LST1: A Gene with Extensive Alternative Splicing and Immunomodulatory Function

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LST1: A Gene with Extensive Alternative Splicing and Immunomodulatory Function

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The gene of the leukocyte-specific transcript (LST1) is encoded within the TNF region of the human MHC. The LST1 gene is constitutively expressed in leukocytes and dendritic cells, and it is characterized by extensive alternative splicing. We identified 7 different LST1 splice variants in PBMC; thus, 14 LST1 splice variants (LST1/A–LST1/N) have been detected in various cell types. These isoforms code for transmembrane as well as soluble LST1 proteins characterized by two alternative open reading frames at their 3′ end. We demonstrate the presence of the transmembrane variant LST1/C on the cell surface of the monocytic cell lines U937 and THP1. Recombinant expression of LST1/C permitted its profound inhibitory effect on lymphocyte proliferation to be observed. In contrast, the alternative transmembrane variant LST1/A, the extracellular domain of which shows no amino acid sequence homology to LST1/C exerted a weaker but similar inhibitory effect on PBMC. These data demonstrate the protein expression of LST1 on the cell surface of mononuclear cells, and they show an inhibitory effect on lymphocyte proliferation of two LST1 proteins although they have only a very short amino acid homology.

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Materials and Methods

Cell separation

PBMC were isolated from buffy coats of healthy volunteer donors by density gradient centrifugation on Lymphoprep (Nycomed, Oslo, Norway). T cells, monocytes, and B cells were separated from PBMC by positive selection with the immunomagnetic bead system using the respective Dynabeads M-450 (CD4), M-450 (CD8), M-450 (CD14), and M-450 (CD19) (Dynal, Oslo, Norway) as described previously (12–14). After selection, the cells were treated with Detachabeads (Dynal) to remove the CD4, CD8, and CD19 Abs from the membrane receptors (15). The purities of the recovered cells were 98% for CD4+ cells, 99% for CD8+ cells, 99.6% for CD14+ cells, and 80–90% for CD19+ cells.

Culture of dendritic cells

Dendritic cells were generated from PBMC as described (16–18). Briefly, mononuclear cells were isolated from leukocyte-enriched buffy coats by

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FIGURE 1. LST1 expression and the control vectors. The vector pQE-16 was used for expression of the control protein DHFR. The clone ΔLST1/C-DHFR was created by cloning ΔLST1/C cDNA into the BglII and HindIII restriction sites of the expression vector pQE-40, which contains the N-terminal part of the DHFR protein. The expression vector pQE-30 was used to generate the ΔLST1/A clone by cloning the BglII-restricted cDNA into the BamHI site of pQE-30. All three expression vectors encode an N-terminal histidine tail (6X His). The DNA and amino acid sequences of ΔLST1/A and ΔLST1/C are indicated.

RNA isolation and RT-PCR

With the use of Trizol, total RNA samples were prepared from cells at each time indicated, according to the manufacturer’s instructions. To remove traces of contaminating DNA, 2 μg of total RNA were digested with 1 UI DNase (Promega, Madison, WI) for 1 h, phenolized, and precipitated as described previously (19). One reverse transcription reaction for every cell stimulation was performed with 2 μg RNA using avian myeloblastosis virus reverse transcriptase, oligo(dT)15, and RNasin (all purchased from Promega) for 1 h at 37°C (19). The revealed cDNA was used for all PCR amplifications of the respective cell stimulations and control amplifications with G3PDH-specific oligonucleotides (Clontech, Palo Alto, CA) using the sense primer 5'-CTGAGTGCCATGCTGCGGTCGAGTGCCGAGTCGACCGTTAAGCTGAGATGCTGCACTTC-3' and of DNase-treated not reverse transcribed RNA were performed. LST1 transcripts were amplified with the oligomers 5'-ATGAGGAACT9TGAGGCAAGTC-3' encoded in exon 5 (nucleotides 2457–2446). For the PCR reaction mixtures, 80 ng reverse transcribed RNA were used in 25 μl PCR buffer containing 1 μM concentrations of each primer, 0.25 mM dNTP (Sigma, Vienna, Austria), and 1 UI Taq polymerase (Boehringer Mannheim, Vienna, Austria). After an initial step of denaturation (5 min at 94°C), 35 PCR cycles were performed (1 min at 94°C, 1 min at 56°C, 2 min at 72°C) followed by a final extension of 10 min at 72°C. PCR products were loaded on a 2% agarose gel together with a PCR m.w. marker (United States Biologicals, Cleveland, OH) and ran on a Bio-Rad gel system. The generated cDNA was used for all PCR amplifications.

