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Research Article

Microsomal glutathione S-transferases: selective up-regulation of leukotriene C_4 synthase during lipopolysaccharide-induced pyresis

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Abstract. Cysteinyl-leukotrienes (cys-LTs) are potent smooth muscle contracting agents, which play key roles in inflammatory and allergic diseases. The committed step in cys-LT biosynthesis is catalyzed by leukotriene C_4 synthase (LTC4S) as well as microsomal glutathione S-transferase type 2 (MGST2) and type 3 (MGST3). Here we report that intraperitoneal injections of lipopolysaccharide in rats lead to a strong increase of LTC4S messenger RNA (mRNA) levels after approximately 1 h, particularly in the heart, brain, adrenal glands and liver, without any significant effect on MGST2 and MGST3 mRNA levels. After 6 h, LTC4S mRNA returns to basal levels, concomitant with a 4.9-, 4.0-, 2.9- and 2.3-fold induction of LTC4S protein in brain, heart, liver and adrenal gland, respectively. Hence, challenge with lipopolysaccharide in vivo causes an organ-selective, local priming for leukotriene C_4 synthesis. Moreover, these data suggest that LTC4S and cys-LTs may be involved in acute systemic inflammatory responses such as fever and tachycardia.

Key words. Leukotrienes; LTC4 synthase; GSH S-transferase; inflammation; fever.

Introduction

Leukotrienes (LTs) C_4, D_4 and E_4, collectively known as cysteinyl-leukotrienes, are powerful lipid mediators of allergy and anaphylaxis. Typically, these compounds elicit smooth muscle contractions at only nanomolar concentrations, particularly in the respiratory tract and microcirculation [1, 2], and specific drugs targeted against cys-LTs were recently marketed as a new asthma medication [3]. Although cys-LTs are mainly regarded as local spasmogenic mediators, Robbiani et al. recently discovered that cys-LTs are involved in the primary immune response orchestrated by T cells and dendritic cells, indicating a more global role of these substances in inflammation and immune regulation [4].

In cellular biosynthesis of cys-LTs, arachidonic acid is converted by 5-lipoxygenase (5-LO), assisted by 5-lipoxygenase activating protein (FLAP), into the unstable epoxide LTA_4 [5]. This intermediate may either be hydrolyzed by a soluble LTA_4 hydrolase into the chemotactic agent LTB_4 [6], or conjugated with GSH to produce LTC_4 in a reaction primarily catalyzed by LTC_4 synthase (LTC4S; EC 2.5.1.37), i.e., the committed step in cys-LT formation [7, 8]. In addition, several cytosolic and microsomal GSH transferases (EC 2.5.1.18), in particular MGST2 and MGST3, may catalyze this reaction [9–12]. Biological effects of cys-LTs are signaled via two types of G-protein-coupled receptors, denoted $CysLT_1R$ and $CysLT_2R$, respectively [13–17].

To date, little is known regarding the in vivo regulation of LTC4S, MGST2 and MGST3, which all belong to a widespread protein superfamily designated MAPEG (mem-

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brane-associated proteins in eicosanoid and glutathione metabolism) [18]. Using immunohistochemistry, it has been reported that cells expressing LTC4S are over-represented in bronchial biopsy specimens from patients suffering from aspirin-intolerant asthma [19]. This over-expression was suggested to be genetically determined at the level of gene promoter polymorphism, but opposite conclusions have also been presented [20, 21]. Studies on isolated cells in vitro have demonstrated that LTC4S expression is regulated by different cytokines, in particular temporal combinations of interleukin (IL)-3, IL-4 and IL-5 [22], as well as transforming growth factor-β (TGF-β) [23]. At the molecular level, recent work by Zhao et al. has shown that cell-specific transcription of the LTC4S gene appears to be regulated by a Kruppel-like transcription factor and Sp1 [24].

Recently, we cloned and functionally expressed rat LTC4S, MGST2 and MGST3, allowing side-by-side comparative investigations of their biochemical properties and expression pattern in vivo [25]. Here we report that intraperitoneal injections of lipopolysaccharide (LPS) in rats lead to a rapid, transient and tissue-selective increase of LTC4S messenger RNA (mRNA), whereas MGST2 and MGST3 mRNA levels are not significantly affected. The change in LTC4S mRNA is followed by a subsequent increase in LTC4S protein. This up-regulation of LTC4S, particularly in the brain, heart and adrenal glands, suggests that LTC4S and cys-LTs may be involved in the local signaling of acute systemic inflammatory responses such as fever, tachycardia, fatigue and general malaise.

