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A Stimulation-Dependent Alternate Core Promoter Links Lymphotoxin α Expression with TGF-β1 and Fibroblast Growth Factor-7 Signaling in Primary Human T Cells

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Lymphotoxin (LT)-α is an inflammatory cytokine that is expressed in three active forms: a secreted homotrimer (LT-α1) and two transmembrane heterotrimers in differing stoichiometries with LT-β (LT-α2β2 and LT-α2β1) (1, 2). Through the interaction of these complexes with different receptors, LT-α has been shown to impact an array of processes, including B cell homing and affinity maturation (3–5), T cell tolerance to self Ags (6–8), inflammation (9, 10), Peyer’s patch and lymph node development (11, 12), and lipid metabolism regulation (13). LT-α expression is limited to lymphocytes, principally by resting and activated T cells and secondarily by NK cells and B cells (14, 15), and is affected by many stimuli (2, 14, 16, 17).

The LT-α (LTA) gene is located in the TNF region near the telomeric end of the class III region of the human MHC (Fig. 1A) and is comprised of four exons (Fig. 1B) (18). Exon 1 (+1 to +163) encodes Most of the 5′ untranslated region (UTR). The translation start site (ATG, +460) is in exon 2 (+451 to +558), which encodes the remainder of the 5′ UTR and the majority of the signal peptide. The mature protein is encoded by exons 3 (+645 to +750) and 4 (+998 to +2038). Exon 4 also encodes a long 3′ UTR. The regulatory region of the LTA gene has not been well defined. The most extensive investigation of a LTA regulatory segment (−915 to +7) by reporter gene assay was conducted in B cell lines (16). Deletion analysis determined those regions necessary for minimal and maximal activity and a region with suppressive activity. The elements involved in CD40 and IL-4 induction of LTA also were mapped. Other studies have determined the regulatory elements necessary for autoinduction (19), as well as for induction by viral proteins (20, 21). Although limited in scope, these studies in conjunction with restricted expression of LTA by specific cell types does hint at complex regulation of this gene.

Further complexity of LTA regulation is suggested by variations in transcript expression and in the starting nucleotide of exon 1. First, the expression of different LTA mRNA transcripts has been shown to deviate dependent on cell type and stimulation condition (22). Eight distinct LTA mRNA transcript variants were expressed differentially among lymphocyte subsets and within each subset on comparison of unstimulated cells versus cells stimulated with either PMA plus ionomycin or PHA. Consistent with these results are data showing that DNase I hypersensitive sites in the LTA locus differ among cell types (23). Second, although a TATA box is located 20 nucleotides upstream of the defined transcription start site (TSS) of LTA exon 1, the starting nucleotide of LTA mRNAs is rather variable. LTA mRNAs have been described that
initiate in the proximal promoter region (−915 to −1; Fig. 1) at positions −379 (GenBank accession no. DQ123821.1 from primary human PBMCs) (22) and −185 (NM_001159740.1), in exon 1 at positions +33 (D12614.1 from a B cell line and NM_000595.2), +35 (DQ123821.1 from primary human PBMCs) (22), +102 (X01393), and +115 (D00102.1 from a T cell line) (24), and in exon 2 at position +454 (BC034729.1 from a lymphoma) (25). Collectively, these data suggest that the nature of LTA transcripts likely is influenced by cell type and by the stimulating agent and that the LTA gene may be transcribed from several alternate core promoters. The latter would not be surprising, as alternate promoter usage has been estimated to occur in ~40% of all human protein coding genes (26).

Several alternatively spliced LTA transcripts have been described. Four alternative splice sites have been identified in exon 4 that result in five unique LTA transcript variants (TVs) (22). Each of these TVs would produce truncated isoforms of LT-α and their functional significance is not known. The same study identified another TV (DQ123822.1) that retains intron 2. Retention of intron 2 introduces a premature stop codon and, thus, this transcript likely would not produce a functional protein product. An alternative splice site also is located in exon 1 at position +19 and its usage has been reported in two transcripts (DQ123821.1 and NM_001159740.1) (22) that initiate significantly upstream of the defined LTA TSS (18). These transcripts contain alternatives to the traditional exon 1 that normally terminates at nucleotide +163. Utilization of the +19 splice site is observed in LTA transcripts from Pan troglodytes as well (XM_0011525887, XM_0011525945, and XM_001153240) (22). Alternative splicing and the potential of multiple alternate core promoter sites, as well as limited cell type expression and transcript variation due to cell type and stimulation condition, demonstrate that LTA transcriptional and posttranscriptional regulation are quite complex.

The LTA gene contains an extensive downstream segment (i.e., the region between the transcription [+1] and translation [+460] start sites; Fig. 1). This segment includes exon 1 (+1 to +163), intron 1 (+164 to +450), and the first nine bases of exon 2 (+451 to +459). Some evidence suggests that the downstream segment plays a role in transcriptional regulation of the LTA gene. Investigation of HIV-1 transactivation of LTA expression showed that a section of exon 1 from +1 to +115 is required for increased LTA expression by the Tat protein (20). Modeling suggested that this portion of the downstream segment can form a stem-loop that resembles a Tat-responsive element. Single nucleotide polymorphisms (SNPs) at +81 (exon 1) and +253 (intron 1) in the downstream segment have been shown to affect the binding of transcription factors, whereas reporter gene constructs containing these SNPs and portions of the LTA downstream segment (+73 to +92 and −307 to +268) have shown that these SNPs impact expression levels (27, 28). Furthermore, a full-length human LTA mRNA transcript (BC034729.1) was described that initiates at the 3′ end of the LTA downstream segment at the fourth base of exon 2 (position +454) (25), indicating the presence of an alternate core promoter site within the downstream segment. These data are clear indications that the LTA downstream segment contains functional elements that regulate, as well as those that initiate, LTA transcription.

Based on the above data, the contribution of the LTA downstream segment to transcriptional regulation was investigated. We demonstrate that in different lymphocyte cell types the LTA downstream segment is a necessary and integral transcriptional regulatory component, that an alternate core promoter consisting of a stimulating protein 1 (Sp1) and initiator (Inr) element is present, and that the alternate core promoter is used under specific stimulation conditions in primary human T cells. Most importantly, this study provides evidence for a direct link between LTA expression and signaling by TGF-β1 and fibroblast growth factor (FGF)-7, having implications for the involvement of T cells and LT-α in a wide variety of processes regulated by TGF-β1 and FGF-7.

