Primary sources and immunological prerequisites for sST2 secretion in humans

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Aims
Serum levels of the soluble growth stimulation gene-2 (sST2) are elevated in heart and pulmonary diseases. However, the relationship of the sST2/interleukin (IL)-33 axis and its triggers as well as its organ distribution is still not known. This study was thus designed to investigate the cellular origin and regulation of sST2 and IL-33 in vitro and in vivo.

Methods and results
sST2 and IL-33 gene expression and protein secretion were analysed in pooled organ-specific cDNAs and in primary cell cultures, respectively, by RT–PCR and ELISA technology. The strongest sST2 mRNA expression was detected in heart and lung tissues, which correlated with spontaneous secretion of sST2 protein in vitro. The inflammatory cytokines IL-1α, IL-1β, and tumour necrosis factor α as well as supernatants of lipopolysaccharide (LPS)-stimulated peripheral blood mononuclear cells led to an enhanced secretion of sST2 in cultured cardiac myocytes and lung alveolar epithelial cells. These cytokines enhanced sST2 secretion via an NFκB-dependent mechanism. In addition, LPS stimulation in humans in vivo induced a short-term inflammatory response that was followed by a massive enhancement of sST2 secretion.

Conclusion
These results identify the primary sources and inflammatory triggers for the enhancement of sST2 secretion and demonstrate a relationship between inflammation and the secretion of a bioactive member of the IL-1R family, both in vitro and in vivo.

Keywords
Soluble growth stimulation gene-2 • Cardiac myocytes • Alveolar epithelial cells • Cell culture • Inflammation

1. Introduction
Growth stimulation gene-2 (ST2) (also known as IL1RL1, DER4, T1, and FIT-1), a novel member of the toll-like/interleukin (IL)-1-receptor-like superfamily, was originally identified as a gene induced by serum or oncogene expression in mouse fibroblasts.1,2 Homologous cDNA was subsequently cloned from humans3 and rats (Fit-1).4 This gene is tightly linked to the IL-1-R genes on mouse chromosome 15 and human chromosome 2.6 Soluble ST2 (sST2) protein and its membrane-anchored counterpart (ST2L) are both transcribed from a single promoter and are regulated at the transcriptional level.4,7 ST2L has recently been identified as the receptor for IL-33. Whereas IL-33 activates Th2 cytokines in T cells,8 mast cells, eosinophils, and basophils, sST2 is known to have immunosuppressive properties by acting as a decoy receptor for this cytokine. In addition, a direct interaction of sST2 with macrophages has been shown to down-regulate pro-inflammatory cytokines9–12 in these cells. Furthermore, the activation of ST2L by IL-33 leads to the down-regulation of Toll-like receptor 4 signalling, thereby desensitizing cells to lipopolysaccharide (LPS) stimulation.13–15 Recent studies have demonstrated that IL-33 is released as pro-IL-33 which is biologically active.16 Furthermore, cleavage of pro-IL-33 by apoptotic caspases leads to its inactivation.17 Interestingly, these authors also show that pro-IL-33 seems not to be secreted actively but passively after cell injury.17

The secretion of sST2 protein was reported to be strongly enhanced in inflammation-associated illnesses. In the clinical setting,
increased levels of sST2 were shown to be augmented in patients with dengue virus infection, sepsis and trauma, lung fibrosis, chronic obstructive pulmonary disease, food allergy, subarachnoid haemorrhage, and heart allograft recipients who developed transplant vasculopathy (personal observation, unpublished results). More recently, we have found a long-lasting exacerbation of sST2 levels after utilization of heart-lung machines in open heart surgery. In another clinical setting, sST2 protein has emerged as a novel biomarker in patients with congestive heart failure, acute dyspnoea, and in patients with recent acute myocardial infarction (AMI). In contrast, a report that questioned the validity of sST2 based acute AMI diagnosis in the setting of the emergency department has recently been published. Relevant to these reports is a patent application that utilized sST2 as a prognostic factor for overall mortality (World Intellectual Property Organization, PCT/US2007/067333).