Materials and methods

Materials

All chemicals were of analytical grade and, unless stated otherwise, obtained from Sigma. LTA_4 was saponified in acetone with 50 mM NaOH (20% v/v) for 1 h at room temperature, or in tetrahydrofuran with 1 M LiOH (6% v/v) for 48 h at 4 °C. Oligonucleotides were purchased from Cybergene AB (Huddinge, Sweden).

LPS challenge of rats and organ preparations

All procedures used in the in vivo assays were in accordance with the guidelines established by the Animal Care and Use Committee of Karolinska Institutet. Male Sprague-Dawley rats (300 g each) were injected with a single intraperitoneal dose of 2 mg/kg LPS (from *Escherichia coli* serotype O26:B26; 30,000 endotoxin units per milligram). This dose causes a significant elevation of body temperature of rats (from 36.6 ± 0.3 °C to 38.3 ± 0.2 °C) and elicits a mild inflammatory response, which can be observed as a transient generalized piloerection and reduced mobility of the animals. Control animals were injected with saline vehicle alone. One and 6 h after injection, LPS-treated and control rats were sacrificed, and tissues were carefully perfused with saline and dissected. Isolated organs and tissues were used fresh or snap frozen in liquid nitrogen and stored at –80 °C until further preparation. Two independent experiments were performed twice with several months in between each set. The four experiments yielded similar results. For statistical analyses, data from two experiments were used.

Analysis of LTC4S, MGST2 and MGST3 mRNA by semi-quantitative RT-PCR

Reverse-transcription polymerase chain reaction (RT-PCR) was conducted with the Gene Amp RNA PCR kit (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol, starting from 1 µg total RNA. Amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as control. From the results of preliminary experiments, 24 PCR cycles for GAPDH, 35 cycles for LTC4S, and 25 cycles for MGST2 and MGST3 were selected as optimal amplification conditions to produce a log-linear relationship between the amount of each mRNA and intensity of PCR product. The PCR reaction contained 0.2 mM dNTP and either 1 µM primers and 0.75 U cloned *Pfu* polymerase for LTC4S, 0.5 µM primers and 1.5 U of *Taq* DNA polymerase for MGST2 and MGST3, or 0.1 µM primers and 1.5 U of *Taq* DNA polymerase for GAPDH. The conditions of the reaction were initial denaturation at 96 °C for 30 s followed by annealing at 55 °C for 1 min, and extension at 72 °C for 90 s; repetitive cycles of 96 °C for 5 s, 50 °C for 30 s, and 72 °C for 60 s; and a final extension at 72 °C for 7 min. For LTC4S the respective annealing temperatures were 50 and 45 °C. Primers for amplification were as follows: LTC4S sense 5′-GAAGAACTTTCCACGTGTCG-3′, LTC4S antisense 5′-GTGCAGCCATTGCCACTAGC-3; MGST2 sense 5′-TTCAATCAAGTTTTTGCAACC-3′, MGST2 antisense 5′-TCTTGGCAACATGAAAGTCC′-3; MGST3 sense 5′-TACCCTCCCTTCCTATTCTTCC-3′, MGST3 antisense 5′-AAAGCAGAGCACACAGTTG-TGC′-3′, GAPDH sense 5′-CCAGTATGATTCTACCC-ACG-3′, GAPDH antisense 5′-CCACCACCCTGTTGC-TGTAG-3′. The expected sizes of amplified LTC4S, MGST2 and MGST3 DNA fragments were 282, 204 and 200 base pairs (bp), respectively, and each of the corresponding PCR products was sequenced to verify its identity. Aliquots of the PCR mixtures (10 µl) were analyzed by electrophoresis using a 1% agarose gel containing 0.5 µg/ml ethidium bromide.