Cloning of LST1 cDNAs

To analyze the splice transcripts expressed in freshly isolated monocytes, we subcloned 2.5 μl of the RT-PCR product that was revealed through amplification of reverse transcribed mRNA from freshly isolated monocytes with the oligonucleotides 5'-pblst1 and 3'-pblst1 into the TA cloning vector pCR II (Invitrogen, Leek, Netherlands). The ligation product was transfected into INVa Fb bacteria (Invitrogen) following the manufacturer’s recommendations.
and IFN-glane 6, lane 3), or after addition of TPO (lane 2), 24 h (lane 2), and LPS (lane 12, lane 10, GM-CSF (lane 11, CD14 RT-PCR of a freshly separated monocyte-enriched cell population (85%

99%

introducing a ORF4 5

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end of the cDNA clone LST1/C (Fig. 3), which is expressed in

The 3 end of the transmembrane region as well as the N-terminal mouse dihydrofolate reductase (DHFR) protein, resulting in expression of a soluble LST1/C-DHFR fusion protein with a histidine affinity tag. The expression vector pQE-30 was used to generate the LST1/A clone (spans the 3’-end of exon 3, exon 4, and 5’)

DNA sequencing and data analysis

Plasmid DNA was prepared according to standard protocols, and DNA sequencing was conducted by the dideoxynucleotide chain termination method using the PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Weiterstadt, Germany) with the Applied Biosystems DNA Sequencing System Model 373A or the Thermo Sequenase fluorescence-labeled primer cycle sequencing with 7-DEACA-DGTT (Amersham, Vienna, Austria) with the Li-Cor 4000L Sequencing System. Sequencing primers were the universal and reverse M13 primers. DNA sequences were analyzed with MacMolly software (Soft Gene, Bocholt, Germany)

Production of a polyclonal rabbit LST1 Ab

The 3’ end of the cDNA clone LST1/C (Fig. 3), which is expressed in U937 cells (2) and spans exon 2, 3, and 5, was amplified with the primer open reading frame 6 (ORF6) 5’-ACT AGATCTTCCGCTGCCTGTT 3’ containing a BglII restriction site (underlined) and the 3’-primer ORF4 5’-ACTAAGCTTGTTGACTGAC 3’ introducing a HindIII site (underlined). The BglII- and HindIII-restricted amplification product that encompasses exon 5 and the region 3′-terminal of the transmembrane region of exon 3 were cloned into the respective cloning sites of the expression vector pQE-40, which contains a 6× His affinity tag at the N terminus as well as the N-terminal mouse dihydrofolate reductase (DHFR) carrier protein (Fig. 1) (Qiagen, Hilden, Germany). This construct fused the 4.1-kDa LST1/C protein encoding the extracytoplasmic region of the 6.8-kDa LST1/C polypeptide (Fig. 4A) to the 24.5-kDa DHFR carrier protein, resulting in expression of a soluble LST1/C-DHFR fusion protein with a histidine affinity tag. The expression vector pQE-30 was used to generate the LST1/A clone (spans the 3’-end of exon 3, exon 4, and 5’

by cloning it into the BamHI site of pQE-30, enabling the purification of the 8-kDa extracytoplasmic region of the LST1/A protein(10.8 kDa). The control vector pQE-16 (Qiagen) was used for expression of the 24.5-kDa DHFR control protein (Fig. 1). The K-12-derived Escherichia coli strain M15[pREP4] was used for high level expression of the proteins LST1/C-DHFR, LST1/A, and DHFR.