Apart from prognostic information obtained from sST2 in humans little is known about organ-specific production and regulation of sST2, ST2L, and IL-33. The source of sST2 in cardiovascular diseases was presumed to be of myocardial origin, showing load induction of ST2 mRNA in neonatal rat cardiac myocytes in vitro. Bartunek et al. however provided first evidence that the adult human myocardium is not the sole source of increased sST2 levels in pressure overload hypertrophy and congestive cardiomyopathy. In addition, data were presented which documented that venous and arterial endothelial cells have the ability to secrete ST2 protein after in vitro IL-1β, tumour necrosis factor (TNFα), and phorbol ester stimulation. On the basis of the relationship between sST2 and indexes of diastolic load, the authors hypothesized that the vascular endothelium senses the haemodynamic and inflammatory status, and hence secretes sST2 into the circulation.

We conclude from the present literature that sST2 has a prominent role in clinical heart failure diagnosis and is associated with an aggravated immune status in humans. Nonetheless, there is no study available that has systematically investigated the origin of sST2 in IL-33. The source of sST2 in cardiovascular diseases was presumed to be of myocardial origin, showing load induction of ST2 mRNA in neonatal rat cardiac myocytes in vitro. Bartunek et al. however provided first evidence that the adult human myocardium is not the sole source of increased sST2 levels in pressure overload hypertrophy and congestive cardiomyopathy. In addition, data were presented which documented that venous and arterial endothelial cells have the ability to secrete ST2 protein after in vitro IL-1β, tumour necrosis factor (TNFα), and phorbol ester stimulation. On the basis of the relationship between sST2 and indexes of diastolic load, the authors hypothesized that the vascular endothelium senses the haemodynamic and inflammatory status, and hence secretes sST2 into the circulation.

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2. Methods

2.1 Culture of human primary cells

Primary human cells were obtained from Lonza (Lonza, Basel, Switzerland) or Innoprot (Innoprot, Leioa, Bizkaia, Spain) and cultured in their respective growth media (Table 1). Peripheral blood mononuclear cells (PBMC) from 10 healthy donors (six males and four females, non-smokers, mean age 29.3 years) were isolated from heparinized whole blood by standard gradient centrifugation with Ficoll-Paque (GE, Healthcare, Uppsala, Sweden). PBMC preparation of healthy donors was approved by the Ethics Committee of the Medical University of Vienna (034/2010). The purity of the isolated PBMC was >98%, analysed with a automated haematology analyser.

![Table 1: Human primary cells and media used](https://example.com/table1.png)

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<table>
<thead>
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<th>Cells</th>
<th>Company</th>
<th>Medium</th>
<th>Company</th>
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<td>Gibco</td>
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<td>DMEM</td>
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<td>Epithelial cell</td>
<td>Innoprot</td>
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<td>Innoprot</td>
<td>Cardiac myocyte</td>
<td>Innoprot</td>
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<td>Innoprot</td>
<td>Cardiac myocyte</td>
<td>Innoprot</td>
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<td>Lung fibroblasts</td>
<td>Innoprot</td>
<td>DMEM</td>
<td>Gibco</td>
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<td>Bronchus epithelial cells</td>
<td>Lonza</td>
<td>BEGM</td>
<td>Lonza</td>
</tr>
<tr>
<td>Alveolar epithelial cells</td>
<td>Innoprot</td>
<td>Alveolar epithelial cell</td>
<td>Innoprot</td>
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*aHuman umbilical vein endothelial cells.
*bHuman dermal microvascular endothelial cells.

(KX-212, Sysmex Corporation, Takatsukadai, Nishi-ku, Japan). RPMI-1640 (Gibco, Gaithersburg, USA) and DMEM (Gibco) media were supplemented with 10% foetal bovine serum (PAA, Linz, Austria), 25 mM l-glutamine (Gibco), and 1% penicillin/streptomycin (Gibco). Cells were cultured at 37°C, 5% CO₂, and at 95% relative humidity. All cells were used for the experiments in passage 2–3.