Materials and Methods

Cells

Jurkat (human leukemia; T lymphocyte), Hut78 (human Sezary syndrome; T lymphocyte), and Ramos (human Burkitt’s lymphoma; B lymphocyte) cells were obtained from the American Type Culture Collection (Manassas, VA). Jurkat and Ramos cell lines were cultured in RPMI 1640 (Invitrogen, Carlsbad, CA, USA) supplemented with 10% FBS (heat-inactivated for Ramos cells), 10 mM HEPES, and 1 mM sodium pyruvate. The Hut78 cell line was cultured in IMEM (American Type Culture Collection) supplemented with 20% FBS.

Human T cells were purchased (Allcells, Emeryville, CA) or were purified from PBMCs isolated from fresh whole blood with lymphocyte separation medium (Ficoll and Hypaque; Mediatech, Manassas, VA). Briefly, whole blood was brought to room temperature and diluted to a third its original volume with PBS. Lymphocyte separation medium was added (2 mL/pot) to the mixture, which was centrifuged at 1000 g for 20 min. The interface layer was removed and cells were pelleted. The PBMCs were washed with 25 mL PBS four times. T cells were isolated by negative selection with the Pan T Cell Isolation Kit II and an LS MACS column on a MidiMACS magnetic separator (Miltenyi Biotec, Bergisch Gladbach, Germany) following the manufacturer’s protocol. The purity of negatively isolated T cells (>95%) was evaluated by flow cytometry using a CD3-PE human-specific Ab (Miltenyi Biotec). Primary T cells were cultured in RPMI 1640 (Invitrogen) supplemented with 10% human AB serum (Valley Biomedical, Winchester, VA), 1% Glutamax-I (Invitrogen), 1% antibiotic-antimycotic (Invitrogen), 10 mM HEPES, and 1 mM sodium pyruvate. All individuals who supplied whole blood for this study signed informed consent and the protocol was approved by the Georgetown University’s Institutional Review Board (no. 1990-168).

Reporter vector construction

A vector containing the full-length LTA promoter was previously created in our laboratory by amplification and cloning an LTA regulatory region (−2434 to +459) from EBV-transformed B cells (International Histoocompatibility Workshop cell line no. 9020) homozygous for LTA allele p′003 (29) (GenBank accession no. NY274909) and inserted into a pGL2 luciferase vector. To create the pGL3 vector containing LTA (−2434 to +459), the LTA insert in pGL2 was recombined with the pGL3 vector via Gateway technology (Invitrogen) following the manufacturer’s protocol. The full-length LTA regulatory region was amplified by PCR. Reactions contained pGL2 luciferase construct containing the full-length LTA regulatory region (200 ng/μl), LTA-specific forward primer 5′-4-2 (5′-CTGACCTATCT-CATCTGTAGATGAGG-3′), and reverse primer 3-3 (5′-GGGAGAACCC-TGCAGAGAAAG-3′) (20 pmol/μl each), dNTPs (5 mM each), reaction buffer (1×), MgCl2 (2 mM), and Platinum Taq DNA polymerase (5 U; Invitrogen). DNA was denatured (95°C, 2 min) for one cycle, amplified (95°C, 30 s; 58°C, 30 s; 72°C, 3.5 min) for 35 cycles, extended (72°C, 5 min) for one cycle, and held at 4°C. The resulting amplicon was cloned into a donor vector (pcr8/GW/TOPO) via TOPO TA cloning. pGL3-basic (Promega, Madison, WI) was converted to a Gateway-compliant destination vector (Stratagene, La Jolla, CA) and ligated with the Gateway reading frame cassette A. The amplicon donor vector was recombined with the pGL3-ΔV in a separate LR clonase reaction using the LR clonase II enzyme mix (Invitrogen). All procedures followed the manufacturers’ protocols.

The LTA regulatory segment deletion vectors [LTA(−915 to +459), (−265 to +459), LTA(+1 to +459), (−2434 to −1)] were created by amplification of the appropriate regulatory segments utilizing the full-length LTA promoter vector as the template. Amplification reactions were performed as described above adjusting extension time for amplicon length and with the primers described above and the following primers in the appropriate combinations to create each construct: LTA−915 forward (5′-TTTTAAAATATGTATTGGCTCTG-3′), LTA−265 forward (5′-TTTGAAGACTCTAGGCTG-3′), LTA+1 forward (5′-CTGACCTATCTACGAC-3′), and LTA−1 reverse (5′-GCGGGCTTCGCAGGTC-3′). The resulting amplicons were cloned into a donor vector (pcr8/GW/TOPO) and then recombined with the pGL3-ΔV as described above. The downstream transcription factor binding site was removed by the Gateway technology.
mutant vectors (LTAMutSp1 and LTAMutInr) were created by site-directed mutagenesis from LTA(+1 to +459) using the QuickChange II site-directed mutagenesis kit (Stratagene) as per the manufacturer’s protocol. Primers were purchased (Invitrogen) and all constructs were confirmed by DNA sequencing.

Luciferase assays
Jurkat T cells, Hut78 T cells, and Ramos B cells (2 × 10⁶ each) were cotransfected with the indicated vector (3.5 μg for Jurkat; 5 μg for Ramos; 3.5 μg for Hut78) along with the pRL-TK Renilla luciferase plasmid (100 ng; Promega). Transfections were performed in triplicate with a Nucleofector II (Axama, Gaithersburg, MD) using kit V (Jurkat and Ramos cell lines) and kit R (Hut78 cell line). Programs X-001, C-013, and V-001 were used for Jurkat, Ramos, and Hut78 cell lines, respectively. Transfected cells were recovered for 4 h and then were treated with PMA (50 ng/ml) plus ionomycin (1 μg/ml) or left untreated. Luciferase was measured 24 h after transfection using the Dual-Luciferase reporter assay software (Genecodes, Ann Arbor, MI). All procedures followed the manufacturers’ protocols. Data were normalized by coexpression of pRL-TK Renilla luciferase and expressed as fold activity (luciferase activity of the indicated construct/Renilla luciferase activity). A reference construct was designated for each set of experiments, and the data represent a ratio of the mean fold activity of the indicated construct/mean fold activity of the reference construct ± SD of three independent experiments. Differences in the ratio mean fold activity with a p value of <0.05 by Student t test were considered significant.

5′ RNA ligase-mediated rapid amplification of cDNA ends
Primary human or Jurkat T cells (2 × 10⁶/ml) were treated with the indicated agent (PMA [50 ng/ml] plus ionomycin [1 μg/ml], recombinant human [rh] LT-α, 5 ng/ml; Ambacam, Cambridge, MA), rhFGF-7 [20 ng/ml; R&D Systems, Minneapolis, MN] or left untreated for 6 h. Total RNA was isolated from 1 × 10⁶ cells with Ribopure (Ambion, Austin, TX) followed by mRNA isolation with Poly(A) Purist (Ambion) as per the manufacturer’s instructions. The mRNA was treated and ligated to the 5′ RACE adapter and the cDNA was synthesized with First Choice 5′ RLM-RACE (Ambion) as per the manufacturer’s protocol. The except that the after the cation intestinal alkaline phosphatase (37˚C, 10 min). Phosphate buffer was then added to the alkaline phosphatase–treated mRNA that includes the distal segment and those in which this segment is only following the manufacturer’s protocol and then washed twice with PBS. LT was detected at the cell surface by analysis of the cells with a FACSort (Becton Dickinson, Franklin Lakes, NJ) using FCS Express 4 software (DeNovo Software, Los Angeles, CA).

