Selenium controls the sex-specific immune response and selenoprotein expression during the acute-phase response in mice

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Selenium modifies inflammatory reactions in rodents and humans. The liver controls metabolism and transport of selenium via heptatically-derived SEPP (selenoprotein P). Intracellular SEPS (selenoprotein S) modifies endoplasmic-reticulum function and immune-cell activity. Polymorphisms in SEPS have been associated with cytokine levels and inflammatory diseases in a subset of clinical studies. In the present study, we hypothesized that sex and selenium represent decisive parameters controlling the immune response and regulation of SEPS expression in vivo. Male and female mice fed a selenium-poor diet were supplemented or not with selenium for 3 days and injected with saline or LPS (lipopolysaccharide) 24 h before analysis. Selenium supplementation mitigated the LPS-induced rise in circulating cytokines in male mice. Serum SepP and selenium concentrations decreased in response to LPS, whereas hepatic SepS was specifically up-regulated despite declining selenium concentrations in the liver. Hepatic SepS induction was mainly controlled by post-transcriptional mechanisms and attributed to hepatocytes by analysing transgenic mice. Notably, selenium supplementation was essential for an optimal SepS induction. We conclude that selenoprotein biosynthesis becomes redirected in hepatocytes during the acute-phase response at the expense of dispensable selenoproteins (e.g. SepP) and in favour of SepS expression, thereby causing declining serum selenium and improving liver function. The selenium status and sex control SepS expression and modify cytokine response patterns in serum, which might explain contradictory results on associations of SEPS genotype and inflammatory diseases in clinical studies.

Key words: lipopolysaccharide (LPS), critical illness, selenium (Se), sexual dimorphism, translation.

INTRODUCTION

Se is an essential trace element needed for the biosynthesis of a small number of mammalian selenoproteins [1]. Se intake and personal Se status are implicated in widespread human pathologies including cancer, cardiovascular disease and neurodegeneration [2]. Recent research has specifically highlighted the importance of Se status for the activity of the immune system [3–5]. Positive effects of Se supplementation have been observed in a number of clinical trials with patients suffering from sepsis [6], HIV infection [7] or autoimmune thyroid disease [8]. The effects of Se are mediated by selenoproteins, a notion that is supported by association studies linking specific selenoprotein genotypes to human diseases [9,10]. To assess an individual’s Se status, GPX (glutathione peroxidase) activity in blood cells (GPX1) or plasma (GPX3), and total Se or circulating SEPP (selenoprotein P) concentrations are measured [11]. SEPP is derived mainly from the liver and seems to represent the best Se marker, accounting for the majority of circulating Se in blood and responding over a broad range to Se intake [12]. Serum Se is an established negative acute-phase reactant, and declining serum Se levels correlate to low circulating SEPP levels in sepsis [13,14]. Most importantly, the lower the Se status during critical illness the more likely that the patients will not survive [6,15–17]. Some, but not all, clinical trials have proven effective in improving the outcome of critically ill patients by Se supplementation, but the best application regimen, most suitable Se compound and the mechanisms of action are a matter of discussion [18–20]. One possible molecular component linking Se status with the immune system was recently identified when single nucleotide polymorphisms in the human gene encoding SEPS (selenoprotein S) were correlated to serum concentrations of pro-inflammatory cytokines, e.g. IL-6 (interleukin-6), IL-1β and TNFα (tumour necrosis factor α) [21]. SEPS encodes an essential factor for retrotranslocation of misfolded proteins from the ER (endoplasmic reticulum) into the cytosol for proteasomal degradation (ERAD; ER-associated protein degradation) [22,23]. Hereby SEPS is connected to a number of physiological pathways that modulate inflammation, e.g. NF-κB (nuclear factor κB) and JNK (c-Jun N-terminal kinase) activation and induction of acute-phase proteins [24]. Insufficient Se supply might cause impaired SEPS expression leading to reduced ERAD activity and cell dysfunction upon ER stress. Accordingly, pro-inflammatory cytokines, glucose deprivation or chemically induced ER stress induce SEPS gene expression in vitro [25–27]. A molecular circuit can be envisaged in which inflammatory stimuli induce SEPS biosynthesis as a positive acute-phase reactant to ensure efficient ERAD activity, stress relaxation and prevention of apoptosis [26]. We wondered about hepatic SEPS regulation in vivo during the acute-phase response since the hepatic Se status and hepatic enzymes involved in selenoprotein biosynthesis are reduced under these conditions [28]. Moreover, we wanted to test the suitability of a short-term Se supplementation strategy as a preventive measure to positively affect the cytokine response pattern and increase selenoprotein expression in order to prepare an individual for an impending infection risk, potentially causing