2.2 RNA isolation and cDNA preparation

Pooled human tissue cDNAs were obtained from Clontech (Clontech, Palo Alto, CA, USA). Cells were seeded in 12-well plates (Corning Incorporated, Corning, NY, USA) and were harvested at 50–80% confluence. RNA was prepared using the TRizol Reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer’s instructions. The purity of the RNA was confirmed with the nano-drop spectrophotometer (Fisher Scientific, Vienna, Austria). cDNAs were transcribed with the GeneAmp Kit using MuLV-reverse transcriptase and oligo(dT) primers (Applied Biosystems, Foster City, CA, USA) as indicated in the instruction manual.

2.3 PCR

Conventional PCRs were performed in a volume of 50 μL containing 0.2 μL Red Hot DNA polymerase (ABgene, Hamburg, Germany), 5 μL 10× reaction buffer IV (ABgene), 3 μL MgCl₂ (25 mM), 4 μL dNTPs (2.5 mM), 2.5 μL of each primer (10 μM), and 31.8 μL H₂O. The PCR included 5 min at 94°C for initial denaturing, followed by 33 cycles of 1 min at 94°C, 45 s at 60°C, 45 s at 72°C, and a final extension step at 72°C for 5 min. Amplicons were subjected to electrophoresis in a 1.5% agarose gel containing ethidium bromide. The specificity of the PCR products was confirmed by sequencing. S2T2 and IL-33 mRNA expression was further quantified by real-time PCR with LightCycler 480 SYBR Green I Master (Roche Applied Science, Penzberg, Germany) according to the manufacturer’s protocol. cDNA sequences of the genes under investigation were obtained from GenBank. Primers were designed using the PRIMER3 software from the Whitehead Institute for Biomedical Research (Cambridge, MA, USA). The following forward and reverse intron-spanning primers were synthesized by VBC Genomics (VBC Genomics, Vienna, Austria): sST2 (forward: 5’-CTCCAAGTTCATCCCTCTG-3’, reverse: 5’-GATCCCAAACCCCATCTCTTGTT-3’), IL3 (forward: 5’-GGAGTGCTTGGCCTTGGTA-3’, reverse: 5’-TGCATTGGAGGGTGTGGAGA-3’), β-2-microglobulin (β2M, forward: 5’-GATGAGGATATGC-3’).
CTGCCG'TGTG-3', reverse: 5'-CAATCGAATGGCGGACT-3'), and delta-aminolevulinate synthase (ALAS, forward: 5'-CCACGTGAAGAG CTATGTA-3', reverse: 5'-ACCCTCAACACAAACCAAA-3'). The relative expression of the target genes was calculated by comparison to the housekeeping gene β2M using a formula described by Pfaffl.32 The efficiencies of the primer pairs were determined as described by Kadi et al.33 (efficiencies: β2M: 1.92; sST2: 1.91; IL-33: 1.88; ALAS: 1.98), and the specificity of the PCR products was confirmed by sequencing.

2.4 Stimulation of primary cultured alveolar epithelial cells and cardiac myocytes in vitro and detection of secreted sST2 and IL-33

Primary human alveolar pulmonary epithelial cells and cardiac myocytes (5 × 10⁴) were seeded in six-well plates (Corning Incorporated) and cultured overnight in fully supplemented growth medium. After starvation in basic medium without serum and growth factors for 12 h, the cells were further cultivated for 24 h under following conditions: untreated (control), 10 ng/mL IL-1α (PeproTech, Inc., Rocky Hill, NJ, USA), 10 ng/mL IL-1β (PeproTech, Inc.), 10 ng/mL TNFα (PeproTech Inc.), 10 ng/mL IL-6 (PeproTech, Inc.), 100 ng/mL LPS (Sigma, Vienna, Austria) stimulation and hypoxic conditions. For hypoxia experiments, the plates were transferred to modular incubator chambers (Vangard International, Inc., Neptune, NJ USA) and flushed with 95% N₂ and 5% CO₂ for 10 min to simulate anoxic conditions. For hypoxia experiments, the plates were transferred to modular incubator chambers (Vangard International, Inc., Neptune, NJ USA) and flushed with 95% N₂ and 5% CO₂ for 10 min to simulate anoxic conditions. The pO₂-value was <2%. To inhibit NFκB activation, we pre-incubated cells with 5 μg/mL of the previously published specific NFκB inhibitor caffeic acid phenethyl ester (CAPE, Eubio, Vienna, Austria)34,35 for 2 h. Cytokine stimulation was then performed for 24 h in the presence of 5 μg/mL CAPE. After centrifugation of the cell culture supernatants, sST2 and IL-33 mRNA protein secretion was measured by ELISA (R&D Systems, Inc., Minneapolis, MN, USA) according to the manufacturer's instructions. All experiments were performed in biological and technical triplicates.