For semi-quantitative analysis of amplified PCR products the fluorescent dye PicoGreen (Molecular Probes, Eugene, OR) was used according to the manufacturer's instructions [26, 27]. Two microliters of amplified DNA in 100 µl TE buffer were mixed with an equal volume of diluted PicoGreen reagent (1:200, v/v in TE buffer). Sam-

ples were incubated for 5 min at room temperature protected from light in a microtiter plate. The fluorescence was measured (λ_{ex} = 485 nm; λ_{em} = 538 nm) in a SpectraMax GeminiXS fluorometer from Molecular Devices (Sunnyvale, CA). The standard curve for the quantitative analysis was obtained with λ DNA standard in TE buffer and was linear from 1 to 128 ng/well.

Immunoblot analysis

Aliquots (75–100 µg) of membrane protein in loading buffer were separated by SDS-polyacrylamide gel electrophoresis (PAGE) through a 15% Tris-HCl, precasted, linear gradient gel (Bio-Rad, Sundbyberg, Sweden) and electroblotted onto poly(vinylidene difluoride) membranes (Pall, Dreieich, Germany). The transfer efficiency was visualized by using prestained molecular weight protein standards (Bio-Rad, Sundbyberg, Sweden). Membranes were then soaked for 1 h at 25 °C in Tris-buffered saline (100 mM Tris-HCl, pH 7.5, 150 mM NaCl) containing 0.1% (v/v) Tween 20 (0.1% TTBS) and 5% (w/v) nonfat dried milk. The membranes were subsequently washed (2 × 5 min) in 0.1% TTBS followed by an overnight incubation at 4 °C with a polyclonal antiserum raised against a peptide of human LTC4S (residues 37–51) or MGST2 (residues 42–55), used at dilutions 1:500 and 1:3,000, respectively. The reactivity and specificity of the human LTC4S and MGST2 antibodies against the respective rat protein were assessed by comparative immunoblot analysis of human and rat recombinant LTC4S or MGST2, respectively. After washing, the blot was incubated for 1 h at 25 °C with a horseradish peroxidase-linked donkey anti-rabbit antibody (1:3,000 dilution) in 0.1% TTBS and 2% (w/v) nonfat dried milk. The washing steps were repeated, and subsequently enhanced chemiluminescence detection was performed according to the manufacturer's instructions (ECL Plus, Amersham Pharmacia Biotech, Uppsala, Sweden). For certain tissues, SDS-PAGE immunoblots were quantitated using a luminescent image analyzer LAS-1000Pro version 2.0 with Image Gauge version 3.46 software (Fujifilm, Stockholm, Sweden).

Analysis of LTC_4 synthase activity

Homogenized brain tissue, obtained from control and LPS-treated (6 h) animals, was centrifuged at 100,000 × g for 60 min to prepare a membrane fraction. Aliquots (100 µl) of 100,000 × g pellets, dissolved in 5 v/w phosphate-buffered saline, pH 7.4, were then incubated with 25–30 µM of LTA_4, 5 mM GSH and 0.2% BSA for 5 min at RT. The reaction was terminated by the addition of 200 µl methanol containing a defined amount of internal standard, prostaglandin B_2 followed by centrifugation at 500 × g for 10 min at 4 °C to remove precipitated proteins. LTC_4 and other metabolites of LTA_4 were resolved by isocratic reverse-phase high-performance liquid chromatography (HPLC) on a Waters Associates Novapak C_{18} column (3.9 × 150 mm) eluted with a mixture of acetonitrile/methanol/water/acetic acid (29:19:52:0.1, v/v, pH adjusted to 5.6 with 10 M NaOH) at a flow rate of 1.0 ml/min. LTC_4 was quantified by measurements of peak area ratios between LTC_4 and prostaglandin B_2.

Statistical analysis

If not otherwise stated, data are expressed as means ± SE of four analyses from two independent experiments. One-way ANOVA (analysis of variance) and Student's *t* test were used for the statistical comparison of LTC4S mRNA levels in various tissues, with the same results. $P < 0.05$ was considered to be significant.