Chromatin immunoprecipitation assays
Primary human T cells (2 × 10⁶/ml) were stimulated (1.5 h) with rhFGF-7 (20 ng/ml; R&D Systems). Chromatin immunoprecipitation (ChIP) assays were performed using the ChIP-IT Express kit (Active Motif, Carlsbad, CA) for Jurkat T cells or the MAChIP ChIP system (Invitrogen) for primary human T cells. Briefly, Jurkat T cells (unstimulated) or primary human T cells (unstimulated or stimulated with rhFGF-7) (5 × 10⁶) were fixed with a solution of formaldehyde (37%) for 5 min at room temperature. Fixed cells were treated and nuclei were extracted as instructed. Chromatin was sheared with a Microson ultrasonic cell disruptor (Misonix, Farmingdale, NY) at a setting of 3.5 for 5 pulses of 20 s each. A sample of sheared chromatin was then separated on a 4% agarose gel and imaged using a Kodak EDAS 290 imaging system (Eastman Kodak, Rochester, NY) to verify adequate shearing. Sheared chromatin was immunoprecipitated with Abs (3 μg) specific for Sp1, TFII-I, and steroid response element (SRE)BP1 (Santa Cruz Biotechnology, Santa Cruz, CA) or with a RNA polymerase II (RNAPII)–specific Ab and control rabbit IgG (ChIP-IT control kit, human; Active Motif). The RNAPII-specific Ab and ChIP-IT control primer set provided with the ChIP-IT kit served as positive controls for ChIP and for the subsequent amplification, respectively. Nonspecific enrichment of chromatin due to Ab was controlled for by using rabbit IgG, and nonspecific amplification of chromatin was controlled for by using the CNAPl negative control primer set (ChIP-IT control kit, human). Absorption of the Ab/protein/DNA complexes to the magnetic beads, reversal of the crosslinks between the DNA and nuclear proteins, and DNA purification were performed as instructed. For Jurkat T cells, a 235 bp segment of the LTA locus that spans +315 to +549 was amplified from the immunoprecipitated chromatin using an MJ Research DNA cycle (Bio-Rad Laboratories, Waltham, MA). Reactions contained immunoprecipitated chromatin (50 ng), LTA–specific forward (5′-ACTG-CATCTGTGGTCCCTCTTCTGTC-3′) and reverse (5′-AGGCGACGAGGACCAGCACGA-3′) primers (10 pM each), dNTPs (5 pM each), reaction buffer (1×), MgCl₂ (2 mM), and Platinum Taq DNA polymerase (5 U; Invitrogen). DNA was denatured (95˚C, 2 min) for one cycle, amplified (95˚C, 20 s; 59˚C, 30 s; 72˚C, 30 s) for 35 cycles, extended (72˚C, 5 min) for one cycle, and held at 4˚C for primary human T cells, a 167-bp segment of the LTA locus that spans +309 to +475 was amplified as above except with the LTA–specific forward (5′-TCTCTTGACTCTGATGTC-3′) and reverse (5′-GGTCACTGCTGCTGCATG-3′) primers, and the annealing temperature was adjusted to 61˚C. The GAPDH locus was amplified with the positive control primer set, and the CNAPl precursor locus was amplified using the negative control primer set (each provided in the ChIP-IT control kit, human) as per the manufacturer’s instructions. Amplification products were separated on 3% agarose gels and imaged using a Fluor Chem HD imaging system and AlphaView software (Alpha Innotech, CA). The final amplon was cloned using TOPO-TOPO 2.1 (Invitrogen) as per the manufacturer’s instructions. Individual bacterial colonies were isolated and grown for 20 h in 2 ml Luria-Bertani (Miller) Broth (EMD Chemicals, Gibbstown, NJ) supplemented with ampicillin (100 μg/ml). Third BamH1 fragment was sheared with a Microson ultrasonic cell disruptor (Misonix, Farmingdale, NY) to verify adequate shearing. Sheared chromatin was amplified (95˚C, 10 min). Lysates were centrifuged and stored at 4˚C. Plasmid DNA from supernatant (5 μl) was amplified by PCR utilizing TOPO TA 2.1–specific M13 forward and M13 reverse primers. Reactions were denatured (95˚C, 10 min) for one cycle, amplified (95˚C, 30 s; 55˚C, 30 s; 72˚C, 30 s) for 35 cycles, extended (72˚C, 5 min) for one cycle, and held at 4˚C. Amplified DNA was purified using Microcon YM-100 columns or Amicon Ultra 0.5 ml 30K Ultracel filters (Millipore, Billerica, MA). Purified amplons (2 μl) were sequenced with the BigDye Terminator sequencing kit (Applied Biosystems, Foster City, CA). Sequencing reactions were electrophotographed on an Applied Biosystems 3730 DNA analyzer. Data were analyzed using Sequencher 4.5 software (GeneCodes, Ann Arbor, MI). All procedures followed the manufacturer’s protocols.

Flow cytometry
Primary human T cells (2 × 10⁶/ml) were treated with the indicated agent rhFGF-7 (5 ng/ml; eBioscience, San Diego, CA), or rhFGF-7 (20 ng/ml; R&D Systems, Minneapolis, MN), or left untreated for 72 h. Cells (1.5 × 10⁶) were harvested at 6, 24, 48, and 72 h and washed twice with PBS. Cells were stained (30 min) on ice with an alpha-foetalprotein/PE-conjugated LT-α antiserum (SREBP1, Abcam, Cambridge, MA), with an LT–specific Ab (MAB1684; Abcam, Minneapolis, MN) followed with an R–PE–conjugated goat anti-mouse IgG (H+L) (Jackson ImmunoResearch, West Grove, PA) or with an R-PE–conjugated goat anti-mouse IgG (H+L) only following the manufacturer’s protocol and then washed twice with PBS. LT was detected at the cell surface by analysis of the cells with a FACSort (Becton Dickinson, Franklin Lakes, NJ) using FCS Express 4 software (DeNovo Software, Los Angeles, CA).