2.5 Generation of PBMC supernatants, quantification of inflammatory cytokines, and exposure of cultured alveolar epithelial cells and cardiac myocytes to these supernatants

PBMC (1 × 10⁶) were cultured (Ultraculture, Lonza) for 24 h with or without 100 ng/mL LPS (Sigma), referred thereafter as supernatants (SN) + LPS and SN − LPS, respectively. Levels of IL-1α, IL-1β, IL-6, TNFα, sST2, and IL-33 in these SN were determined by ELISA (R&D Systems) according to the manufacturer's instructions. Primary human alveolar pulmonary epithelial cells and cardiac myocytes (5 × 10⁴) were seeded in six-well plates (Corning Incorporated) and cultivated overnight in fully supplemented growth medium. After starvation in basic medium without serum and growth factors for 12 h, the cells were further incubated with PBMC derived SN + LPS or medium alone for 24 h. After centrifugation of the cell culture supernatants, sST2 and IL-33 protein secretion was measured as described above. All samples were assayed in triplicates. Sensitivity of the ELISA assays were as follows: for ST2 5 pg/mL, for IL-33 3 pg/mL, for TNFα 0.7 pg/mL, for IL-1α 1 pg/mL, for IL-1β 1 pg/mL, and for IL-6 0.7 pg/mL. Intra- and interassay coefficients of variation were as follows: for ST2 4.8 and 6.3%, for IL-33 7%, for TNFα 4.9 and 7.6%, for IL-1α 2.3 and 4.9%, for IL-1β 3.8 and 5.2%, and for IL-6 2.8 and 3.6%, respectively. According to the manufacturer, no significant cross-reactivity or interference between different cytokines was observed.

2.6 Human endotoxin model

The study was approved by the Ethics Committee of the Medical University of Vienna (EK725/2007) and conforms to the principles outlined in the Declaration of Helsinki, including current revisions and the Good Clinical Practice guidelines. Seventeen healthy volunteers (seven males and ten females) at the age of 18–45 were enrolled after informed consent was obtained. They were not permitted to smoke more than 10 cigarettes per day. In our study, collective the mean age was 23.3 (± 2.05) years and 12 were non-smokers and 5 smoked <10 cigarettes per day. A complete health examination, including physical examination, electrocardiogram, and laboratory screening, was performed in all subjects before the first study day. Alcohol consumption before the study day was an exclusion criteria and an alcohol breath test on the study day was performed. Healthy volunteers were allowed to drink one cup of tea or coffee. ECG, heart rate, blood pressure, oxygen saturation, body temperature, lung function (forced vital capacity and forced expiratory volume after 1 s) and alcohol breath test were performed at baseline measurements, before LPS administration. Two nanogram per kilogram *Escherichia coli*-derived LPS (US Standard Endotoxin, U.S.P. United States Pharmacopeial Convention, Inc., Rockville, MD, USA) dissolved in NaCl 0.9% was administered slowly intravenously to induce a systemic inflammatory response. Following LPS administration heart rate, blood pressure, oxygen saturation, and body temperature were measured every 30 min. Owing to LPS administration, an increase of body temperature, heart rate, and blood pressure could be detected with a maximum after ~3–4 h. Some subjects suffered from headache and nausea. After ~6 h the symptoms declined. Venous blood was collected at 4 and 24 h post-intravenous administration of *E. coli* LPS for further analysis. Serum samples were aliquoted and deep frozen at −80°C. Serum levels of IL-1α, IL-1β, IL-6, TNFα, IL-33, and sST2 were measured by ELISA as described above.

2.7 Statistical analysis

Results are presented as mean ± SEM, if not mentioned otherwise. Owing to the relatively small sample size, the Mann–Whitney U-test was used to calculate significance. Since this is a descriptive study with no main a priori hypothesis; a P-value of 0.05 was deemed to be significant.