Abbreviations used: ER, endoplasmic reticulum; ERAD, ER-associated protein degradation; GPX, glutathione peroxidase; IL, interleukin; LPS, lipopolysaccharide; MCP1, monocyte chemoattractant protein 1; SEPP, selenoprotein P; SEPS, selenoprotein S; TNFα, tumour necrosis factor α; Tnfrsd11b, thioredoxin reductase; TXRF, total-reflection X-ray fluorescence analysis.

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septicaemia. This scenario can be envisaged for a regular patient in the clinic facing, for example, a surgical intervention [29]. To this end, we have analysed the LPS (lipopolysaccharide)-based murine model of septic shock and studied both male and female mice to determine whether the regulation of selenoprotein expression differs between the sexes [30]. Our findings indicate mitigating effects of Se supplementation on the LPS-dependent rise of certain cytokines in blood, especially in male mice. This response is paralleled by specific changes in selenoprotein expression in hepatocytes in favour of improved ER function during the acute phase and at the expense of SepP production. We conclude that availability of Se is of prime importance for hepatic SepS expression in vivo. This connection might help to explain some of the discrepant findings on the given or missing associations of SEPS genotype with inflammatory diseases in recent clinical studies, for these analyses failed to correct for the actual Se status of the probands.

EXPERIMENTAL

Materials

All chemicals were of analytical grade and obtained from Sigma–Aldrich. Oligonucleotides were from Invitrogen, restriction endonucleases were from New England Biolabs and Taq polymerase was from Qiagen.

Animal experimentation

Wild-type C57BL/6j mice were obtained from Charles River and were bred on regular lab chow (Altromin). A time-course analysis of selenoprotein expression after a single injection of LPS was conducted as described previously [28]. In addition, transgenic mice on a C57BL/6 background carrying a removable gene for the selenocysteine-specific tRNA (floxed Trsp) [31] were generously given by Delph Hatfield (Section on the Molecular Biology of Selenium, Basic Research Laboratory, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, U. S. A.) and were bred, genotyped and studied as described in [32]. In these mice, selenoprotein expression can become specifically impaired in hepatocytes by crossing these mice with a strain expressing Cre recombinase under the control of an albumin promoter (Alb-Cre) [33]. In a third experiment, groups of male and female mice (n = 6 per group) were transferred at the age of 45 days (P45) to an Se-poor diet (C1045; Altromin) containing 0.1 p.p.m. Se on average. Then, the Alb-Cre mice received 0.1 p.p.m. Se during the entire experiment [34]. Our findings indicate mitigating effects of Se supplementation on the LPS-dependent rise of certain cytokines in blood, especially in male mice. This response is paralleled by specific changes in selenoprotein expression in hepatocytes in favour of improved ER function during the acute phase and at the expense of SepP production. We conclude that availability of Se is of prime importance for hepatic SepS expression in vivo. This connection might help to explain some of the discrepant findings on the given or missing associations of SEPS genotype with inflammatory diseases in recent clinical studies, for these analyses failed to correct for the actual Se status of the probands.

Enzyme assays

Hepatic Gpx and TxnRd (thioredoxin reductase) activities were determined as described in [30,34]. Briefly, livers were homogenized on ice in 250 mM sucrose, 20 mM Hepes, and 1 mM EDTA, pH 7.4, and cleared by centrifugation. Protein concentrations were determined in the supernatants by a modified Bradford assay using IgG as a standard (Bio-Rad Laboratories). NADPH consumption by GSH reductase was recorded at 340 nm as a measure of oxidized GSH formation in the Gpx reaction with t-butylhydroperoxide as substrate [30]. TxnRd activity was determined by monitoring the NADPH-dependent reduction of DTNB [5,5′-dithiobis-(2-nitrobenzoic acid)], determined as the increase in A412 as described in [34].

