mRNA levels 4-fold, whereas LPS induced TLR1 expression 2-fold. To confirm that sST2 inhibited LPS-induced proinflammatory gene expression in the same RNA samples, mRNA levels of IL-12 p40 and IL-12 p35 were also assessed. Both IL-12 p40 and p35 mRNA levels were almost undetectable in unstimulated cells or cells treated with sST2. LPS induced expression of both IL-12 p40 and p35, and sST2 repressed this LPS-induced expression (p40, 2.6-fold and p35, 5.5-fold).

**LPS-induced mortality in vivo is blocked by sST2**

The observation that sST2 could inhibit LPS-induced proinflammatory cytokine production in vitro suggested that this molecule might have therapeutic potential as an LPS antagonist. To test this hypothesis, a murine LPS shock model was used. Mice were injected i.p. with LPS and then treated 1 h later with either sST2 or hlgG. Although only 4 of 12 mice in the control hlgG-treated group survived over a 4-day period, mice treated with sST2 had a significantly enhanced survival rate (10 survived of 12; p < 0.01) (Fig. 5A). Treatment with sST2 clearly down-regulated the levels of serum IL-6, IL-12, TNF-α, and IFN-γ in LPS-challenged mice (Fig. 5B). No significant differences in IL-10 levels were seen between sST2- and hlgG-treated mice. Because there is evidence that IL-18 may be involved in controlling the onset of LPS shock (29), we also measured serum IL-18 in the two groups. IL-18 levels were not altered by treatment with sST2 (Fig. 5B).
Anti-ST2 Ab exacerbates LPS shock

The above-described data demonstrate that sST2 has potent anti-inflammatory activity and blocks LPS shock in vivo. Therefore, it might be expected that inhibition of endogenous sST2 action would exacerbate mortality. We tested this hypothesis by examining the effects of an anti-ST2 Ab that has been used previously to block Th2 responses in vivo (6). Mice were injected i.p. with either anti-ST2 Ab or an isotype control on day −1, 0, and +1 of LPS challenge. Although 9 of 25 from the control group succumbed to LPS-induced shock, the level of mortality in the anti-ST2 Ab-treated group was significantly higher (20 of 25; p < 0.01) than in the control group (Fig. 6A). Levels of the proinflammatory cytokines IL-6, TNF-α, and IFN-γ were also elevated in sera from mice treated with anti-ST2 Ab compared with the control group (Fig. 6B). The level of IL-10 was similar in both groups (3.5 vs 3.8 ng/ml).

Discussion

Data presented here demonstrate for the first time that sST2, the product of a gene previously found to be selectively expressed in BALB/c mice were given three i.p. doses of an anti-ST2 polyclonal Ab (250 μg/mouse) or an isotype control and challenged i.p. with LPS (18 mg/kg). Two hours after LPS treatment, serum was collected from six mice in each group and pooled. Levels of IL-6, TNF-α, and IFN-γ were quantitated by ELISA (presented as the mean of triplicates ± SD), and survival in each group was monitored (n = 25). *p < 0.05; **p < 0.01, anti-ST2 vs isotype control.

FIGURE 6. Anti-ST2 Ab exacerbates LPS-induced mortality and augments proinflammatory cytokine production in vivo. Age- and sex-matched BALB/c mice were given three i.p. doses of an anti-ST2 Ab or an isotype control. Serum was collected before LPS treatment, at 0 h, and at +1 h. Levels of IL-6, TNF-α, and IFN-γ were quantitated by ELISA (presented as the mean of triplicates ± SD), and survival in each group was monitored (n = 25). *p < 0.05; **p < 0.01, anti-ST2 vs isotype control.
factor, because LPS-induced NO and IL-10 levels were not altered by sST2 treatment, and we were unable to detect significant levels of TGF-β1 in sST2-treated BMMs (data not shown). Furthermore, kinetic studies showed that sST2-mediated suppression was apparent 4 h after LPS treatment at the protein level. This is consistent with the effect of sST2 in vivo, where IL-6, IL-12, IFN-γ, and TNF-α levels were suppressed 2 h after administration of sST2. Similarly, IL-6, TNF-α, and IFN-γ levels in the serum were markedly elevated in mice treated with anti-ST2 Ab 2 h after LPS challenge.

In conclusion, apart from acting as a marker for Th2 cells and directly modulating Th1/Th2 balance by influencing Th2 cell functions (6, 7), the gene encoding T1/ST2 may also have an important regulatory role in the inflammatory response by acting directly on macrophages. Fig. 7 summarizes the regulatory pathway of sST2 in LPS-induced inflammatory responses. LPS activates macrophages via TLR4, inducing production of proinflammatory cytokines including IL-1β and TNF-α, which activate fibroblasts and other cell types to produce sST2. Then sST2 binds to macrophages via a yet undefined receptor and directly and selectively represses the expression of proinflammatory cytokines, including IL-6, IL-12, and TNF-α, possibly through the down-regulation of TLR4. Thus, sST2 may function as an important mediator in this negative feedback loop for preventing uncontrolled inflammatory reactions. Consistent with this is our demonstration that ST2-IgG, an effective surrogate of ST2, markedly blocked LPS-induced shock and mortality in mice treated with ST2-IgG 2 h after LPS treatment, and we were unable to detect significant levels of LPS-induced NO and IL-10, because LPS-induced NO and IL-10 levels were not altered by sST2 treatment.

This scheme does not exclude the existence of ST2 as a dimeric molecule that may signal differently.

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References

1. Tominaga, S. 1989. A putative protein of a growth specific cDNA from BALB/c 3T3 cells is highly similar to the extracellular portion of murine interleukin 1 receptor. FEBS Lett. 238:301.
8. Kumar, S., M. D. Miminch, and P. R. Young. 1995. ST2/T1 protein functionally binds to two secreted proteins from Balb/c 3T3 and human umbilical vein endothelial cells but does not bind interleukin 1. J. Biol. Chem. 270:27905.