3. Results

3.1 Pooled human lung tissue displays highest mRNA sST2 transcription in vivo: impact for primary cell culture studies in vitro

To investigate which human organ system contains the highest sST2 and IL-33 concentration, we obtained pooled human cDNAs and performed conventional PCR. We found high expression of sST2 in the small intestine, the heart, the kidney, and the lung (Figure 1A, upper panel). All cDNAs were normalized to the housekeeping gene β2M (Figure 1A, lower panel). To quantify the expression levels of sST2, the same cDNAs were analysed by real-time PCR (Figure 1B). The highest sST2 mRNA expression was found in lung, followed by kidney, heart, small intestine, brain prostate, and pancreas. In contrast to sST2, the expression of IL-33 was much less organ specific (Figure 1C). Except for skeletal muscle, PBMC and kidney, which showed very low expression levels of IL-33, all other tissues expressed IL33 at similar levels. We could only detect a weak relative enhanced IL-33 expression in testis (3.8-fold), brain (4.4-fold), small intestine (4.3-fold), and heart (3.6-fold) (Figure 1C). We could not observe any correlation between sST2 and IL-33 expression.
3.2 Human cell type-specific sST2 mRNA expression and secretory capacity in vitro

To validate pooled human organ mRNA data, we sought to determine whether human sST2 mRNA expression in primary cell culture would yield similar results. By real-time PCR (Figure 2A), we detected low sST2 mRNA levels in PBMC and endothelial cells. All cell types derived from human skin and the kidney were negative for sST2 expression. Moderate sST2 mRNA expression was detected in cardiac myocytes, however, not in cardiac fibroblasts. The highest sST2 mRNA expression was again observed in cells derived from lung. Whereas lung fibroblasts showed no expression, we detected highest levels of sST2 mRNA transcription in bronchial and alveolar epithelial cells (Figure 2A). No correlation in the sST2 and IL-33 expression pattern was observed as determined by real-time PCR.
To investigate whether high mRNA content in a particular tissue would correlate with increased secretion of sST2, primary cells from various organs (endothelium, skin, renal, heart, lung) were cultured, and sST2 and IL-33 release were assayed by ELISA. Figure 2C shows that sST2 mRNA content correlated significantly with spontaneous release of sST2 protein. The highest sST2 content was observed in SN derived from lung alveolar epithelial cells, lung bronchus epithelial cells, and cardiac myocytes. Secretion of IL-33 protein was not detected in any of these cell types (data not shown).

3.3 Human cell type-specific sST2 secretory capacity is augmented by IL-1α, IL-1β, and TNFα but not by IL-6, hypoxia, and LPS stimulation via an NFκB-dependent mechanism in vitro

The finding that cultured heart and lung epithelial cells spontaneously secrete sST2 in vitro led us to investigate whether inflammatory cytokines, endotoxin (LPS) treatment or hypoxic conditions is able to augment sST2 secretion in vitro. As shown in Figure 3A and C, IL-1α, IL-1β, and TNFα significantly enhanced the secretion of sST2 by lung epithelial cells as well as cardiac myocytes into the culture medium when compared with control conditions. No sST2 secretion was detected in pulmonary and cardiac fibroblasts (data not shown). IL-33 secretion was not detected in any of these cell types (data not shown).

To identify the underlying molecular mechanisms leading to enhanced sST2 secretion in vitro, we inhibited NFκB activation by the specific NFκB inhibitor CAPE. As shown in Figure 3B and D, this led to a complete attenuation of the enhanced sST2 secretion induced by inflammatory cytokines in both alveolar epithelial cells (B) and cardiac myocytes (D). One representative experiment of three is shown. Error bars represent one standard deviation calculated from three replicates for each set of values (*P < 0.001, §P < 0.05).