Results
The LTA downstream segment is critical to full regulatory region activity and can act as an independent regulatory unit
Because evidence has suggested a role for the downstream segment (+1 to +459) (Fig. 1) in transcriptional regulation of the LTA gene (20, 25, 27, 28), its impact on transcriptional activity was examined via luciferase reporter gene assay. Constructs that contain various segments of the LTA regulatory region (Fig. 2A) were analyzed in Jurkat T cells and in Ramos B cells under basal and under PMA plus ionomycin–stimulated conditions. In addition to the downstream segment, a distal segment (~2434 to ~916) that had not been previously investigated for regulatory activity also was examined. Luciferase activity of the construct that contains the distal segment [LTA(~2434 to ~459)] was ~40% less than that of the constructs that lack the distal segment [LTA(~915 to ~459) and LTA(+1 to +459)] under basal conditions in Jurkat T cells (Fig. 2B). However, there was no significant difference in activity among these constructs after stimulation of Jurkat T cells with PMA plus ionomycin (Fig. 2D). Furthermore, Ramos B cells showed no significant difference in the activity of the construct that includes the distal segment and those in which this segment is
absent under both basal (Fig. 2C) and stimulated conditions (Fig. 2E). To confirm the reduction in activity in unstimulated T cells when the distal segment is present, a second T cell line (Hut78) was analyzed. Again, the presence of the distal segment resulted in a significant reduction in activity when compared with the construct that lacked the distal segment (Supplemental Fig. 1).

**FIGURE 1.** The LTA locus and regulatory segment designations. (A) Schematic representation of the ~15-kb TNF region of the class III region of the human MHC showing the location of the LTA locus in relation to the TNF and LTB loci. Gene orientation is represented by the arrows. (B) Schematic representation of the LTA locus. Nucleotide numbering is based on the LTA transcription start site (TSS; +1) as determined by S1 nuclease mapping (18). The boundaries of the exons (black boxes) are labeled below each box. The boundaries of the regulatory segments are marked with a line. The translation start site (ATG) begins at nucleotide +460 in exon 2. Representation is not drawn to scale.

**FIGURE 2.** The LTA downstream segment is an essential regulatory component and alone is sufficient for LTA expression. (A) Schematic representation of the full-length LTA regulatory region (~2434 to +459) showing the relative positions of the distal (~2434 to ~916), proximal (~915 to ~1), and downstream (+1 to +459) segments. Also depicted is a schematic of the LTA regulatory region constructs used in luciferase assays. Luciferase activity of the LTA regulatory region constructs in either unstimulated Jurkat T cells (B) and Ramos B cells (C) or PMA (50 ng/ml) plus ionomycin (1 µg/ml)–stimulated Jurkat T cells (D) and Ramos B cells (E). EV was used to measure background luciferase activity. Luciferase activity was normalized by coexpression of pRL-TK Renilla luciferase and expressed as fold activity (luciferase activity of the indicated construct/Renilla luciferase activity). Data represent a ratio of the mean fold activity of the indicated construct/mean fold activity of a designated reference construct [LTA(~2434 to +459)] ± SD of three independent experiments. The p values were determined by Student t test. **p ≤ 0.001, ***p ≤ 0.0001.
These data suggest that the distal segment (−2434 to −916) participates in LTA gene regulation and that a T cell–specific repressor element is present within this region. The repressor element apparently is active in unstimulated T cells and is relieved upon stimulation. In another study, it was demonstrated that a portion of the proximal segment (−915 to −265) reduces expression in B cell lines (16). We observed the same effect of this region on expression in B cells (Supplemental Fig. 2); however, this region had no impact on expression in T cells. These data in conjunction with the demonstration that among lymphocyte subsets there are differences in LTA transcript profiles (22) and in LTA locus DNase hypersensitivity sites (23) further illustrate that there is cell type specificity to LTA transcriptional regulation.

The constructs that lack the LTA downstream segment [LTA (−2434 to −1) and LTA (−915 to −1)] exhibited a reduction in luciferase activity of ≥50% when compared with the corresponding constructs that include this segment [LTA (−2434 to +459) and LTA (−915 to +459), respectively] in T cells and in B cells under basal and under stimulated conditions (Fig. 2B–E). In some instances, deletion of the downstream segment completely abrogated luciferase activity to the background levels of the control construct vector without insert (EV). Interestingly, the construct containing only the downstream segment [LTA(+1 to +459)] demonstrated activity equivalent to that of the construct containing the proximal and downstream segments [LTA (−915 to +459)] in both cell types and under both conditions (Fig. 2B–E). This result was confirmed in a third cell line (Hut78; Supplemental Fig. 1). First, the dramatic reduction in expression for the constructs without the downstream segment suggests that the downstream segment is a critical component required for full activity of the LTA regulatory region in different lymphocyte cell types and under different conditions. Second, the ability of the LTA downstream segment to regulate expression at a level equivalent to the proximal and downstream segments suggests that the downstream region contains all of the elements necessary for full transcriptional expression of the LTA gene. Furthermore, these data suggest that an alternate core promoter is present in the downstream regulatory segment that is able to coordinate and initiate transcription independent of the known core promoter that includes the TATA box (−26 to −21) in the proximal (−915 to −1) regulatory segment.

Stimulation-dependent transcription initiation occurs within the LTA downstream segment in vivo in primary cells and indicates the presence of an alternate core promoter

Based on the above data, it was hypothesized that an alternate core promoter in the LTA downstream segment allowed for luciferase expression from the construct [LTA(+1 to +459)] that contained only this regulatory segment. To determine whether an alternate core promoter in the LTA downstream segment directs transcription initiation of the LTA gene in vivo, LTA-specific 5′ RLM-RACE was performed using primary T cells from three unrelated individuals. Under basal conditions, five unique TVs were identified. LTA TV1 (21%), LTA TV2 (29%), and LTA TV3 (35%) that initiated at positions −43, −41, and −29, respectively, were the primary transcripts in resting T cells from one of the individuals (Fig. 3). Each of these TVs is identical in composition apart from differing TSS. Exon 1 of these transcripts includes a portion of the proximal regulatory segment (TSS to −1) plus exon 1 (+1 to +163), which is spliced to exon 2 (+451 to +558) and then to exon 3 (+645 to the LTA primer site used for amplification). LTA TV4 was the predominant transcript (98 and 100%) found in the other two individuals under basal conditions. This TV is similar in composition to LTA TV1, TV2, and TV3 except for its TSS (+6) that is within exon 1. The final transcript, LTA TV6, was present in two individuals as a minor (2%) and as a secondary (15%) species under basal conditions. The TSS (−29) and composition of this TV is similar to LTA TV3, except that exon 1 ends at position +19. The use of this alternative splice site has been reported previously and is common in LTA transcripts from P. troglodytes (22).