Results and discussion

The committed step in the production of cys-LTs, i.e., the conjugation of LTA_4 with GSH to form LTC_4, is primarily catalyzed by LTC4S [28–30]. In addition, several cytosolic and microsomal GSH transferases, in particular MGST2 and MGST3, may catalyze this reaction [9–12]. Indeed, LTC_4 synthase activity in human umbilical vein endothelial cells, previously attributed to LTC4S [31, 32], has now been shown to originate from abundant expression of MGST2 [33, 34]. LTC4S is predominantly expressed in certain myeloid cells such as monocytes/macrophages, eosinophils, basophils and mast cells, all of which possess the entire enzyme machinery required for LT biosynthesis, for review see [8]. MGST2 is found in, e.g., liver, kidney, endothelial cells, adrenal gland and small intestine but has also been detected in human mast cells [11, 33, 35]. MGST3, on the other hand, appears to be ubiquitously expressed [12].

Tissue distribution of LTC4S, MGST2, and MGST3 in the rat

Preliminary experiments revealed that Northern blot analysis was not sufficiently sensitive to detect mRNA expression of LTC4S in rat tissues. We therefore used RT-PCR followed by a semi-quantitative analysis of amplified PCR products by the fluorescent PicoGreen dye for the measurement of mRNA levels of LTC4S, MGST2 and MGST3 in rat tissues. With this technique we found that LTC4S mRNA is expressed at low levels in 5 out of 10 organs examined, with a rank order ileum > lung ≥ spleen > colon > skeletal muscle, and to a lesser extent in liver and kidney, whereas barely detectable mRNA signals were obtained in heart, brain and adrenal gland (fig. 1). This expression profile agrees well with data for the human enzyme [36]. Protein expression of LTC4S, as assessed by immunoblotting, was detectable in all tissues except the spleen and data for the heart, brain, liver and adrenal gland are shown in figure 4.

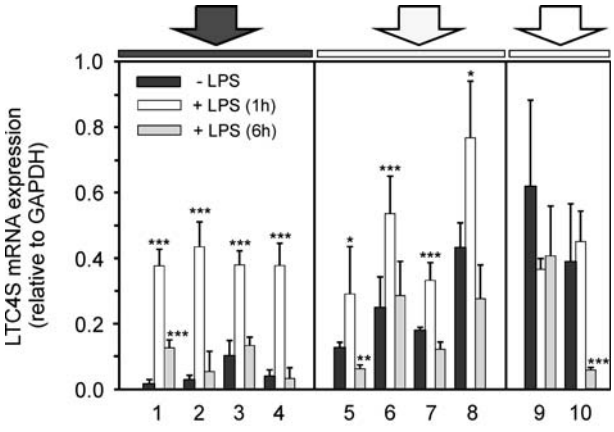

Figure 1. Effects of in vivo LPS challenge on the mRNA levels of rat LTC4S. Total RNA was prepared from tissues of control- or LPS-treated rats as described in the experimental procedures. RT-PCR was performed for LTC4S and GAPDH during the linear phase of amplification. The tissues are indicated by the following numbers: 1. Heart, 2. Brain, 3. Liver, 4. Adrenal Gland, 5. Kidney, 6. Colon, 7. Skeletal Muscle, 8. Lung, 9. Ileum, 10. Spleen. The histogram depicts a quantitative analysis of LTC4S mRNA in the respective tissues from animals without LPS treatment (filled bars), 1 h after LPS treatment (open bars), or 6 h after LPS treatment (shaded bars). All values for mRNA levels are normalized to the corresponding mRNA amount of the housekeeping gene GAPDH. The histogram is divided in three sections, which indicate tissues displaying strong LTC4S up-regulation (filled arrow), modest increase (grey arrow) and no detectable change or decrease (open arrow) of LTC4S mRNA during LPS challenge. Bars depict mean values ± SE of four analyses from two separate experiments. The statistical significance of changes relative the untreated controls is expressed: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.01$.