Determination of circulating cytokines

Aliquots of serum (10 μl/sample) were analysed by a multiplex analyser system (Luminex 200; Luminex Corporation) in combination with LINCOplex™ cytokine immunoassays (Linco Research) as described in [30]. The tests for murine Il-6, Mcp1 (monocyte chemoattractant protein 1) and TnFα were conducted according to the manufacturer’s instructions, and calibration curves were generated with seven commercial standard samples for each cytokine covering the concentration range 0.0–1000 pg/ml. The intra- and inter-assay precision of these cytometric bead assays are typically below 10% [35].

Expression of recombinant SepS

SepS coding sequence was amplified from a murine liver cDNA library (forward primer, AAAGCTTAGACGAGAGAGGACGCG; reverse primer, CTCGAGCGCAGATGATCCGGC) with proper restriction sites for inframe cloning into pcDNA3.1-V5-His-TOPO (Invitrogen). The correct sequence and tag position were verified by DNA sequencing. The resulting construct was transfected (or not) into human hepatoma HepG2 cells by calcium phosphate. Cells were harvested 48 h after transfection. The resulting cell lysates containing or without recombinant SepS were used as positive and negative controls in Western blot analyses respectively.

Western blot analyses

Serum samples were collected and protein homogenates were prepared from different tissues and separated by SDS/PAGE on 10% (SepP) or 15% (SepS) gels before being transferred on to a nitrocellulose membrane (Protran®; Schleicher & Schuell) as described in [28]. Membranes were stained by PonS (Ponceau S) in order to control complete transfer and equal loading of the lanes. Relative serum albumin concentrations were determined by densitometric quantification of the major band at 67 kDa. A polyclonal antiserum (α-SepS) was generated in rabbits by immunization with a synthetic SepS-specific peptide (Ac-GRSYKRNSGRPQEEDGPGC-amide, corresponding to residues 125–142 of murine SepS, Mus musculus histocompatibility H47 mRNA, GenBank® accession number NM_024439.3). Antibodies specific for murine SepP have been characterized previously and were used as described in [28]. V5-specific antibodies were purchased from Invitrogen and used according to the manufacturer’s instructions.

Se determination by TXRF (total-reflection X-ray fluorescence analysis)

Aliquots of tissue homogenates (100 μl) were digested with nitric acid (4 volumes, 65%) and spiked with an internal gallium standard (Sigma–Aldrich). Mouse serum samples (10 μl) were diluted with distilled water (40 μl) and spiked as above. Samples were applied to quartz glass carriers and left to dry. TXRF analyses were performed as recommended using a Picofox S2 instrument (Bruker AXS). The accuracy of Se determination was verified with a mouse serum pool and a specified human serum sample (Seronorm). The inter-assay coefficient of variation was
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Figure 1  Tissue-specific acute-phase regulation of SepS expression in vivo