**FIGURE 3.** Transcription initiation in the LTA downstream segment in vivo is stimulation dependent. (A) Distribution (percentage) of the LTA mRNA TVs isolated from primary human T cells of three unrelated individuals (P1, P2, P3) under the indicated stimulation conditions. Data represent a single experiment. In two cases (P1, basal; P2, LTA−2) the experiment was repeated, as the transcript profile differed from the other two individuals for that stimulation condition. Sample number (n) represents the total number of cloned LTA mRNA transcripts sequenced from each individual under each condition. (B) A schematic representation (not to scale) of the LTA locus from −141 to +750 serves as a reference for the TVs and shows exons 1–3 (open boxes) and the translational start site (+460, ATG). The LTA sequences present in each LTA TV are indicated (filled boxes). The start and end sites of each TV that differ from the previously defined exon/intron boundaries are indicated.
Because no transcripts were identified that initiated within or after the LTA downstream segment under basal conditions from primary T cells, and because similar results were observed using the Jurkat T cell line under basal and under PMA plus ionomycin–stimulated conditions (data not shown), we hypothesized that transcription initiation mediated by the LTA downstream segment in vivo might occur under specific stimulation conditions. Therefore, the sequence near the 3’ end of the downstream segment was analyzed for binding sites to transcription factors whose activity could be induced by specific stimuli. Three putative binding sites of interest were identified (Fig. 4A): an NF-κB (position +302 to +314) binding site, Smad binding sites (+366 to +376 and +383 to +393), and a SRE (positions +356 to +370). Activation of the transcription factors that bind to these elements can be specifically stimulated by LT-α3, TGF-β1, and FGF-7, also known as keratocyte growth factor, respectively.

LT-α expressed as LT-α3 and as LT-αβ2 activates NF-κB signaling pathways by binding to the associated receptor (TNFR1 or TNFRII and LT-βR, respectively) (2, 30, 31), and LT-α3 has been shown to autoregulate the expression of its gene via a NF-kB site (−98 to −88) in the proximal promoter segment (19). For these reasons and because a potential NF-κB site (+302 to +314) may be present in the LTA downstream regulatory segment, rhLT-α3 was chosen as a stimulating agent. Four transcripts were identified after stimulation with rhLT-α3 (Fig. 3). As hypothesized, LTA TV5 was found expressed in primary T cells from two of the three individuals at an average frequency of ~23% after rhLT-α3 stimulation. The transcription start site of this TV varied slightly between positions +433 to +436 within a potential pyrimidine-rich Inr element near the 3’ end (+450) of the LTA downstream regulatory segment, continued into exon 2 and then to exon 3. Similar results were obtained with the Jurkat T cell line (data not shown). The predominant transcripts (LTA TV5 and LTA TV6) constituted ~76% of the combined transcript total found in the three individuals. LTA TV6 (TSS −29) was seen previously under basal conditions in primary T cells, whereas LTA TV5 was a novel transcript. LTA TV5 is identical to LTA TV2 with respect to its TSS (−41), but it is similar in composition to LTA TV6, as it contains the alternative exon 1 that utilizes the splice site at +19. LTA TV5 and TV6 usage did vary among the individuals examined. For instance, LTA TV6 was present at a frequency of 20 and 76.5% in individuals 1 and 3, respectively, and it was not present in the third individual. LTA TV2 (seen previously under basal conditions) was present as a minor transcript (1%) in two of the individuals. Although not the primary site of LTA autoregulation, stimulation of primary human T cells with rhLT-α3 did show that transcription initiation occurs in the LTA downstream segment in vivo and suggests that an alternate core promoter is located near the 3’ end of this regulatory segment. Furthermore, these data suggest that the LTA downstream segment alternate core promoter is stimulation dependent, as activation of NF-κB and its subsequent possible occupancy of the putative NF-kB binding site (+303 to +311) after rhLT-α3 stimulation is the likely trigger that promotes transcription.

Multiple Smad binding sites (core binding sequence GTCT) (32) potentially are located near the 3’ end of the LTA downstream segment. Two of the Smad binding sites have high matrix similarity values (MatInspector; http://www.genomatix.de) and are located from +366 to +376 and from +383 to +393 (Fig. 4A). The Smad transcription factor signaling cascade is activated by TGF-β1 binding to its receptor (32–34). Therefore, rhTGF-β1 was chosen as a stimulating agent. As suspected, the major transcript expressed by primary human T cells from the three individuals after stimulation with rhTGF-β1 was LTA TV5 (TSS +433 to +436 in the LTA downstream segment) that was found previously as a secondary transcript after rhLT-α3 stimulation (Fig. 3). Similar results were obtained with the Jurkat T cell line (data not shown). This transcript was present at or near 100% in two of the three individuals and comprised 82.5% of the LTA transcripts from the third individual. Note that stimulation of primary human T cells with rhTGF-β1 also causes a substantial increase in the expression of LT at the cell surface (Fig. 5). LTA TV6 again was observed as a minor transcript (1.2%) in T cells from one individual and as a secondary transcript (16.3%) in T cells from another individual. A novel minor (1.2%) transcript, LTA TV7, also was identified from one individual. LTA TV7 is identical in composition to LTA TV5 and TV6, except that a different TSS (+6) is used. These data confirm that transcription initiation occurs in the LTA downstream segment in vivo and further suggest the presence of an alternate core promoter near the 3’ end of this regulatory segment. Additionally, these data suggest that this LTA downstream segment alternate core promoter is a major promoter site under specific stimulation conditions. In this instance, transcription most likely is triggered by TGF-β1–mediated Smad activation and subsequent binding to the putative Smad binding site(s).

A potential SRE (+356 to +370) (35, 36) located near the 3’ end of the LTA downstream segment also was of interest (Fig. 4A). SREs are located in the regulatory regions of many of the genes that control lipid and cholesterol biosynthesis and are bound by SREBP transcription factors (35–37). FGF-7 has been demonstrated to activate SREBP, which, in turn, induces genes involved in lipid and cholesterol biosynthesis.
in lipogenesis (38, 39). The primary receptor for FGF-7 is an iso-
form of FGF receptor 2 (FGFR2) (40, 41), and it was determined
that primary human T cells express FGFR2 (data not shown).
Therefore, rhFGF-7 was chosen as a stimulating agent. Again,
similar to rhTGF-
\[\beta_1\], LTA TV8 was the major transcript expressed
by primary human T cells from the three individuals after stim-
ulation with rhFGF-7 (Fig. 3), and this stimulation also led to
a substantial increase in the expression of LT at the cell surface (Fig.
5). This transcript was present at or near 100% in two of the three
individuals and comprised 92.1% of the LTA transcripts from the
third individual. Three transcripts that were seen previously under
other stimulation conditions also were present as minor species:
LTA TV5 (0.7%) in one individual and LTA TV3 (4.5%) and LTA
TV4 (3.4%) in another individual. These data suggest that tran-
scription initiation from the LTA downstream regulatory segment is
stimulated by FGF-7–mediated activation of SREBP and subse-
quent binding to the putative SRE. More importantly, these data
provide further confirmation for the presence of an alternate core
promoter site near the 3′ end of the LTA downstream segment that is
a major promoter site under specific stimulation conditions in vivo.