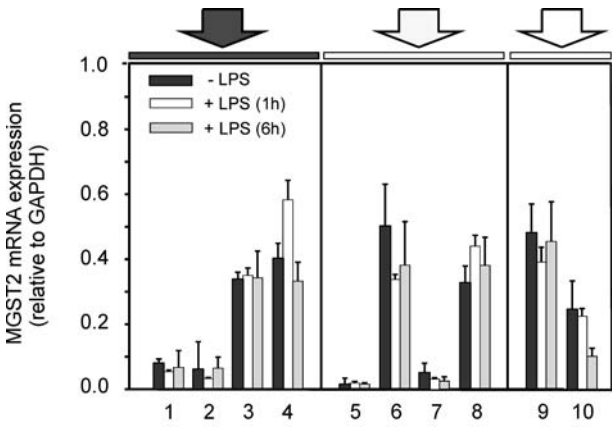

Figure 2. Effects of in vivo LPS challenge on the mRNA levels of rat MGST2. Total RNA was prepared from tissues of control- or LPS-treated rats and subjected to RT-PCR for MGST2 and GAPDH during the linear phase of amplification. The tissues are indicated by the following numbers: 1. Heart, 2. Brain, 3. Liver, 4. Adrenal Gland, 5. Kidney, 6. Colon, 7. Skeletal Muscle, 8. Lung, 9. Ileum, 10. Spleen. The histograms depict a quantitative analysis of MGST2 mRNA in the respective tissues from animals without LPS treatment (filled bars), 1 h after LPS treatment (open bars), or 6 h after LPS treatment (shaded bars). All values for mRNA levels are normalized to the corresponding mRNA amount of the housekeeping gene GAPDH. The panels are divided as in figure 1 to allow comparison between tissues displaying strong LTC4S up-regulation (filled arrow), modest increase (grey arrow) and no detectable change or decrease (open arrow) of LTC4S mRNA during LPS challenge. Bars depict mean values ± SE of four analyses from two separate experiments.

For MGST2, the expression pattern of mRNA is colon ≥ ileum > adrenal gland ≥ liver ≥ lung and some mRNA also in the spleen (fig. 2), which is also consistent with the human enzyme [11]. MGST2 protein, however, could only be detected in liver, ileum, colon, brain and adrenal gland but not in spleen and lung membranes, perhaps reflecting differences in post-transcriptional regulations (fig. 4 and data not shown). On the other hand, rat MGST3 mRNA was ubiquitously expressed with a rank order colon ≥ ileum > adrenal gland ≥ liver > heart = kidney ≥ spleen ≥ brain > lung ≥ skeletal muscle (fig. 3). With the exception of the liver, displaying very low mRNA levels in man but high in rat, expression of rat MGST3 mRNA matches that of the human enzyme [12]. At present, there is no anti-MGST3 antibody available, which prevents studies of the protein distribution.

Rat LTC4S, but not MGST2 or MGST3, is upregulated in a tissue-selective manner during LPS-induced systemic inflammation

To investigate the potential effects of an inflammatory stimulus in vivo on the expression of LTC4S, MGST2 and MGST3 in the rat, we used intraperitoneal injections of LPS, an established and extensively used animal model

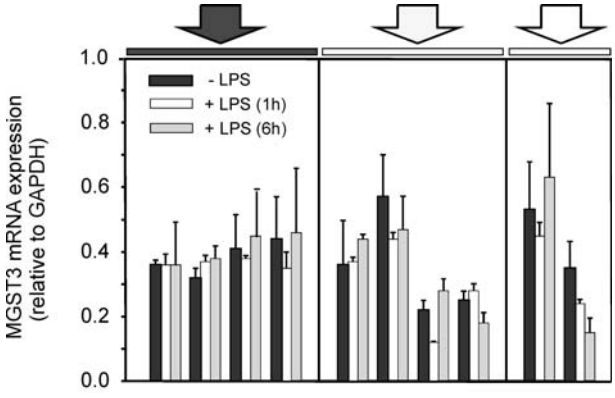

Figure 3. Effects of in vivo LPS challenge on the mRNA levels of rat MGST3. RT-PCR was performed on total RNA from tissues of control- or LPS-treated rats for MGST3 and GAPDH during the linear phase of amplification. The tissues are indicated by the following numbers: 1. Heart, 2. Brain, 3. Liver, 4. Adrenal Gland, 5. Kidney, 6. Colon, 7. Skeletal Muscle, 8. Lung, 9. Ileum, 10. Spleen. The histograms depict a quantitative analysis of MGST3 mRNAin the respective tissues from animals without LPS treatment (filled bars), 1 h after LPS treatment (open bars), or 6 h after LPS treatment (shaded bars). All values for mRNA levels are normalized to the corresponding mRNA amount of the housekeeping gene GAPDH. The panels are divided as in figure 1 to allow comparison between tissues displaying strong LTC4S up-regulation (filled arrow), modest increase (grey arrow) and no detectable change or decrease (open arrow) of LTC4S mRNA during LPS challenge. Bars depict mean values ± SE of four analyses from two separate experiments.