(A) The SepS antiserum (α-SepS) yields strong signals in cells transfected with recombinant V5-tagged SepS (lanes 2–5), but not in control cells (lanes 1 and 6). Shown are the position of immunoactive SepS co-localized with V5 signals and the theoretical size of the recombinant protein verifying suitability of the antibodies for SepS analysis. (B) Time-course analysis of hepatic (hep.) SepS protein (upper panel) and mRNA (lower panel) concentrations after LPS injection. Male mice (n = 4 per group) received a single injection of saline (C) or LPS (100 μg/mouse) and were killed after 6 h (all controls) or after the indicated periods of time. Samples were analysed by two Western and two Northern blots, of which a representative result is shown. Liver protein extracts from the individual mice were analysed by Western blotting (upper panel) and SepS amounts were quantified by densitometry. Ponceau S (PonS) staining served to control for efficient protein transfer on to the membrane and equal loading of the lanes. RNA (lower panel) was isolated from the same livers and used for Northern analysis of SepS and β-actin (β-act). *P < 0.05 and **P < 0.01 (one-way ANOVA with Dunnett’s post-hoc test). (C) Tissue specificity of LPS effects on SepS up-regulation. Protein extracts (n = 3) from brain, spleen and testes of the experimental mice in (B) were analysed by Western blotting at different time intervals after injection of LPS. Recombinant SepS served as a positive control (+). No significant effects of LPS on SepS protein concentrations were observed in these tissues. (D) Effects of LPS on SepS expression in wild-type (wt) and transgenic (KO) mice. Protein extracts from livers of treated (LPS) and control (C) mice were analysed by Western blotting. Recombinant SepS protein served as a positive control (+). Transgenic mice (n = 5) with albumin-promoter-driven Cre expression causing genetic deletion of the Sec-specific tRNA in hepatocytes express little SepS compared with wt (n = 5) animals (wt C compared with KO C). SepS expression is not stimulated by LPS in livers of KO mice (n = 3) compared with saline-injected KO controls (n = 2), indicating that hepatocytes account for increased SepS expression in liver of wt mice during an acute-phase response in vivo. **P < 0.01 (one-way ANOVA with Bonferroni’s post-hoc test). β-act, β-actin; n.s., not significant; rel., relative.

below 10 % for samples containing Se concentrations greater than 100 μg/l.

Statistical analysis

Results are expressed as means ± S.E.M. Comparisons of two groups were done with Student’s t test. Differences between treatment groups were analysed by one-way ANOVA and Dunnett’s or Bonferroni post-hoc tests for significance, as indicated in the Figure legends, using Graphpad Prism v. 4.0 software (Graphpad). Statistical significance was defined as P < 0.05 (*), P < 0.01 (**) or P < 0.001 (***)..

RESULTS

Liver-specific induction of SepS during the acute-phase response in vivo

SepS has been shown to be dynamically regulated by divergent stressors in vitro [25–27]. Therefore we aimed to test whether SepS expression also changes during the acute-phase response in vivo. To this end, a SepS-specific antiserum (α-SepS) was raised in rabbits and tested in Western blot analyses. A single band of approx. 27 kDa was detected from cells transfected with a recombinant tagged SepS but not in control cells (Figure 1A). The identity of the band was verified by antibodies directed against the V5 tag of the recombinant SepS protein. In vivo, a single injection of LPS induced SepS expression in liver, mainly at the protein level (Figure 1B). Relative transcript concentrations of SepS were determined by densitometric analysis of the Northern blots and remained constant in relation to β-actin signals during the study period of 24 h after LPS injection (Figure 1B). In comparison with liver, no effects on SepS protein concentrations were observed in brain, spleen or testes of the same mice over the time period analysed (Figure 1C). In order to identify the cell type responsible for the up-regulation of SepS protein levels in liver, a transgenic mouse line with hepatocyte-specific genetic inactivation of selenoprotein expression was studied [36]. LPS had no effects on SepS protein concentrations...
in the livers of these mice (Figure 1D). These results indicate that hepatocytes are responsible for the LPS-dependent induction of SepS in liver during the acute-phase response.

Se-dependent sex-specific modulation of the immune response

Se has been shown to modulate the LPS-induced oxidative stress response in murine macrophages in vitro [37]. Moreover, upon influenza virus infection, mice with altered selenoprotein expression display a different cytokine response compared with controls [4]. Yet the selenoproteins are sex-specifically expressed [30,38]. We thus hypothesized that both sex and Se are important parameters for selenoprotein regulation and immune response. Therefore we conducted a short-term Se supplementation experiment to specifically elucidate the importance of Se availability for the cytokine response and hepatic SepS expression. To this end, male and female mice were raised on an Se-poor diet during the 3 weeks before challenge to develop a marginal Se status. Then, groups of animals (n = 6 per sex) were supplemented or not with selenite in drinking water (100 μM) for 2 days prior to NaCl (control) or LPS injection (100 μg/animal, intraperitoneal) yielding four experimental groups (far right-hand side). Acute-phase responses of cytokines, Se and selenoproteins were studied 24 h after injection of NaCl or LPS.

