Published in final edited form as:
Arterioscler Thromb Vasc Biol. 2010 March ; 30(3): 395–402. doi:10.1161/ATVBAHA.109.191395.

Mouse Aorta Smooth Muscle Cells Differentiate Into Lymphoid Tissue Organizer-Like Cells on Combined Tumor Necrosis Factor Receptor-1/Lymphotoxin *β*-Receptor NF-*κ*B Signaling

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Abstract

Objective—Mouse aorta smooth muscle cells (SMC) express tumor necrosis factor receptor superfamily member 1A (TNFR-1) and lymphotoxin *β*-receptor (LT*β*R). Circumstantial evidence has linked the SMC LT*β*R to tertiary lymphoid organogenesis in hyperlipidemic mice. Here, we explored TNFR-1 and LT*β*R signaling in cultured SMC.

Methods and Results—TNFR-1 signaling activated the classical RelA NF-*κ*B pathway, whereas LT*β*R signaling activated the classical RelA and alternative RelB NF-*κ*B pathways, and both signaling pathways synergized to enhance p100 inhibitor processing to the p52 subunit of NF-*κ*B. Microarrays showed that simultaneous TNFR-1/LT*β*R activation resulted in elevated mRNA encoding leukocyte homeostatic chemokines CCL2, CCL5, CXCL1, and CX3CL1. Importantly, SMC acquired features of lymphoid tissue organizers, which control tertiary lymphoid organogenesis in autoimmune diseases through hyperinduction of CCL7, CCL9, CXCL13, CCL19, CXCL16, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1. TNFR-1/LT*β*R cross-talk resulted in augmented secretion of lymphorganogenic chemokine proteins. Supernatants of TNFR-1/LT*β*R–activated SMC markedly supported migration of splenic T cells, B cells, and macrophages/dendritic cells. Experiments with *ltbr*$^{-/-}$ SMC indicated that LT*β*R-RelB activation was obligatory to generate the lymphoid tissue organizer phenotype.

Conclusion—SMC may participate in the formation of tertiary lymphoid tissue in atherosclerosis by upregulation of lymphorganogenic chemokines involved in T-lymphocyte, B-lymphocyte, and macrophage/dendritic cell attraction.

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Supplement information is available on the ATVB web site (http://atvb.ahajournals.org). Microarray data have been deposited in NCBI GEO (www.ncbi.nlm.nih.gov/geo/) and are accessible through GEO series accession number GSE19139. GEO makes supplement files available for FTP download by series accession at the following FTP site: ftp://ftp.ncbi.nih.gov/pub/geo/DATA/supplement/series.

Disclosure
None.

Keywords

smooth muscle cells; immune response; chemokines; signaling pathways; tumor necrosis factor receptor; lymphotoxin-β receptor

Most studies of atherosclerosis have focused on intima lesions, which are composed of lipid-laden macro-phage/foam cells, T cells, and smooth muscle cells (SMC). Plaque leukocytes[1,2] interacting with SMC[3–6] are thought to initiate adaptive immune responses toward arterial wall-derived antigens. However, evidence that macrophage/foam cells/dendritic-like cells (DC)[7] and T cells may mediate antigen-dependent T-cell responses within plaques remains circumstantial. The anatomic location for the initiation of antigen-dependent B-cell responses is similarly puzzling,[8,9] and impacts of antigen-specific T and B cells on disease progression remain to be delineated.[8] T-cell and B-cell responses typically require antigen-presenting DC in T-cell areas, follicular DC in activated germinal centers of B-cell follicles, and high rates of T-cell recirculation, none of which has been shown to occur in atherosclerotic plaques.[1,2,8,10] Although immune responses toward atherosclerosis may occur in lymph nodes or spleen, evidence to support this possibility is limited.[8] Thus, how and where (auto)immune reactions generate self-reactive T cells and B cells to trigger plaque instability and myocardial infarction are all important issues that remain to be defined.