LTA downstream segment–mediated expression is dependent
on an Sp1 binding site and an Inr element

Certain elements can act alone or in concert as a core promoter in
the absence of a TATA box (42,43). Further scrutiny of the 3′ end
of the LTA downstream segment indicated the presence of two
elements that could function either independently or cooperatively
as an alternate core promoter: a Sp1 binding site (+401 to +417)
and a polypyrimidine tract (+422 to +446) that is similar to an Inr
element (44) that binds TFII-I (45) (Fig. 4A). Sp1 and TFII-I both
can direct preinitiation complex (PIC) formation that recruits
RNAPII and initiates transcription from TATA-less promoters (46,
47). To examine the contribution of these sites to LTA downstream
regulatory segment–mediated expression, the impact of each site
on luciferase activity in an unstimulated T cell line was deter-
mined. Mutation of either the putative Sp1 site (LTA MutSp1) or
the Inr (polypyrimidine tract) (LTA MutInr) significantly reduced
luciferase activity regulated by the downstream segment by
∼75 and 50%, respectively (Fig. 4B). These data indicate that both the
putative Sp1 site and Inr element are integral components needed
for full transcriptional activity mediated by the LTA downstream
regulatory segment. Furthermore, these data suggest that these
regulatory elements may act cooperatively to form the alternate
core promoter site near the 3′ end of the LTA downstream segment
because mutation of either element reduced, but did not com-
pletely abrogate, expression.

Sp1, TFII-I, and RNAPII bind in vivo to the LTA locus in the
region containing the proposed alternate core promoter

To establish whether Sp1 and TFII-I transcription factors bind to
the LTA downstream regulatory segment in vivo, ChIP assays were
performed using the Jurkat T cell line. If either or both of these

**FIGURE 5.** FGF-7 and TGF-\[\beta_1\] stimulation increase the expression of the membrane-bound form of LT on primary human T cells. Shown
are representative examples of the expression of LT by primary human T cells from patient 1 (A–
D) and patient 2 (E, F) that were left unstimu-
lated (gray shading) or were stimulated (no
shading) with FGF-7 (A, C, E) or with TGF-\[\beta_1\]
(B, D, F). Stimulated T cells stained only with
PE-conjugated goat anti-mouse IgG secondary
Ab were used as the negative control (black
shading). All examples show cell surface
staining with a LT-\[\alpha\]–specific Ab. Comparable
staining was achieved with a LT-\[\beta\]–specific Ab
followed by the secondary Ab (not shown).
Expression of LT was substantially increased
after 6 h stimulation (A, B, E, F) and signifi-
cantly increased after 48 h stimulation (C, D).
T cells isolated from patient 3 (not shown)
displayed similar levels of LT expression before
and after stimulation. LT expression was found
to increase steadily from 6 to 48 h where ex-
pression levels peaked. LT expression levels
were in decline 72 h after stimulation (not
shown).
transcription factors participate in the LTA downstream segment promoter site, it would be anticipated that RNAPII also would be recruited to this region. Therefore, a RNAPII-specific immunoprecipitation was included. An LTA region that includes the potential Sp1 site and Inr element (Fig. 6A) was successfully amplified after Sp1- and TFII-I–specific ChIP (lanes 1 and 2) of genomic DNA extracted from unstimulated Jurkat T cells. Amplification of this LTA region also was achieved after immunoprecipitation with an RNAPII-specific Ab (lane 3). GAPDH promoter amplification (positive control, lane 7), and the absence of CNAP1 promoter amplification (negative control, lane 11) after RNAPII-specific ChIP illustrate assay specificity. These data show that this segment of the LTA gene contains binding sites for Sp1 and TFII-I and recruits RNAPII.

Because transcripts that initiate from the LTA downstream segment alternate core promoter were found only after specific stimulation of T cells, it was somewhat unexpected that major components of a PIC were bound to this alternate core promoter in the Jurkat T cell line without prior stimulation. Therefore, transcription factor binding was assessed in primary human T cells. In the Jurkat T cell line, which constitutively expresses the LTA gene and can act in vitro as an independent regulatory unit. In primary human T cells, the downstream segment was determined to be of consequence in vivo to stimulation-specific LTA expression based on the transcripts characterized in this study.

The ability of the LTA downstream segment to act as an independent regulatory unit and the report of a transcript (BC034729.1) (25) with a TSS (+454) that is in the LTA downstream segment near the 3′ end led us to hypothesize that an alternate core promoter was present. In our study, a transcript (LTA TV8) was identified from primary T cells of three unrelated individuals and from Jurkat T cells (data not shown) with a TSS that varied slightly from +433 to +436 in the locus (+1 to +459) in an analysis of LTA regulatory region activity in T and B cell lines. The downstream segment was found to be an essential component of LTA transcriptional regulation. First, the significant reduction of luciferase activity for all constructs that lacked this segment regardless of lymphocyte cell type or activation state demonstrates that the downstream segment is required for maximal LTA regulatory activity. Second, the ability of the downstream segment alone also to yield maximal luciferase activity again regardless of lymphocyte cell type or activation state indicates that this segment contains all of the elements necessary for full transcriptional expression of the LTA gene and can act in vitro as an independent regulatory unit. In primary human T cells, the downstream segment was determined to be of consequence in vivo to stimulation-specific LTA expression based on the transcripts characterized in this study.

**Discussion**

Previous investigations of LTA regulatory region activity have included various portions of the LTA locus from −915 to +268 (16, 20, 27, 28). In the present study, we focused on the region between the transcription and translation start sites, the downstream segment (+1 to +459) in an analysis of LTA regulatory region activity in T and B cell lines. The downstream segment was found to be an essential component of LTA transcriptional regulation. First, the significant reduction of luciferase activity for all constructs that lacked this segment regardless of lymphocyte cell type or activation state demonstrates that the downstream segment is required for maximal LTA regulatory activity. Second, the ability of the downstream segment alone also to yield maximal luciferase activity again regardless of lymphocyte cell type or activation state indicates that this segment contains all of the elements necessary for full transcriptional expression of the LTA gene and can act in vitro as an independent regulatory unit. In primary human T cells, the downstream segment was determined to be of consequence in vivo to stimulation-specific LTA expression based on the transcripts characterized in this study.