After protein purification according to standard protocols of the manufacturer (Qiagen), two rabbits were immunized with 2.2 mg of the LST1/C-DHFR protein using Freund’s adjuvant. The rabbits were injected s.c. on days 0, 28, 56, and 70 with a protein solution containing 880 µg/ml LST1/A in Western blot analyses. This protein A purified Ab did not detect LST1/A in Western blot analyses. This protein A purified Ab did not detect LST1/A in

FiguRe 2. DNA gel electrophoresis of LST1-specific RT-PCR. Positively selected macrophages, B cells, and T cells as well as differentiated dendritic cells were used before or after incubation with various cytokines. No clear difference in alternative splicing of the LST1 mRNA can be detected between these cell types directly after separation or after cultivation for the times indicated. A. Lane M, DNA weight marker; lane 1, CD4+ cells stimulated with PHA for 96 h; lane 2, dendritic cells; lane 3, CD197+ cells incubated with LPS for 15 h; lane 4, CD197+ cells incubated with LPS and IFN-γ for 48 h; lane 5, CD8+ cells cultivated with thrombopoietin (TPO) for 48 h; lane 6, CD14+ cells stimulated with GM-CSF for 24 h. B, RT-PCR of a freshly separated monocyte-enriched cell population (85%

99%

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by cloning it into the BamHI site of pQE-30, enabling the purification of the 8-kDa extracytoplasmic region of the LST1/A protein(10.8 kDa). The control vector pQE-16 (Qiagen) was used for expression of the 24.5-kDa DHFR control protein (Fig. 1). The K-12-derived Escherichia coli strain M15[pREP4] was used for high level expression of the proteins LST1/C-DHFR, LST1/A, and DHFR.

After protein purification according to standard protocols of the manufacturer (Qiagen), two rabbits were immunized with 2.2 mg of the LST1/C-DHFR protein using Freund’s adjuvant. The rabbits were injected s.c. on days 0, 28, 56, and 70 with a protein solution containing 880 µg/ml LST1/A in Western blot analyses. This protein A purified Ab did not detect LST1/A in Western blot analyses. This protein A purified Ab did not detect LST1/A in

FiguRe 3. A. LST1 splice variants expressed in PBMC. Cloning and sequencing of the RT-PCR product from PBMC enabled identification of seven alternative LST1 splice variants. The schematic structure starts with exon 2, which encodes the start codon, whereas exon 3 contains a putative transmembrane region of 23 hydrophobic amino acids. Thus, LST1/J, LST1/M, LST1/DE, LST1/L, and LST1/N constitute putative soluble isoforms. Because exon 4 is 23 bp long, a different ORF is used in exon 5 in the variants LST1/A, LST1/J, and LST1/L, which span this exon in contrast to the remaining cDNAs. B. LST1 splice variants not expressed in PBMC. Five alternative noncoding exon 1 (1A, 1A’, 1B, 1C, and 1D) forms have been identified, and the splice variants LST1/B, LST1/C, and LST1/G constitute transmembrane variants.
FIGURE 4. A. The LST1 genomic sequence. Protein-encoding exon sequences of LST1 are boldface; the putative noncoding alternative exons 1A-1D and the noncoding part of exon 2 are underlined. Exon 1A, exon 1B, and 5’ mark alternative shorter variants of the respective exon sequences. The start and stop codons of the LST1 cDNAs and its short upstream ORF are outlined. The ORF encoding a nonapeptide is located at the 5’-end of exon 2, whereas the putative start codon of all LST1 cDNAs identified thus far can be found at its 3’-end (position 1101). The stop codons of the two different ORFs are outlined in exon 5 (LST1/A at position 2469 and for LST1/C at 2378) which are used according to the presence or absence of exon 4 in the various cDNAs. The 3’-end of exon 5 encodes the polyadenylation signal AATAAA. The polymorphic PvuII restriction site and the polymorphic TNF microsatellites TNFd3172 LST1: COMPLEXLY SPLICED GENE WITH IMMUNOMODULATORY FUNCTION by guest on April 14, 2017 http://www.jimmunol.org/ Downloaded from
FIGURE 5. LST1 surface expression. FACS analyses were assessed with the primary Ab LST1–25-Affi and a PE-conjugated Fab fragment of a goat anti-rabbit Ig as secondary Ab. Black areas, results obtained by sole labeling with the secondary Ab. The control (A) compares single labeling of the cells with double-labeling of U937 cells with the HLA-G rabbit Ig as primary Ab. No difference between these controls can be seen, proving the specificity of the LST1–25 Ab. LST1 surface expression of the cell lines U937 (B), THP1 (C), and Molt-4 (D) and after cultivation of U937 cells with LPS (E) and THP1 with a combination of IL-2 and IFN-γ (F) is shown.

All positive results were controlled with the LST1–25-Affi Ab as primary Ab. The following FITC-conjugated mAbs (Becton Dickinson, Mountain View, CA) were utilized in a third labeling step: Leu/CD8, Leu-3/CD4, Leu-12/CD19, and Leu-M3/CD14. After each incubation, cells were washed in FACS buffer and then analyzed after three final washings. To determine cell purities, the same respective FITC-conjugated mAbs were used.