We and others[11–13] reported that T-cell and B-cell aggregates emerge in adventitia of aorta segments adjacent to atherosclerotic lesions of apolipoprotein E-deficient (apoE$^{-/-}$) mice. These aggregates were precursors of well-structured aorta tertiary lymphoid organs that showed a high degree of organization akin to lymph nodes.[14] These data provided evidence that aorta tertiary lymphoid organs may organize antigen-dependent T-cell and B-cell (auto) immune responses toward atherosclerosis.[14] In addition, medial SMC underlying intimal plaques became activated and expressed the lymphorganogenic chemokines CXCL13 (B-lymphocyte chemoattractant) and CCL21 (secondary lymphoid tissue chemokine).[14,15] Moreover, aorta tertiary lymphoid organ integrity depended on the lymphotoxin β-receptor (LTβR).[15–17] Together, these data indicate that media SMC were activated by plaques to express features of lymphoid tissue organizers (LTO) through an LTβR-dependent NF-κB signaling pathway.[14,18–21]

Mesenchymal cell-derived LTO have been studied during embryonic lymphoid organ neogenesis, when they interact with CD3^{-}/CD4^{+} hematopoietic cells referred to as lymphoid tissue inducer cells to give rise to secondary lymphoid organs.[10] It has been proposed that LTO also may be functional in adult animals in chronic inflammatory diseases and infection. Activated synoviocytes in rheumatoid arthritis and intestinal fibroblasts in inflammatory bowel disease recapitulate features of embryonic LTO by controlling tertiary lymphoid organ neogenesis.[10,13–21] Importantly, action of LTO in tertiary lymphoid organs is often associated with tissue destruction and significant disease pathology.[14] Here, we examined the effects of tumor necrosis factor (TNF) and of an agonistic anti-LTβR mAb (α-LTβR) in cultured mouse aorta SMC. Our data show that SMC stimulated by TNF and α-LTβR, but not with either TNF or α-LTβR alone, adopt a phenotype that strikingly resembles LTO.

Materials and Methods

Mice

Mice on the C57BL/6J background were from The Jackson Laboratories and housed and fed as reported.[14] *Ltbr*$^{-/-}$ mice[21] were a kind gift from Klaus Pfeffer, Institute for Medical Microbiology, Heinrich-Heine University of Düsseldorf, Germany. Animal procedures were approved by the Animal Care and Use Committee of Thuringia.

Cell Culture

SMC were obtained from aortae of 8- to 12-week-old C57BL/6 mice by sequential dissection and enzyme digestion.[14] Aortic endothelial cells were harvested by scraping using a Teflon policeman before digestion. SMC were used at passages 1 to 3 and purity was ≥99% as shown by α-smooth muscle actin positivity of cytospins. Cells were stimulated with 10 μg/mL agonistic rat anti-mouse-LTβR mAb (α-LTβR)[22] or 1 ng/mL mouse recombinant TNF (R&D Systems) as indicated.

Assays

Quantitative reverse-transcription polymerase chain reaction was performed as described using primers as reported in the Data Supplement, available online at http://atvb.ahajournals.org.[14] Enzyme-linked immunosorbent assays were performed as recommended by the supplier (R&D Systems). Splenocyte migration was analyzed according to Guo et al[23] and detailed in the supplementary materials. FACS and microarray analyses were performed as described previously and are detailed in the supplementary materials.[14,24]

Results

SMC TNFR-1 and LTβR Signaling Differentially Activates Classical and Alternative NF-κB Pathways