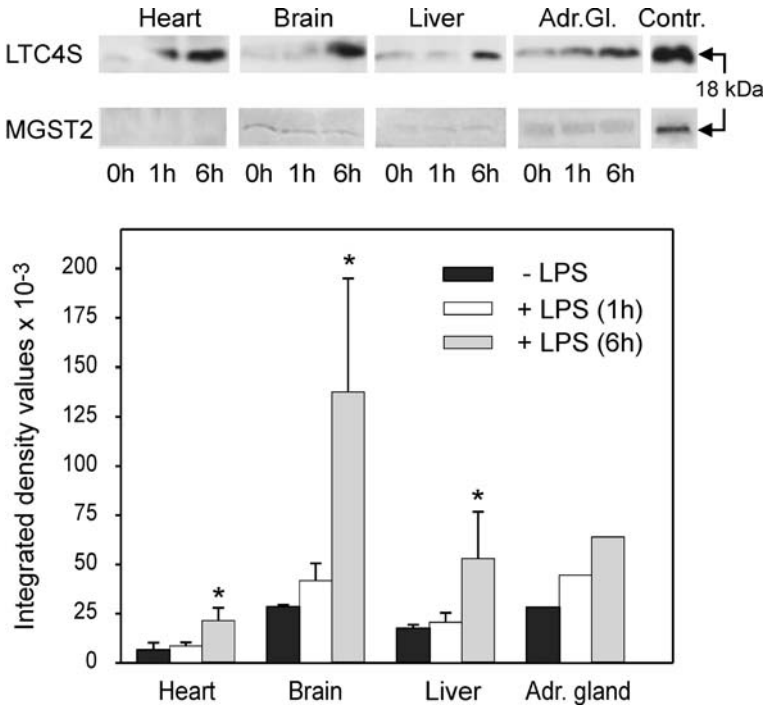

Figure 4. Induction of rat LTC4S protein after in vivo LPS challenge. 100 µg of protein was subjected to immunoblot analysis as described in the experimental procedures. The top panel shows two series of immunoreactive bands corresponding to LTC4S and MGST2. The bottom panel depicts a histogram obtained by densitometric analysis of several immunoblots, as described in the experimental procedures. Integrated density values of each blot were calculated for unstimulated and LPS-challenged tissues. Bars depict mean values ± SE of four determinations from two independent experiments (except adrenal gland, which is a single determination of pooled material from two experiments). Note that the three LTC4S immunoreactive bands for each organ come from a single immunoblot and cannot be directly compared with the histogram. The statistical significance of changes relative the untreated controls is expressed as $*P < 0.05$.

for general inflammation and fever. LPS activates cells of the innate immune system to produce proinflammatory cytokines, in particular tumor necrosis factor-α (TNF-α), IL-1β, and IL-6, which in turn elicit both a local inflammatory reaction at the site of administration as well as systemic responses such as pyresis, tachycardia and general fatigue. At high doses leading to endotoxemia, the host reaction may be lethal by generating disseminated intravascular coagulation and multi-organ failure resulting in a septic shock syndrome.

When rats were challenged with LPS (2 mg/kg, i.p.), LTC4S mRNA levels were greatly increased within 1 h in heart, brain, liver and adrenal gland (fig. 1). In addition, LPS treatment increased LTC4S mRNA expression 2–3-fold in all other tissues, except for ileum and spleen, in which the expression level remained largely unchanged or even decreased. 6 h after administration of LPS, LTC4S mRNA in the various tissues returned to levels comparable to those observed in the control rats. However, the time course for changes in mRNA levels differed in some experiments. Thus, in the heart, mRNA was still elevated, although at a lower level as compared to the value at 1 h (fig. 1). In two (out of four) experiments, a similar result was also noticed for brain and liver with mRNA levels after 6 h equal to, or above, those observed after 1 h, indicating that the peak in mRNA occurs at different time points, usually between 1 and 6 h (data not shown). These slight variations presumably reflect variable reactions to LPS and differences between animals. In contrast to the effects on LTC4S, the mRNA levels of MGST2 and MGST3 remained largely unchanged over 6 h post-LPS administration (figs 2 and 3). Furthermore, no effects on MGST2 protein expression could be detected (fig. 4 and data not shown), which in turn suggests that MGST2 is not up-regulated during systemic inflammatory reactions and fever. Thus, one may speculate that MGST2 does not significantly contribute to LTC_4 formation during host-defense reactions, but is perhaps rather involved in biosynthesis of LTC_4 for other, basal 'housekeeping' purposes.