Lymphocyte proliferation assay after stimulation with PHA and candidin

PBMC were isolated on Lymphoprep as described. Cells were resuspended in RPMI 1640 medium containing 1% l-glutamine (ICN, Asserelegem, Belgium), 1% penicillin/streptomycin (Sigma, Bornem, Belgium), and 10% autologous (assays 1 and 2) or pooled human AB serum (ICN) in assays 3–5. Triplicate cultures were set up in a 200-μl volume in 96-well round-bottom plates (Nunc, Meraldeke, Belgium) at a concentration of 5 × 10^5 cells/ml. Cultures were incubated either in medium alone or with optimally stimulating doses of PHA (1/100 dilution of reconstituted Bacto-hemagglutinin P (Difco Bacto)) or 50 μg/ml candidin (Stallergenes, Fresnes, France) as a control. To evaluate an immunomodulatory activity of the LST1 variants, 5–50 ng of the renaturated proteins ∆LST1/C-DHFR, ∆LST1/A, or the buffer used for elution of these proteins from the Ni-NTA column as negative control were added either to medium alone, or together with PHA or candidin at the onset of triplicate cultures. After incubation for 6 days in a humidified 5% CO_2, 95% air atmosphere, DNA synthesis was measured by adding 0.5 μCi [3H]thymidine (40–60 Ci/mmol; Amersham, Arlington Heights, IL) during the final 12 h of culture. Cells were harvested with a semiautomatic cell harvester, and thymidine incorporation was assayed in a liquid scintillation counter (Beckmann LS 1801, Galway, Ireland). Proliferation is given as the mean of triplicates ± SE.

heavy chains only in Western blot analyses. Because HLA-G is not recognized on the cell surface of U937, Molt-4, and THP-1 cells with this rabbit antiserum, this primary Ab was used as control Ab in our experiments (Fig. 5).

Single-step purification and solubilization of the LST1 proteins

All functional assays with the LST1 proteins described in this work were conducted with solubilized proteins. For solubilization of the LST1 proteins ∆LST1/C-DHFR, DHFR, and ∆LST1/A, a single-step purification/ refolding procedure based on modification of existing solid phase solubilization procedures was performed (20). Briefly, the pellet from 1 liter incubation for 6 days in a humidified 5% CO_2, 95% air atmosphere, DNA synthesis was measured by adding 0.5 μCi [3H]thymidine (40–60 Ci/mmol; Amersham, Arlington Heights, IL) during the final 12 h of culture. Cells were harvested with a semiautomatic cell harvester, and thymidine incorporation was assayed in a liquid scintillation counter (Beckmann LS 1801, Galway, Ireland). Proliferation is given as the mean of triplicates ± SE.

Flow cytometric measurement of surface Ag expression

To determine surface LST1 expression, cells (5–10 × 10^5) were suspended in FACS buffer (PBS containing 1% BSA), labeled with the protein A-purified primary Ab LST1–25 (see above) for 30 min at 4°C followed by a second labeling step with PE-conjugated Fab fragments of a goat anti-rabbit Ig (Southern Biotechnology, Birmingham, AL). In parallel, a single labeling without the primary Ab was performed as control as well as a rabbit Ig control as secondary Ab. No difference between these controls can be seen, proving the specificity of the LST1–25 Ab. In parallel, a single second labeling step with PE-conjugated Fab fragments of a goat anti-rabbit Ig as secondary Ab. The following FITC-conjugated murine mAbs (Becton Dickinson, Mountain View, CA) were utilized in a third labeling step: Leu/CD8, Leu-3/CD4, Leu-12/CD19, and Leu-M3/CD14. After each incubation, cells were washed in FACS buffer and then analyzed after three final washings. To determine cell purities, the same respective FITC-conjugated mAbs were used.

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The results were performed with the buffer in which the pro-

cultured human pooled AB serum. Various final concentrations of the rena-
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Proliferative responses of PBMC to allogeneic stimulation were deter-

dined in one-way MLR, as described (21). Briefly, 5 × 10^4 responder

PBMC were cocultured with 5 × 10^4 mitomycin C-treated (Sigma, St.