As mouse aorta SMC express TNFR-1 and LTβR in vivo and in vitro,[14] we examined their signaling. TNF increased p100 protein levels and triggered rapid and complete IκBα degradation, indicating that TNF activated the classical NF-κB pathway (Figure 1A, left). In contrast, α-LTβR induced processing of the p100 inhibitor to the p52 subunit of NF-κB and triggered a moderate decrease in IκBα levels, indicating that α-LTβR predominantly activated the alternative NF-κB signaling pathway (Figure 1A, right). Furthermore, TNF strongly induced nuclear translocation of RelA within 30 minutes, as shown by Western blotting of nuclear extracts (Figure 1B, left). In contrast, α-LTβR also induced RelA translocation to the nucleus, albeit to a lesser extent, but predominantly induced nuclear translocation of RelB at late time points (Figure 1B, right). Early TNFR-1–induced and delayed LTβR-induced nuclear translocation of RelA and RelB, respectively, was confirmed by immunofluorescence staining of SMC (Figure I). Electrophoretic mobility shift assay supershift analysis confirmed TNF-induced RelA but not RelB NF-κB binding within 30 minutes (Figure 1C, left). In contrast, α-LTβR induced both RelA and RelB complexes, although with slower kinetics (Figure 1C, right). These data show that in SMC TNFR-1 signaling activated the classical NF-κB pathway, whereas LTβR signaling resulted in the activation of classical RelA and alternative RelB NF-κB pathways. Cross-talk of TNFR-1 and LTβR signaling was reported in embryonic fibroblasts to occur through upregulation of RelB and p100 levels by TNF in combination with enhanced p100-to-p52 processing by α-LTβR.[25–28] To examine TNFR-1/LTβR signaling cross-talk, we analyzed cytoplasmic and nuclear p100, p52, RelB, and RelA levels after treatment with TNF, α-LTβR, or both. Whereas TNF treatment (24 hours) markedly increased cytoplasmic p100 and RelB levels without inducing nuclear translocation, combined TNFR-1/LTβR stimulation resulted in p100 degradation and significantly increased nuclear accumulation of p52 and RelB compared to LTβR signaling alone. In contrast, nuclear RelA levels were only marginally increased on combined TNFR-1/LTβR activation (Figure 1D). Thus, TNFR-1 and LTβR in SMC differentially engage the classical and alternative NF-κB pathways, respectively, and they synergize to enhance nuclear translocation of p52 and RelB.

TNFR-1 and LTβR Signaling Induces Differential Transcription Responses

To assess kinetics of TNFR-1– dependent vs LTβR-dependent transcription responses, preliminary microarray screening experiments were performed in single-array experiments.

SMC were stimulated with TNF or α-LTβR, and microarrays were prepared at 2, 6, and 24 hours following MIAME guidelines (www.ncbi.nlm.nih.gov/geo/info/MIAME.html; data were deposited in National Center for Biotechnology Information's gene expression omnibus, GEO accession number GSE19139). TNF induced a stronger response at 2 and 6 hours when compared to 24 hours. In contrast, α-LTβR induced a muted response at 2 and 6 hours and a robust response at 24 hours (Figure IIA–F; Table I). Thus, TNF-induced and LTβR-induced transcription followed kinetics that paralleled their NF-κB signaling kinetics (compare Figure 1A–D and Figure IIA–F). Accordingly, we chose the 24-hour time point to explore transcription after TNFR-1 and LTβR activation in detail. SMC were stimulated with each agonist alone or with a combination of TNF/α-LTβR, and microarrays were prepared. TNF induced 86, α-LTβR induced 23, and both agonists induced 177 genes (Figure III; Table II). These data show that the combination of TNF/α-LTβR elicited transcription of 85 previously untranscribed genes at 24 hours. Moreover, for 40 genes both agonists hyperinduced mRNA expression (Figure 2A; Table III). Three groups of TNF/α-LTβR–induced genes were apparent: TNF-dominated group (Figure 2A; no hyperinduction; TNF>α-LTβR); α-LTβR–dominated group (Figure 2A; no hyperinduction; α-LTβR>TNF); and hyperinduction group (TNF/α-LTβ; $R \geq 1.5$-fold the sum of gene expression after single stimulation; Figure 2A; Table III). Thus, concomitant activation of TNFR-1/LTβR hyperinduced transcription of 40 genes and induced transcription of 85 mRNA that were not significantly expressed in quiescent SMC or SMC exposed to each agonist alone.