3.4 Supernatant derived from LPS-stimulated PBMC augments sST2 secretion by primary cardiac myocytes in vitro

In order to mimic a situation similar to inflammation in vivo, we consequently exposed experimentally alveolar epithelial cells and cardiac myocytes to SN derived from LPS-stimulated PBMC. Briefly, we...
prepared PBMC from 10 individual donors (see Methods) and cultivated them for 24 h with or without LPS (100 ng/mL). As depicted in Table 2, LPS stimulation of PBMC caused a massive increment of IL-1α, IL-1β, IL-6, and TNFα (all P < 0.01), whereas sST2 and IL-33 were not detectable in the SN. In a further attempt, we found that this SN caused a highly significant increment of sST2 secretion, when co-incubated with alveolar epithelial cells (Figure 4A) and cardiac myocytes (Figure 4B). These data indicate that endotoxin triggering causes an inflammatory secretory response in white blood cells that has the ability to augment sST2 secretion in alveolar epithelial cells and cardiac myocytes in vitro. IL-33 secretion was not detectable (data not shown).

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Untreated (pg/mL)</th>
<th>+ LPS (pg/mL)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>0.04 (+ 0.13)</td>
<td>49.05 (+ 28.95)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IL-1β</td>
<td>0.05 (+ 0.15)</td>
<td>1257.55 (+ 586.26)</td>
<td>&lt;0.001</td>
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<tr>
<td>IL-6</td>
<td>13.34 (+ 14.21)</td>
<td>4218.51 (+ 1823.49)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TNFα</td>
<td>0.18 (+ 0.41)</td>
<td>1673.6 (+ 70.36)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-33</td>
<td>0.00</td>
<td>0.00</td>
<td>/</td>
</tr>
<tr>
<td>sST2</td>
<td>0.00</td>
<td>0.00</td>
<td>/</td>
</tr>
</tbody>
</table>

Table 2 Cytokines released from PBMC with or without LPS treatment

Figure 4 Treatment of lung alveolar epithelial cells and cardiac myocytes with PBMC derived SN either untreated or treated with LPS. Lung alveolar cells and myocytes were cultured with SN derived from PBMC cultures from 10 individual donors either untreated or treated with LPS. Twenty-four hours after treatment sST2 secretion was determined by ELISA. Treatment of alveolar epithelial cells (A) and cardiac myocytes (B) with PBMC SN stimulated with LPS led to a strong increase in sST2 secretion. The small diagrams show the mean values of all 10 donors. Error bars represent one standard deviation calculated from three replicates for each set of values.
3.5 Human endotoxin model

Finally, we investigated whether our in vitro observation of augmented sST2 secretion caused by inflammation can be corroborated in a human endotoxin model. As shown in Figure 5, a significant augmentation of IL-6 (Figure 5A) and TNFα (Figure 5B) could be observed within 4 h post-LPS stimulation. Twenty-four hours after LPS administration secretion levels of IL-6 and TNFα were again back to baseline levels. Secretion of IL-1α and IL-1β was not detectable at both time points (data not shown). Similar to the effect of inflammatory cytokines observed in vitro, this short pro-inflammatory response was followed by a massive augmentation of sST2 secretion within 24 h (Figure 5C). IL-33 secretion was not regulated by the application of LPS (Figure 5D).

4. Discussion

Our findings represent the first human comparative in vitro as well as in vivo investigation in relation to the sST2/IL-33 axis. Human lung, heart, and brain tissue harbour the highest mRNA transcripational content in comparison to other organ systems. Pulmonary alveolar epithelial and cardiac myocytes evidenced excessive spontaneous secretion of sST2 into the culture medium when compared with controls. IL-1α, IL-1β, TNFα, and SN derived from LPS-stimulated PBMC caused a significant increment of sST2 specifically in alveolar epithelial cells and cardiac myocytes that was shown to be dependent on NFκB activation. Finally, in a human in vivo endotoxin model, we demonstrated that LPS stimulation induces a short-term boost of inflammatory cytokines (IL-6 and TNFα) that is followed by a highly significant increment of sST2 within 24 h. We deduce from these results that human myocytes and pneumoepithelial cells are important sources of circulatory bioactive sST2 and that endotoxin triggering, via induction of inflammatory cytokines in PBMC, augments sST2 secretion in alveolar epithelial cells and cardiac myocytes.