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![Image](https://example.com/image.png)
portion of the downstream segment in vivo, that RNAPII is recruited to the same region in vivo, and that mutation of either the pyrimidine tract (Inr) or the Sp1 site significantly reduced luciferase activity directed by the LTA downstream segment in vitro assays. Taken together with the identification of LTA TV8 from three separate individuals, these data clearly indicate that an alternate core promoter is formed by the combination of the Sp1 site and the Inr element that cooperatively direct transcription initiation of the LTA gene from the downstream segment. A similar configuration appears to serve as the core promoter for the 5'-lipooxygenase gene (58).

The LTA downstream segment alternate core promoter was demonstrated to be stimulation dependent, as LTA TV8 was found only after specific stimuli were used to activate both primary human T cells and the human Jurkat T cell line (data not shown). Stimulation with rhLT-α3, rhTGF-β1, and rhFGF-7, but not with PMA plus ionomycin (only tested in Jurkat cells; data not shown), induced expression of LTA TV8. Interestingly, ChIP assays suggest that somewhat different mechanisms are used to pre-engage the downstream segment alternate core promoter for transcription in primary human T cells versus the transformed human Jurkat T cell line. In primary T cells, Sp1 appears to constitutively occupy its binding site, as it was detected in both basal and rhFGF-7-stimulated nuclear lysates. Only after stimulation with rhFGF-7 was TFII-I and RNAPII binding detected to the LTA downstream segment. These data suggest that Sp1 may serve to keep the downstream segment alternate core promoter accessible in the basal state and to direct nucleation of the PIC after specific cellular stimulation. In support of this mechanism, the Sp1 site was found to have a greater impact when mutated on luciferase expression mediated by the LTA downstream segment than the Inr element. In contrast, the alternate core promoter in the Jurkat T cell line apparently is constantly occupied by the PIC, as Sp1, TFII-I, and RNAPII binding were detected under basal conditions, yet LTA TV8 was detected only after specific stimulation. These data indicate that an assembled PIC is formed at the LTA downstream segment alternate core promoter in transformed T cells, but it is stalled until specific signals direct the transition to elongation. This difference is not unexpected because even under basal conditions transformed cells could be considered to be in a more activated state compared with primary cells. Genome-wide studies have shown that RNAPII containing PICs permanently bind to certain gene promoters, initiate transcription, and await signals that prompt elongation (59, 60). Sp1 being prebound to the downstream segment alternate promoter in primary T cells may serve a similar purpose that enables this highly inducible gene to bypass the complex process of chromatin remodeling, allowing for rapid nucleation of the PIC and response to specific extracellular signals (42, 61).

Several alternate core promoters appear to guide LTA expression. The initiation sites of the transcripts identified in this study suggest that there are three active core promoters in the LTA regulatory region, likely serves as the alternate core promoter for these transcripts. The third active core promoter was characterized in our study. It is located in intron 1, consists of an Sp1 site and an Inr element, and orchestrates transcription of LTA TV8 under specific stimulation conditions. Other core promoters may be present in the LTA locus as suggested by the starting nucleotide of LTA cDNA sequences reported in GenBank. For instance, the transcript that initiates at position −185 (NM_001159740.1) lies within a potential Inr element (−188 to −182; CTCAGCT) and is downstream of a possible Sp1 binding site (−229 to −222; CCCCAGCCC), whereas the transcripts that initiate at positions +102 (X01393) and +115 (D00102.1) are located downstream of another potential Sp1 binding site (+75 to +83; CCGCCCCGCG). Use of these possible alternate core promoters also may depend on specific cellular stimulation.

Data from our study suggest that the composition of exon 1 of LTA mRNA transcripts may play a major role in the regulation of LT-α expression at the level of translation. It was striking that the preponderance (94.8%) of LTA transcripts (LTA TV1–TV4) identified in this study from resting (basal) primary human T cells regardless of the TSS included sequences to the end (163) of the original exon 1 that encodes most of the 5’UTR (see Fig. 3). Under stimulated conditions, however, there was a consistent transition to LTA transcripts (99%) that excluded all or most of exon 1 either by utilizing the alternate core promoter in the downstream segment (LTA TV8) or the alternate splice site at +19 (LTA TV5–TV7), respectively. A comparable transcript distribution was obtained with the Jurkat T cell line under basal versus stimulated conditions (PMA plus ionomycin, rhLT-α3, and rhTGF-β1; data not shown). A portion (+1 to +115) of exon 1 has been shown to be necessary for HIV-1 Tat transactivation of LTA expression (20) and, in that study, modeling suggested that this portion of exon 1 can form a very stable stem-loop when transcribed into RNA that has similarities in both sequence and secondary structure to the HIV-1 Tat responsive element. When included in LTA transcripts under basal conditions, this section of the 5’UTR could form secondary structure in the mRNA that serves to minimize LT-α expression by simply impeding the progress of the 40S ribosomal subunit via secondary structure or by binding a protein that blocks translation as in the case of dsRNA-dependent protein kinase R binding to Tat responsive element (62–64). Utilization of the alternative splice site at +19, which appears to be a major alternative splice site in both humans and in P. troglodytes (22), or utilization of the LTA downstream segment alternate core promoter would exclude mRNA sequences requisite for secondary structure formation. These data suggest that the composition of the 5’UTR has an important role in regulation of LTA expression and that in transcripts under stimulated conditions exclusion of sequences necessary to form secondary structure in the 5’UTR facilitates translation of the LTA mRNA and increases LT-α expression. Thus, protein levels could dramatically change on cellular stimulation with little change in LTA mRNA levels simply by altering the composition of exon 1.

The principal outcome of LT-α signaling via either the TNFRs or the LT-βR is activation of NF-κB intracellular signaling pathways.
(2, 30, 31) that target LT-α–responsive genes, including LTA itself (19). LTA autoregulation requires an NF-κB binding site (~98 to −88) located in the proximal promoter segment upstream of the TATA box. Interestingly, NF-κB also can promote transcription initiation by cooperation and direct interactions with TFII-I (65) and with Sp1 (66, 67). Because a potential NF-κB binding site might be present ~125 nucleotides upstream of the LTA downstream segment alternate core promoter, the contribution of the alternate promoter to LTA autoregulation was assessed. Consistent with the study by Messer et al. (19), the preponderance of transcripts (LTA TV2, TV5, and TV6) identified in this study initiated downstream of the NF-κB binding site in the proximal regulatory segment. However, the LTA downstream segment alternate promoter did make a secondary contribution (~23% of the transcripts were LTA TV8) to autoregulation of LTA expression in two of the three individuals examined. The reason for the lack of transcripts initiating from the LTA downstream segment alternate promoter under these conditions in one of the individuals is unclear. One possibility is that genetic differences in the LTA regulatory region between individuals might influence the preference for a particular promoter (27–29, 68). Note that there also was disparity in the preferred alternate core promoter used under basal conditions among the three individuals. In the Jurkat T cell line, the downstream segment alternate promoter made an equal contribution (57%) to LTA autoregulation (data not shown). The increased percentage of transcripts from the downstream segment alternate promoter in Jurkat T cells versus primary T cells likely is a consequence of a pre-engaged PIC at the LTA downstream segment alternate promoter in the transformed T cell line, but not in primary T cells under basal conditions. Although not the primary target, these data do indicate that NF-κB via LT-α, stimulation can activate transcription from the downstream segment alternate core promoter in primary and transformed human T cells and that a functional NF-κB site likely is present in the LTA downstream regulatory segment.