The decreasing serum Se and SepP concentrations. Accordingly, SepS protein levels increased selenocysteine-loaded tRNA in order to support the biosynthesis of selenoproteins. In general, ample Se as a substrate is needed in the form of Se-poor conditions, but not under Se-supplemented conditions (results not shown) as studied previously in detail [28]. LPS-dependent down-regulation of serum Se and SepP concentrations were especially pronounced in the supplemented mice with higher Se status. These results verify that serum Se and SepP represent robust negative acute-phase reactants. Hepatic Se concentrations were similarly strongly affected by the LPS stimulus in both sexes (Figure 4C). In comparison, LPS affected hepatic Gpx activity in Se-supplemented male mice only (Figure 4D). TxnRd activities in liver were not significantly affected by LPS under these conditions (Figure 4E). The activity of kidney-derived Gpx3 remained largely unaltered by LPS in the Se-supplemented or Se-poor male or female mice (Figure 4F).

Importance of sex and Se for SepS expression and LPS-dependent maximal induction

In general, ample Se as a substrate is needed in the form of selenocysteine-loaded tRNA in order to support the biosynthesis of selenoproteins. Accordingly, SepS protein levels increased significantly in Se-supplemented mice compared with Se-poor animals (Figure 5). In contrast with the declining hepatic Se and circulating SepP concentrations [28], LPS injection increased SepS expression in liver in both male and female animals (Figures 1B and 1D, and Figure 5). Notably, a robust induction was observed in the Se-poor animals, indicating that certain mechanisms within the hepatocytes can support SepS expression, even against overall low Se status, in a prioritized manner. In male mice, SepS expression was strongly induced by LPS under Se-poor conditions, but not under Se-supplemented conditions when SepS apparently already had reached peak concentrations (Figure 5). In contrast, maximal SepS expression in females was...
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Figure 3 Inflammatory cytokines in control and Se-supplemented mice after LPS injection

Serum samples from control and experimental female (left-hand panels) and male (right-hand panels) mice (n = 6 per group) were analysed for circulating concentrations of Il-6, Mcp1 and TNFα (Tnf-α). LPS injection increased serum cytokine concentrations in mice independent of sex. Supplementation of Se mitigated the profound immune response in male, but not in female, mice, which was especially pronounced in the case of Il-6 and Mcp1. *P < 0.05 and **P < 0.01 (Student’s t test). n.s., not significant.

DISCUSSION

The acute-phase response in sepsis is a life-threatening process aiming to reprogramme the organism’s metabolism for defence against invading pathogens. Under such conditions, serum iron, for example, becomes strongly reduced as a physiologically relevant reaction to deprive the proliferating microbes from one of their essential trace elements [41]. Likewise, Se in serum is known to decline as a negative acute-phase reactant [39,42]. The physiological purpose for this phenomenon is currently unknown, but the absence from the circulation might again deprive the pathogens of an essential component [43]. Alternatively, it might improve the ability of immune cells to fight invaders by, for example, oxidative burst processes whose effects would otherwise be dampened by selenoproteins [44]. These theories still need experimental proof, but are in line with the reduced serum Se and SEPP concentrations under inflammatory conditions in humans [6,13–16] and declining serum Se and SepP levels in experimental rodents [28,39]. But what processes control the negative acute-phase responses of serum Se and SEPP? Comparing LPS-dependent SepP decline in the serum of Se-supplemented and Se-poor mice (Figure 4B), it becomes obvious that a loss of SepP into other tissues or an extensive binding on to the surface of the blood vessels is unlikely, otherwise a stronger and more uniform decline would have been observed, especially in the male and female animals with low Se status. Since SepP expression depends on the Se status, the declining Se concentrations in liver (Figure 4C) argue more in favour of a reduced hepatic SepP biosynthesis rate during the acute-phase response. This notion is in line with the down-regulation of important factors involved in hepatic SepP biosynthesis under inflammatory conditions [28] and the rapid and specific degradation of another hepatic selenoprotein, Sep15, during acute ER stress [45]. These findings imply that the reducing Se concentrations in serum rather represent a side-effect secondary to an altered metabolic program in liver. This interpretation is corroborated by the improved survival odds of sepsis patients with high Se status and with the positive effects of Se supplementation in critically ill patients [6,15].
Figure 4  Biomarkers of Se status after Se supplementation and LPS injection