Louis, MO) allogeneic PBMC for 6 days at 37°C in a humidified 5% CO_2,

air atmosphere at a volume of 150 μl culture medium in U-bottom

96-well microtiter plates (Costar, Cambridge, MA). The medium was com-

posed of RPMI 1640 supplemented with 2 mM L-glutamine (Biochrom,

Berlin, Germany), 100 U/ml penicillin (Biochemie, Vienna, Austria), 100

μg/ml streptomycin (Grüenthal, Stolberg, Austria), and 10% heat-inacti-

vated human pooled AB serum. Various final concentrations of the rena-
tured proteins ΔLST1/C-DHFR and DHFR were added at the onset of triplicate cultures, whereas the buffer used for elution of these proteins from the Ni-NTA column was used as negative control. During the last 12–16 h, each well was exposed to 2 μCi [3H]thymidine and harvested, as described above. The proliferation was displayed as mean of triplicates ± SE.

Results

Detection of various LST1 transcripts in PMNC and dendritic cells

RT-PCR analysis of a freshly isolated monocyte-enriched cell popu-

lation (85% CD14^+ cells) was used to detect four different LST1 splice variants 220, 265, 286, and 381 bp long (Fig. 2). Constitutive LST1 transcription was observed in positively selected CD14^+, CD8^+, and CD19^+ cells, as well as in dendritic cells (Fig. 2).

No difference in size of the LST1 transcripts after stimulation

After stimulation of the PBMC, B cell, and T cell populations, no significant change in length of the LST1 mRNAs was detected by RT-PCR in comparison with freshly isolated cells (Fig. 2). After stimulation with various cytokines and agents (IL-3, IL-4, G-CSF, GM-CSF, TPO, IFN-γ, PHA, or LPS) for up to 96 h, the same transcripts 221, 265, 286, and 381 bp long were amplified in the monocyte-enriched cell population tested.

Sequencing of different protein-encoding transcripts in PBMC

To characterize the transcripts amplified by RT-PCR, the ampli-

fication products of an unstimulated monocyte-enriched cell pop-

culation (80% CD14^+ monocytic cells) were subcloned and se-

quenced. By analyzing 30 different cDNA clones, we identified 7 different transcripts (Fig. 3A), of which 243-, 309-, and 358-bp mRNAs had not been detected by gel electrophoresis. Only two of these seven different transcripts had been previously described (LST1/A and LST1/DE (Fig. 3)). According to the proposed nomenclature of de Baey et al. (11), we named the newly detected variants LST1/J (248 nt), LST1/K (297 nt), LST1/L (182 nt), LST1/M (225 nt), and LST1/N (202 nt) (Fig. 3A). Because we amplified only the protein-coding sequences of the LST1 trans-

cripts (exons 2–5), we could not distinguish between the variants LST1/D (exons 1A, 2, 5') and LST1/E (exons 1C, 2, 5') differing only in the noncoding exon 1. Therefore, the transcript spanning exon 2 and 5' was called LST1/DE. We confirm the presence of a third alternative splicing acceptor site of exon 5 in our cDNA clones that had only been presumed previously (Fig. 4A) (11). However, expression of the splice variant LST1/C (2, 11) was not identified in PBMC. The sequences of the isoforms LST1/ J–LST1/N have been submitted to the EMBL database under the respective submission numbers Y18486–Y18490.

LST1 transcripts encode different proteins

Only two of the transcripts expressed in PBMC contain exon 3 (LST1/A, LST1/K), which encodes a stretch of 23 hydrophobic amino acids spanning the transmembrane region (2). Thus, all the remaining transcripts probably constitute soluble cytoplasmic iso-

forms, because they do not contain a signal sequence necessary for secretion. Only the cDNA clones LST1/A, LST1/DE, and LST1/J encompass exon 4. As exon 4 spans 23 nt, its presence in LST1 transcripts leads to a frame shift. Thus, an alternative open reading frame (ORF) is present in the C terminus of the clones spanning exon 4 in comparison with the remaining transcripts, LST1/K–LST1/N. The two different ORFs encode putative soluble as well as transmembrane isoforms, which further increase the LST1 protein spectrum.

Mixed leukocyte reaction

Proliferative responses of PBMC to allogeneic stimulation were deter-

mined in one-way MLR, as described (21). Briefly, 5 × 10^4 responder

PBMC were cocultured with 5 × 10^4 mitomycin C-treated (Sigma, St.