Differentiation to LTO-Like SMC

We inspected those genes that showed a statistically significant hyperinduced response[29–31] (Figure 2A; Table III). TNF or α-LTβR, when added alone, induced small or no increases in *cxcl13* and *ccl19* mRNA levels (Figure 2); however, when incubated with TNF and α-LTβR, there was a marked supra-additive increase in *cxcl13* and *ccl19* mRNA (Figure 2A; lower heat map at right). Similar responses were observed for myeloid homeostatic chemokines *ccl2* (MCP-1), *ccl5* (RANTES), *ccl7* (MCP-3), *ccl9* (MIP-1γ), *cxcl1* (Groα), *cxcl10* (IP10), *cxcl16* (SR-PSOX; scavenger receptor for oxidized low-density lipoprotein), and for the interferon-γ–inducible genes *gbp3*, *gbp6*, and *mpa2l* (Figure 2). Hyperinduced mRNA included adhesion molecules *vcam1* and *icam1* (Figure 2A; Table III). In addition, matching the upregulated genes with public data banks, we observed a large interferon signature (www.interferome.com; for ease of reading, only the top 40 genes are displayed as heat map in Figure 2C; see Table III). This indicates that multiple agonists may affect the LTO phenotype of SMC in addition to TNF/α-LTβR. We next analyzed the induced transcriptomes in a more global way. When gene ontology terms related to immune responses were analyzed, significant numbers of genes were upregulated (Figure 2D). Moreover, when gene ontology terms chemokine activity and cytokine activity were inspected, 10 chemokine genes were hyperinduced (Figure 2D). Notably, and not represented in Figure 2, SMC constitutively express other lymphorganogenic genes at high levels, including *tnfr1*, *ltbr*, and *cxcl12* (stromal-derived factor 1), whose signal intensities are available at the GEO data bank (GEO accession number GSE19139). Interestingly, chemokine receptor CXCR7 known to bind CXCL12 was found to be the only chemokine receptor to be constitutively expressed by SMC at high levels. Finally, genes in gene ontology terms related to inflammation were markedly induced (Figure 2D). These data show that cross-talk of TNFR-1 and LTβR signaling hyper-induces genes that are known to control a wide range of immune responses, lymphorganogenesis, lymphocyte homeostasis, stromal–lymphocyte interaction, and autoimmunity. To examine the specificity of the α-LTβR reagent (Figures 1, 2) we used SMC prepared from *ltbr*$^{-/-}$ mice.[32,33] The *ltbr*$^{-/-}$ SMC responded toward TNF but not toward α-LTβR (Figure IV). Applying rather strict filter criteria, a small group of genes was downregulated by TNF, α-LTβR, and, to a larger extent, by a combination of TNF/α-LTβR including lipoprotein lipase (*lpl*), BMP, and activin membrane-bound inhibitor involved in second heart field mesoderm signaling (*bambi*), and

pregnancy-associated plasma protein A (*pappa*) associated with atherosclerosis and vascular injury (Figure 2E). These data demonstrate that genes controlling lymphorganogenesis and a variety of known arterial wall biology-related genes in vivo either are constitutively expressed in SMC or are hyperinduced by a combination of TNF/α-LTβR. These transcription responses appear to be SMC-specific, because similar experiments using aortic endothelial cells did not show comparable transcription responses or an LTO phenotype, although several chemokines were induced in both cell types (Figure V; Table IV). Although we found in preliminary experiments that both human aorta and saphenous vein SMC and human umbilical vein endothelial cells expressed both TNFR-1 and LTβR at levels that were similar to those expressed by their mouse aorta counterparts, available reagents to activate the human LTβR yielded weak responses. Unfortunately, this observation precludes a comprehensive examination of a synergistic activation response of TNFR members in primary human vascular cells at this time.

Prolonged Hyperinduction of mRNA by TNF/α-LTβR Signaling

To verify major microarray data, we analyzed mRNA levels of selected genes by quantitative reverse-transcription polymerase chain reaction. For each mRNA, there were small effects when each agonist was added alone but pronounced actions of both agonists (Figure 3). Distinct mRNA showed different kinetics and quantitative responses (Figure 3). Vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 FACS analyses revealed a marked TNF effect for the surface expression of these adhesion molecules (Figure VI). Although *ccl21* was found to be expressed by medial SMC in hyperlipidemic mouse aortae in vivo,[14] it was not detectable in SMC. This observation is consistent with data from mouse fibroblasts,[31,34] suggesting that *ccl21* expression cannot be recapitulated under these culture conditions.

Hyperinduction of De Novo Synthesis and Prolonged Secretion of CCL5, CX3CL1, CXCL13, and CCL19

We chose selected lymphorganogenic genes to examine protein hyperinduction. CCL5 determined at 6 hours was undetectable in control or in α-LTβR–stimulated SMC but was secreted in TNF-stimulated SMC at low levels and progressively increased in TNF/α-LTβR–stimulated SMC (Figure 4). For CXCL13 and CCL19, the kinetics of chemokine accumulation were comparable to CCL5 with a pronounced hyperinduction. Similarly, CX3CL1 was absent at 6 hours but it became detectable after 24 hours of TNF/α-LTβR stimulation, further increasing up to 72 hours (Figure 4).