(A) Selenite supplementation increased serum Se concentrations in male and female mice (n = 6 per group). LPS injection reduced serum Se strongly in the Se-supplemented mice of both sexes, whereas the effect was not significant in Se-poor animals. (B) Serum levels of SepP increased after Se supplementation as determined by Western blot analysis with antibodies against murine SepP (α-SepP). Upper panel: Ponceau S (PonS) staining verifies constant loading of the serum samples and comparable serum protein amounts in the lanes. The results of Western blotting of pooled serum samples are shown. Lower panel: quantification of the effects was based on additional Western blot analyses with individual samples (n = 6 per group). A marginal, but significant, effect of LPS was observed on albumin concentrations in the serum of male mice (*P < 0.05; Student's t test). Albumin concentrations in female mice were not affected. LPS caused a significant decline in circulating SepP, especially in the Se-supplemented male and female mice (**) P < 0.01; Student's t test). (C) Se concentrations in livers mirror changes in circulating SepP upon selenite and LPS treatment. Se supplementation increased hepatic Se concentrations in all groups of mice. LPS injection caused a strong decline in hepatic Se concentrations in the Se-supplemented male and female mice, but not in Se-poor animals. (D) Hepatic Gpx activity increased upon Se supplementation in both female and male mice and declined in response to LPS in male mice only. (E) Hepatic TxnRd activity was similar in male and female mice and remained unaffected by short-term selenite supplementation or LPS treatment in these experiments. (F) Gpx3 activity was determined from the serum samples. Only male mice displayed significantly increased Gpx3 activities in response to the short-term oral Se supplementation. LPS had no consistent effect on circulating Gpx3 under these experimental conditions in male or female mice. *P < 0.05, **P < 0.01 and ***P < 0.001 (one-way ANOVA with Bonferroni's post-hoc test). hep., hepatic; rel. relative; n.s., not significant.

In this light, the concurrently rising SepS concentration in liver as a positive acute-phase reactant might offer a causal explanation on the fate of the missing serum Se, especially in Se-poor organisms. Of note, the effects of LPS on the biosynthesis of SepP and SepS are elicited largely independently of their transcript concentrations. We have shown previously that LPS impairs expression of central components of the selenoprotein biosynthesis machinery and reduces hepatic SepP production without affecting SepP mRNA concentrations [28]. In the present study, we report a similarly strong effect on SepS expression in liver, albeit in the opposite direction, i.e. increased protein levels again without respective LPS effects on SepS transcript concentrations (Figure 1B). Moreover, using the transgenic mice with cell-type specific impaired selenoprotein biosynthesis, both effects proceed simultaneously in the same cells, i.e. in hepatocytes. Obviously, hepatocytes shuttle their available Se into SepS and away from the biosynthesis of other less vital selenoproteins during the acute-phase response. Such
Figure 5  Sex-specific regulation of hepatic SepS in response to selenite and LPS treatment

LPS injection induced maximal SepS concentrations in Se-supplemented mice, indicating the importance of the Se status for SepS expression. A Western blot of pooled homogenates from the livers of the experimental mice is depicted (upper panel) in order to provide an overview. SepS protein concentrations were determined from two additional Western blot analyses from samples of individual mice that are shown (bottom panel) together with the quantification (histogram). Characteristic sex-specific differences were observed with respect to both basal SepS expression levels and responsiveness to Se and LPS. *P < 0.05, **P < 0.01 and ***P < 0.001 (Student’s t test). rel., relative.