Louis, MO) allogeneic PBMC for 6 days at 37°C in a humidified 5% CO_2,

95% air atmosphere at a volume of 150 μl culture medium in U-bottom

96-well microtiter plates (Costar, Cambridge, MA). The medium was com-

posed of RPMI 1640 supplemented with 2 mM L-glutamine (Biochrom,

Berlin, Germany), 100 U/ml penicillin (Biochemie, Vienna, Austria), 100

μg/ml streptomycin (Grüenthal, Stolberg, Austria), and 10% heat-inacti-

vated human pooled AB serum. Various final concentrations of the rena-

FIGURE 6. Proliferative response of PHA-, candidin-, and alloantigen-

stimulated lymphocytes. Mean values and SE of lymphocyte proliferation

in response to PHA (A) and candidin (B), as well as in MLR (C) are

presented with or without addition of various concentrations of DHFR

(right columns), ΔLST1/C-DHFR (left columns), and ΔLST1/A (middle columns). The controls were performed with the buffer in which the pro-

teins were dissolved.
LST 1/C is expressed on the cell surface of U937 and THP1 cells

To analyze whether cells express the LST1 protein at the cell surface, we used LST1-specific antisera raised against recombinant LST1 polypeptides in FACS analysis. The Ab LST1-25 showed specific binding of the proteins LST1/C and DHFR and did not detect the LST1/A protein in Western blot analysis (Fig. 4B). FACS analysis revealed no surface expression of the LST1/C protein in PBMC from healthy individuals, and it was not induced after in vitro stimulation of the cells with the following substances or combinations for up to 72 h: PHA, TNF-α, IL-2 plus IFN-γ, IL-4, IL-4 plus LPS (data not shown). We also observed no constitutive or inducible (PHA, IL-2, IFN-γ) LST1/C cell surface expression in the T-lymphocytic cell line Molt 4. The histiomonocytic cell line U937, however, showed constitutive expression of the LST1/C protein (Fig. 5), which was not clearly up-regulated by incubation of these cells with LPS. All positive results were confirmed by performing the flow cytometric measurements with the LST1/C-specific LST1-25-Affi Ab. Constitutive expression of the LST1/C protein was also observed in the monocytic cell line THP1 (Fig. 5).

Inhibition of lymphocyte proliferation exerted by LST1

To identify immunomodulatory activities of the variants LST1/A and LST1/C-DHFR, the proliferative response of PBMC stimulated with PHA, candidin, or allogeneic stimulator cells in the MLR was tested with regard to these proteins. The PHA-induced proliferation rate of PBMC (Fig. 6A) was significantly reduced by the addition of 10 or 50 ng/ml LST1/C-DHFR at the beginning of the culture period from 100% to 56 and 15%, respectively. In contrast, DHFR and LST1/A showed no significant inhibitory effect on PHA-induced stimulation. Thus, the LST1/C portion is responsible for the observed inhibition whereas the recombinant proteins LST1/A and DHFR that were isolated by the same procedure had no effect.

The candidin-induced proliferation of lymphocytes was significantly inhibited by LST1/C-DHFR and to a lesser extent by LST1/A, whereas no significant change was effected by DHFR (Fig. 6B). A highly significant immunosuppressive response was also detected when LST1/C-DHFR was added to MLCs, revealing a dose-dependent inhibition of the alloantigen-induced proliferation of lymphocytes. A concentration of 1 ng/ml reduced proliferation by 38%. In the presence of 50 or 100 ng/ml LST1/C-DHFR, the proliferative response was totally blocked (Fig. 6C). Addition of DHFR to the MLR did not show a significant inhibition. Only at the highest concentrations (50 and 100 ng/ml) was proliferation reduced.

Discussion

Our studies show constitutive transcription of the LST1 gene in peripheral mononuclear cells, T cells, B cells, and dendritic cells. RT-PCR analysis and subsequent cloning and sequencing of the cDNA from a monocyte-enriched PBMC cell population led to the identification of seven different splice variants (LST1/A, LST1/DE, LST1/J–N). Only two of them (LST1/A and LST1/DE) have been previously detected (11). The LST1/A mRNA is also present in several human tissues and cell lines, such as placenta, thymus, liver, lung, kidney, tonsil, Hep G2 and Hep 3B, U937, and Jurkat, whereas the LST1/D isoform has been detected only in brain tissue and mononuclear cells (11). The transcripts identified in our studies increase the number of LST1 splice variants to 14, which cover all but 2 of the possible polypeptides based on the known exon/intron organization (Fig. 3). However, we cannot exclude the presence of further splice variants in PBMC. The same is true of CD4+, CD8+, CD19+, and dendritic cells in which transcription of at least four different splice variants were detected by RT-PCR and agarose gel electrophoresis. These data confirm the complex mRNA expression pattern of this gene and show the constitutive expression of several LST1 splice variants in PBMC and dendritic cells.