Challenge with LPS resulted in a significant increase in LTC4S protein in heart, brain, adrenal gland and liver (fig. 2). A quantitative densitometric analysis revealed that LPS stimulation for 6 h increased the amounts of immunoreactive LTC4S protein 4.9-, 4.0-, 2.9- and 2.3-fold in brain, heart, liver and adrenal gland, respectively.

Considering the fact that all organs were carefully perfused, the time-lag between mRNA and protein synthesis (fig. 5), and the organ selectivity of the response, it seems likely that LPS challenge leads to up-regulation of LTC4S in resident

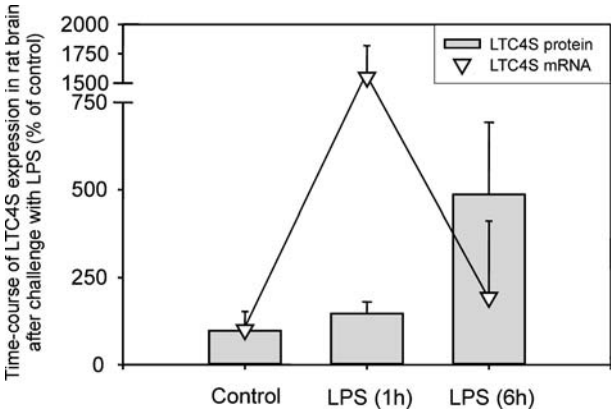

Figure 5. Time courses for the increases in LTC4S mRNA and protein in rat brain during LPS challenge. LTC4S mRNA and protein were analysed as described in the methods section and legend to figures 1–4. The mRNA levels peaked and returned to basal values within 1–6 h, whereas the increase in protein was not robust until 6 h.

cells of the tissues rather than blood-borne cells that have adhered to the vascular wall (cf. fig. 6). These cells may be resident mast cells, macrophages or even parenchymal cells, as indicated by the recent observation that LTC4S mRNA can be upregulated in rat hepatocytes and in the choroid plexus of the cerebral ventricles in mouse brain [37, 38]. Parenchymal cells are rarely, if ever, equipped with all enzymes required for LT biosynthesis, implying that the substrate LTA_4 must be delivered via transcellular routes, e.g., via neighboring activated leukocytes (fig. 6). Such cell-cell interactions have been demonstrated for transcellular biosynthesis of cys-LTs along a platelet-neutrophil or endothelial cell-neutrophil axis [31, 32, 39, 40] and was recently shown to occur in vivo [41].

We also tested whether the increase in LTC4S protein resulted in increased LTC_4 synthase activity in whole rat brain. Thus, we incubated aliquots (100 µl) of 100,000 × g pellets (dissolved in 5 v/w phosphate-buffered saline, pH 7.4) of homogenized brain tissue obtained from control and LPS treated (6 h) animals with 25–30 µM of LTA_4, 5 mM GSH and 0.2% BSA for 5 min at RT. Under these conditions, we observed an increased synthesis of LTC_4 in membrane preparations from the LPS-treated rats as compared to samples from control rats, i.e., 494 ± 65 pmol/ml (n = 5) versus 388 ± 97 pmol/ml (n = 4) which, however, did not reach statistical significance. The observed difference between the overall increase in LTC4S mRNA/protein, on the one hand, and the enzyme activity, on the other, is perhaps due to the fact that LTC4S activity is unstable and known to be affected by a number of factors [42]. Therefore, activity measurement may be unreliable to use as an index of low levels of enzyme expression in complex tissues. It is also possible that MGST2, which was not sensitive to LPS, accounts for some of the activity. Nonetheless, our enzyme activity data are consistent with the results of mRNA and protein measurements.