a post-translational regulatory circuit nicely complements the diverse molecular pathways known that finally translate ER stress into the inflammatory response, i.e. rearranging the protein biosynthesis pattern via eIF2α (eukaryotic initiation factor 2α) phosphorylation, translational frameshifting and activation of XBP1 (X-box-binding protein 1) and proteolytic generation of nuclear ATF6 p50 (the active form of activating transcription factor 6) [24]. In contrast to these effects, which relay a stress signal from ER into the cytoplasm and nucleus, increased biosynthesis of SepS directly improves the functioning of the critical cellular compartment, i.e. ER, via its important role during the removal of misfolded proteins during ERAD [25,27,46]. It is the nature of SepS as a selenoprotein to depend on a sufficiently high Se status for increased biosynthesis. Apparently, the redirection of Se away from other selenoproteins towards SepS expression fulfils this demand both under adverse circumstances, as is the case in a poorly supplied individual, and during the acute-phase response. The concurrent decline of serum Se might represent the price for the prioritized hepatic SepS biosynthesis under inflammatory conditions, i.e. a side-effect.

However, an efficient selenoprotein biosynthesis usually profits from increased Se supply. Such support can be provided by a respectively tailored immunonutrition programme [47]. The positive effects of Se supplementation in the clinic imply that a readily available Se source, e.g. sodium selenite, improves acute-phase response and outcome [6]. Although the best regimen and selenocompound in terms of application route and dosage is a matter of discussion, a low Se status during septicaemia has uniformly been proven as a negative prognostic marker for survival odds [48,49]. Therefore we have attempted a short-term preventive Se-supplementation effort aiming to improve Se status and selenoprotein biosynthesis rapidly via oral application in our experiments. The results clearly indicate that this effort was successful and, in particular, the cell-type-specific up-regulation of SepS in hepatocytes relied stringently on the Se status. Both male and female mice took advantage of an increased Se supply in order to maximize SepS expression albeit with sexual dimorphic dynamics. In response to supplementation, maximal SepS levels were already observed under basal conditions in male, but not in female, mice. Likewise, concentrations of circulating cytokines were mitigated by Se, especially in male mice.

Even though these mechanistic insights have been deduced from rodent models and not human patients, the interrelation of Se status and SepS expression might help to explain divergent findings in genetic association studies of SEPS genotype and inflammatory diseases. In this respect, strong associations were reported between the well-characterized −105 SEPS promoter polymorphism [21] and gastric cancer susceptibility [50], coronary heart disease and stroke [51] and pre-eclampsia [52]. Together with the initial characterization of the interrelation of SEPS and inflammatory cytokines [21], a general effect of SEPS genotype on inflammatory diseases had been assumed. Therefore a lack of SEPS genotype–disease association, as was recently reported for type 1 diabetes, rheumatoid arthritis and inflammatory bowel disease (ulcerative colitis) [53] was surprising. The generalized conclusion might be misleading since the results have not been corrected for by sex and the actual Se status of the probands. Our results highlight the importance...
of these parameters for the immune response, cytokine patterns and SEPS induction. Congruent results on the interaction of genotype, sex and Se status were recently reported for SEPP isoform expression in human cancer [54]. Since the observed inflammatory effects on SEPP and SEPS biosynthesis are mainly exerted at the post-transcriptional level, the Se status and the concentration of selenocysteine-loaded tRNA are decisive parameters for the disease-specific response. According to our results, it seems advisable to determine the Se status along with the SEPS genotype in future studies to be better able to compare the genotype–disease association in men and women and gain knowledge on the potential benefit of a personally tailored Se supplementation strategy in a specific pathology. The preventive measure proposed in the present study to increase the Se status quickly via an orally available supplement might prove useful in the clinic to ensure an optimal physiological ER stress response by high SEPS expression prior to a potential challenge. At the same time, the unfavourable low circulating Se concentrations can be avoided. Respective clinical studies taking the Se status and sex into account appear necessary and are highly recommended.

AUTHOR CONTRIBUTION
Mette Stoedter, Kostja Renko and Lutz Schomburg designed the experiments, performed the analyses and interpreted the data. Antonia Hög contributed to the analyses and data interpretation. All authors wrote and approved the final manuscript.

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