Although splicing of the LST1 RNA seems to be complex, no difference can be found in freshly isolated PBMC, B and T lymphocytes or after stimulation of these cells in vitro for up to 96 h (Fig. 2). We found no change in expression of the different splice variants in CD8+ cells after stimulation with IFN-γ or PHA for up to 48 h. These results contrast the results of de Baey et al. (11), who observed a loss of the putative membrane-anchored isoforms containing exon 3 in the monocytic cell lines U937 and MonoMac 6 and exclusively detected the LST1/A cDNA after activation of a CD8+ T cell clone with IFN-γ. These differences can be explained by the different primers used. The primer pair of de Baey et al. coded in the most 5′ of the alternative noncoding exon 1 sequences (exon 1A) and in exon 5′, which allowed detection only of LST1 transcripts controlled by the first promoter of the LST1 gene causing the alternative exons 1A′, 1B, 1C, and 1D not to be amplified. In contrast, the oligonucleotides of our RT-PCR studies were located in exon 2 and exon 5′. All splice variants analyzed thus far span these exons, because exon 2 contains the start codon and exon 5′ contains the stop codons of both alternative ORFs.

Also, preferential expression of various isoforms in certain cells does exist. No surface expression of the LST1/C variant was seen in peripheral monocytes, T cells, and B cells in our FACS studies, whereas the monocytic cell lines THP1 and U937 show constitutive expression of this protein. These data are in good correlation with the fact that LST1/C mRNA was detected in U937 cells but not in PBMC. No clear influence on the cell surface expression has been observed by treating THP1 and U937 cells with IFN-γ, IL-2, or GM-CSF.

Because the LST1 gene is located within the TNF complex in close vicinity to the genes for TNF-α, TNF-β, and lymphotoxin-β and its transcription is induced by IFN-γ, an immunomodulatory function has been suspected (2). Also, a positive as well as a negative association with bone marrow and renal transplant rejection was found with two different alleles of TNFd microsatellites which are located in intron 4 (9, 10). To examine an immunomodulatory effect of LST1, we expressed the two LST1 variants LST1/A and LST1/C as soluble molecules with an N-terminal His epitope in E. coli (Fig. 1). These two isoforms only share nine amino acids at the N terminus but differ completely in the remaining molecule. They represent the two groups of LST1 proteins with regard to the two alternative ORFs used in the last exon caused by presence or absence of exon 4. The DHFR sequence does not exert an immunomodulatory effect enabling its use for elongation of the 4.1 kDa protein LST1/A, which was indispensable for its expression. Therefore, the 8-kDa LST1/C-DHFR molecule inhibited the PHA- and candidin-induced proliferation highly significantly. This inhibition was dose dependent and exerted at low concentrations (1–10 ng/ml), whereas a significant effect was exerted by LST1/A only with regard to the stimulation of PBMC with candidin but not with PHA.

These results show that PBMC do not present the LST1/C protein on their cell surface, whereas they respond to the presence of...
this protein in soluble form. Interestingly, only two monocytic cell lines expressed LST1/C on their cell membrane, but it was not detected on peripheral monocytes of 24 individuals or after in vitro stimulation of these cells with various cytokotens. This is in good correlation with the failure to amplify the LST1/C transcript in these cells. The LST1/M variant (Fig. 3), however, which constitutes the soluble homologue of LST1/C, is transcribed in PBMC. This demonstrates the complex splicing of the LST1 transcripts and its distinct regulation in different cell types.

Although ΔLST1/A and ΔLST1/C show only a short amino acid homology of nine amino acids, both isoforms inhibit the proliferation at a varying extent. Therefore, alternative splicing seems to result in LST1 isoforms of a probably varying immunosuppressive function. However, further studies of the various LST1 variants must be done to analyze the regulation of alternative splicing and the difference in function of all 10 LST1 polypeptides identified.

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References