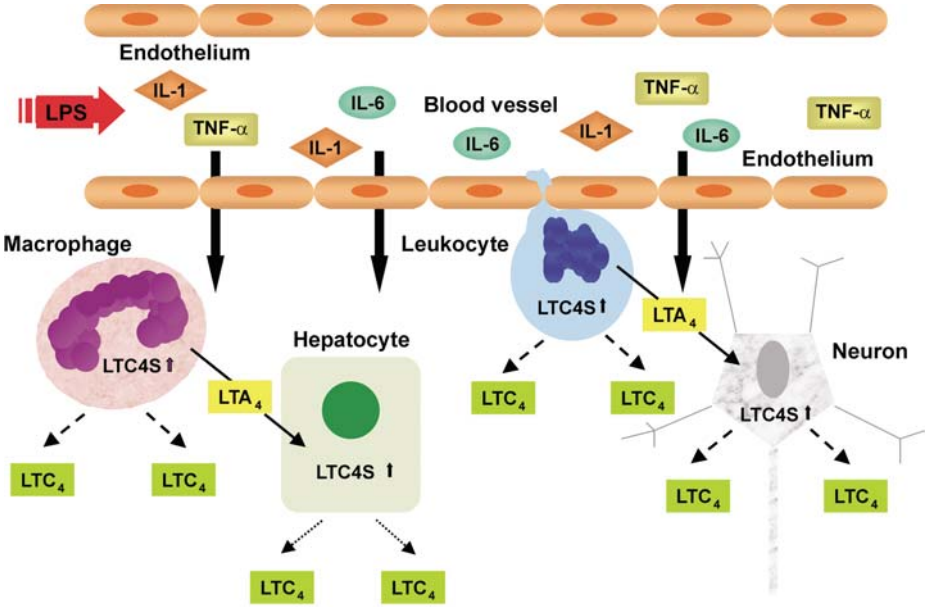

Figure 6. Model for increased cys-LT biosynthesis triggered by systemic inflammation. LPS induces up-regulation of proinflammatory cytokines (1), such as IL-1, IL-6 or TNF-α. These cytokines then trigger, by yet undefined pathway(s), the induction of LTC4S either in bone marrow-derived cells such as macrophages and leukocytes or in certain parenchymal cell types, e.g. hepatocytes and neurons. The myeloid cells can synthesize LTC_4 themselves, whereas parenchymal cells are devoid of the complete enzyme machinery to synthesize LTs. The latter cell types can still contribute to increased LTC_4 biosynthesis via a so-called transcellular biosynthesis, which involves uptake of LTA_4 (yellow box) from surrounding myeloid cells. The release of LTC_4 (green box) and its conversion into LTD_4 and LTE_4, may then lead to signs and symptoms of systemic inflammation.

LTC4S and cys-LTs may be involved in organ-specific and systemic inflammatory responses

It is well known that cys-LTs may be formed in response to allergic stimuli and chronic tissue injury and contribute to local chronic inflammation, particularly in the airways of asthmatics [3]. Induction of LTC4S by cytokines has been studied in several isolated mammalian cell types and cell lines [22, 23]. However, due to the complexity of whole living organisms, it is very difficult to translate such in vitro findings to human pathophysiology, as exemplified by the recent finding that LPS actually decreases LTC4S expression in a human macrophage cell line THP-1 [43]. In fact, very few, if any, previous studies have tried to assess the relevance of these studies for the in vivo situation. Our data indicate that up-regulation of LTC4S may also be triggered by systemic inflammatory signals and prime certain tissues for increased cys-LT biosynthesis (fig. 6), which in turn may elicit local inflammatory circuits provoking vasospasm and edema formation, particularly in the heart, brain, liver and adrenal gland. Moreover, in this way, LTC4S and cys-LT formation may directly, or via secondary signals, contribute to the general signs of systemic inflammation, e.g., fever, tachycardia, hypotension and fatigue. In this context, it is interesting to note that the recently characterized $CysLT_2R$ [15–17], the biological role of which is presently unknown, is also abundantly expressed in the central nervous system, heart and adrenal glands, and may thus transduce tissue-specific responses of cys-LTs during systemic inflammation. Of note, the CysLT2R is also abundantly expressed at the surface of human endothelial cells, which is a strategic location for such a signalling function [44]. Moreover, cytokines released under these conditions may well upregulate the expression of both CysLT1R and CysLT2R, thus priming target tissues to become more responsive to cys-LTs.

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