Certain stimuli, however, were found to preferentially and specifically target expression from the LTA downstream segment alternate core promoter. One of these stimuli was TGF-β1, for which LTA TV8 comprised ~94% of the total LTA transcripts. TGF-β1 signaling through the type I receptor leads to the phosphorylation of Smad proteins that translocate to the nucleus to regulate transcription (32–34). Smads can bind directly to DNA via the SBE (GTCTAGAC) or to single or multiple copies of the sequence GTCT (32, 69). Smad proteins also can activate TGF-β1–responsive genes by interacting with a variety of DNA binding partners, such as Ap-1 (70, 71), Sp1 (72), and TFII-I (73). In this study, a Sp1 site and an Inr element were shown to be key in expression regulated by the LTA downstream segment, and Sp1 and TFII-I were shown to bind this segment in vivo. Because determining whether the putative Smad binding sites in the LTA downstream regulatory segment contribute to TGF-β1–mediated LTA transcriptional activation was not in the scope of this investigation, either mechanism could be responsible for activation of transcription from the LTA downstream segment alternate core promoter. Nonetheless, transcriptional activation of LTA by TGF-β1 is not surprising because both cytokines, for instance, play a critical role in IgA production (74, 75). Both LT-α and LT-βR knockout mouse display IgA deficiency (2, 74) that is apparently the result of impaired homing of B cells to the lamina propria (3, 4), whereas TGF-β1 induces Ig isotype switch to IgA (76–78). Thus, the coordinated expression and action of these cytokines by lamina proprial T cells and stromal cells (3, 79) would provide a microenvironment conducive for homing and maturation of IgA precursor B cells in the gut.

FGF-7 was the other stimulating agent that preferentially and specifically targeted expression from the LTA downstream segment alternate core promoter, as LTA TV8 comprised 97.5% of the transcripts isolated under this condition. Although it is easy to conceive of a situation that requires the coordinated expression of LT-α and TGF-β1, what circumstances would necessitate the coexpression of LT-α and FGF-7? One prospective scenario for cooperation between this cytokine and growth factor is in lipid and cholesterol synthesis. It was intriguing that primary human T cells express FGFR2 (data not shown) and that a potential SRE (+356 to +370) was located directly upstream of the LTA downstream segment alternate core promoter. SREs are bound by SREBP transcription factors that regulate the expression of enzymes involved in lipid and cholesterol synthesis (35–37). FGF-7 is a potent growth factor for cells of epithelial origin that express the FGF-7 receptor (FGFR2-IIIb) (80) and has been found to increase expression of lipogenic enzymes and transport proteins, as well as the transcription factor SREBP1 (38, 39). One of the receptors for LT signaling, LT-βR, is expressed primarily by epithelial and stromal cells (81), and administration of LT to epithelial cells in culture has been shown to increase lipid and cholesterol levels in the cell membrane (82). The impact of LT may be due in part to regulation of lipase genes, as disruption of LT signaling pathways via the LT-βR leads to hyperlipidemia and hypercholesterolemia and impacts the expression of the hepatic lipase gene (13). These data and our findings suggest that signaling via FGFR2 on T cells by growth factors, such as FGF-7, may regulate LTA expression via the putative SRE to coordinate the necessary signals that regulate sterol and lipid biosynthesis during epithelial cell growth, proliferation, and differentiation to provide the building blocks for cell membranes.

Another circumstance that likely requires the coordinated actions of FGF-7 and LT-α is in the development and maintenance of the thymus. Signaling via both the FGFR2-IIIb and the LT-βR has been shown to be necessary for proliferation and differentiation of thymic epithelial cells (TECs), particularly in the medulla (83, 84). The thymic medulla is a dynamic structure in which there is continual turnover and replenishment of the TECs that necessitates persistent orchestration of TEC proliferation and differentiation. In LT-βR, FGF-7, and FGFR2-IIIb knockout mice, there is a breakdown of thymic microarchitecture and development, as well as severely impaired proliferation and differentiation of TECs (83–85). Part of their impact appears to be due to regulation of the expression of medullary TEC-specific genes necessary for a differentiated phenotype (84, 86). Thus, the coordinated expression and actions of LT-α and FGF-7 would maintain the integrity of the thymic medulla. Finally, FGF-7 and LT-α could cooperate in the process of wound healing. It is well established that FGF-7 plays a substantial role in the wound repair process, especially in the skin and intestinal tract. FGF-7 not only protects the epithelium to allow repair and lessen the potential for infection, but it also promotes proliferation, migration, and differentiation of epithelial cells facilitating re-epithelialization of the wound (87, 88). It also is apparent that epidermal T cells have an active role in, and are necessary for, normal wound healing (89, 90). For instance, epidermal T cells isolated from acute wounds are active and promote healing in skin organ culture, whereas epidermal T cells isolated from chronic wounds are unresponsive (91). Furthermore, T cell depletion studies have shown that mechanical strength and collagen deposition are reduced during wound healing (90). However, what specific role might LT produced by epidermal T cells have in the wound healing process? Although no specific role for LTA has been ascribed to wound healing, it could be ascertained that LT-α signaling performs functions in the wound healing process similar
to those critical to liver regeneration (92, 93), to development of secondary lymphoid tissues (2), and to regulation of lipid and cholesterol synthesis necessary for cell growth as discussed above.

This study has shown that LTA expression is a complex process. Eight novel LTA transcript variants were identified that likely originate from three different alternate core promoters. It was demonstrated that one of these alternate core promoters is located in the LTA downstream segment, is composed of a Sp1 binding site and Inr element that binds TFIID, and is stimulation dependent. Most importantly, this study has much broader implications, as it opens the door to examining the role of LT-α signaling by lymphocytes in processes typically associated with signaling by the TGF-β receptor and by the FGF-7 receptor, FGFR2.

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Disclosures

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