The present work helps us to interpret the finding of massively enhanced sST2 secretion after heart surgery and sST2 as a biomarker for cardiac heart failure (CHF). CHF is characterized by a progressive weight loss and an aggravated immune system in vivo. Especially, the inflammatory cytokines TNFα and IL-1β have been implicated to play a pivotal role in the development of this disease. Most interesting, some studies have shown, similar to
sST2 literature,27 that increased serum content of sTNFRs, correlated significantly with CHF mortality. Since both of these diseases are associated with systemic endotoxemia,38–40 we speculate that the activation of the innate immune system and sST2 producing cells interact correspondingly.

The human innate immune response is mediated by epithelial cells and immune cells and constitutes the first defence against invading pathogens. Exposure to the endotoxin LPS, which is recognized by TLR-4 on a variety of human cell types, is known to trigger the release of pro-inflammatory cytokines such as TNFα and IL-1 that is mediated by intracellular signalling pathways and recruitment of pro-inflammatory transcription factors such as NFκB.41,42–44 The core pathway of LPS signalling is well described and involves the activation of protein kinase cascades initiated by TNF receptor-associated factor 6 (TRAF6) upon interaction between myeloid differentiation factor-88 (MyD88) and IL-1 receptor-associated kinase (IRAK)45,46 and leads to NFκB activation. In human peripheral blood leucocytes, LPS-triggered activation of distinct signalling pathways results in a massive, yet temporal, dysregulation of the secretome/transcriptome, which has previously been characterized by profiling methods.47,48 To understand the exact nature of sST2/IL-33 axis in relation to inflammatory responses, we utilized a human endotoxin model. In agreement with van’t Veer et al.,49 we observed a strong augmentation of sST2 serum levels within 24 h post-LPS infusion, which was accompanied by a brief increment of IL-6 and TNFα. In contrast to the settings of our endotoxin model, these authors also used shorter time points and showed that maximum TNFα secretion (up to 1500 pg/mL) was already detected as early as 2 h after LPS application. Of utmost importance is the fact that this group did not identify the cellular source of sST2 in humans. In our study, we therefore investigated whether LPS stimulation had an effect on the sST2 and/or IL-33 secretory capacity in various primary human cells including PBMC. We found that LPS treatment and experimental hypoxic conditions had no effect on spontaneous sST2 secretion in cultured cells

Figure 6 Scheme of the human endotoxin model. Endotoxin stimulation leads to inflammation and the production of inflammatory cytokines such as IL-1α, IL-1β, IL-6, and TNFα. Inflammatory cytokines stimulate both lung alveolar epithelial cells and cardiac myocytes to an enhanced sST2 production via an NFxkB-dependent mechanism. Secreted sST2 enters the circulation and consequently contributes to the attenuation of immune responses in organs exposed to environmental and autologous antigenic triggers.

in vitro. In contrast, alveolar epithelial cells and cardiac myocytes secreted massive amounts of sST2 after exposure to IL-1α, IL-1β, and TNFα and SN derived from LPS-stimulated PBMC. If we interpret our in vivo and in vitro data correctly, the following immunological scenario can be hypothesized in humans: in clinical situations when the immune system is confronted with a ‘danger’ signal50 (e.g. endotoxin, myocardial infarction, inhaled noxious substance) circulating PBMC secrete IL-1α, IL-1β, IL-6, and TNFα which consequently leads to NFxKB-dependent increased sST2 secretion in alveolar epithelial cells and cardiac myocytes (Figure 6).

How do we construe the immunological relevance of lung and heart cells as primary sources of sST2? The lung, in particular, with its enormous surface area of ~150 m² and highly fragile gaseous exchange surfaces, poses a colossal challenge to the fine tuning of the human immune system after endotoxin exposure. In this respect, several studies documented the importance of sST2 in the induction of endotoxin tolerance.14,51 Our finding of massive spontaneous sST2 secretion by alveolar pneumoepithelial cells is in accordance with this speculation. In the case of LPS stimulation, sST2 was shown to attenuate acute lung injury in an experimental animal model.22

In conclusion, we believe that lung- and heart-derived sST2 might serve as an ‘autologous rescue system’ in order to attenuate innate and adaptive immune responses in organs exposed to environmental and autologous antigenic triggers. These results identify the primary sources and immunological prerequisites for the enhancement of sST2 secretion into the human circulation.

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