Activated SMC Promote Migration of Naive Splenic T Cells, B Cells, and Macrophages/DC Through Soluble Chemotactic Molecules

To examine whether the LTO SMC phenotype (Figures 1–4) resulted in biological activity toward leukocytes, supernatants of SMC were examined in a migration assay using naive splenocytes from young C57BL/6J mice as targets. There was no difference in chemotactic activity between cell-free medium and unstimulated SMC, indicating that quiescent SMC did not elaborate significant chemotactic activity toward total splenocytes (Figure 5, upper left). However, TNF, α-LTβR, and both TNF/α-LTβR caused elaboration by SMC of marked migration activity toward total splenocytes (Figure 5, upper left). In view of our hypothesis that SMC may acquire an LTO-like phenotype, we next examined the leukocyte lineage composition of migrated cells by FACS using CD3 for total T cells, CD19 for B cells, and CD11b for macrophages/DC. For splenic T and B lymphocytes, there was a significant effect for TNF and α-LTβR, and a supra-additive effect of the combination of TNF/α-LTβR (Figure 5, upper right and lower left). Moreover, splenic $CD11b^+$ macrophages/DC strongly responded to supernatants of SMC stimulated with TNF, α-LTβR, and both agonists (Figure 5, lower

right). These data demonstrate that activated—but not quiescent—SMC elaborate soluble chemotactic molecules toward T cells, B cells, and macrophages/DC.

Discussion

Here, we show that mouse aorta SMC stimulated with TNF/α-LTβR differentiate into a phenotype that strikingly resembles LTO.[10, 18–20, 35] The LTO phenotype resulted from cross-talk of the classical and alternative NF-κB signaling pathways, most likely through RelA and RelB dimer complex-mediated transcription at target gene promoters. The majority of genes with additive, synergistic, and newly recruited mRNA induction patterns mediate inflammation, leukocyte adhesion, autoimmunity, lymphocyte homeostasis, or lymphoid organ neogenesis in vivo6,31,36–41 (Table V). These data strongly support our hypothesis that activated SMC may participate in the immunologic characteristics of the diseased arterial wall by defining a signaling pathway, its resulting transcription responses, and the elaboration of chemotactic activity toward 3 leukocyte lineages involved in inflammation and tertiary lymphoid organogenesis (Figure 6).

During development[10] and maintenance of secondary lymphoid organs, hematopoietic cells identified as lymphoid tissue-inducer cells interact with mesenchymal LTO to coordinate lymphoid organ growth and organization. LTO in these organs include myofibroblastic reticular cells in T-cell areas.[10] Yet, the identity of the lymphoid tissue-inducer cells in the artery wall that interact with medial SMC-differentiated LTO remains to be uncovered.[14] Our data provide the first comprehensive delineation of transcriptional cross-talk after combined TNFR-1/LTβR activation in any cell type and confirm and extend previous in vivo data of activated SMC by defining a selected set of genes that might mediate tertiary lymphoid organogenesis in atherosclerosis.

Conclusion

The hyperinduction of SMC genes coding for lymphorganogenic chemokines provides strong support for the cross-talk via the NF-κB signaling network model proposed by Basak and Hoffmann.[26–28] The conclusion that the alternative LTβR NF-κB pathway was critical in determining the LTO-like phenotype was based on pharmacological (α-LTβR) and genetic (*ltbr*$^{-/-}$ SMC) evidence together with the finding that TNFR-1 activation triggered the classical but not the alternative NF-κB pathway, whereas LTβR activation triggered both.

Given the complexities of NF-κB signaling cross-talk in SMC and the known activity of NF-κB not only in SMC but also in endothelial cells and macrophage/foam cells, it is not surprising that the role of NF-κB in atherogenesis has produced controversial results in hyperlipidemic mouse models.[42–46] The remarkable ability of SMC to modify their phenotype in vivo depending on environmental conditions has been widely recognized.[3–5] Our data provide evidence for a new role of media SMC, ie, to function as LTO that translate inflammatory cues from plaques and convey them as lymphorganogenic signals to the adventitia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Sources of Funding

This work was supported by the German Research Organization (HA 1083/15-1; WE2224/5; SP 713/4-1) to A.J.R.H., R.G., F.W., and R.S.

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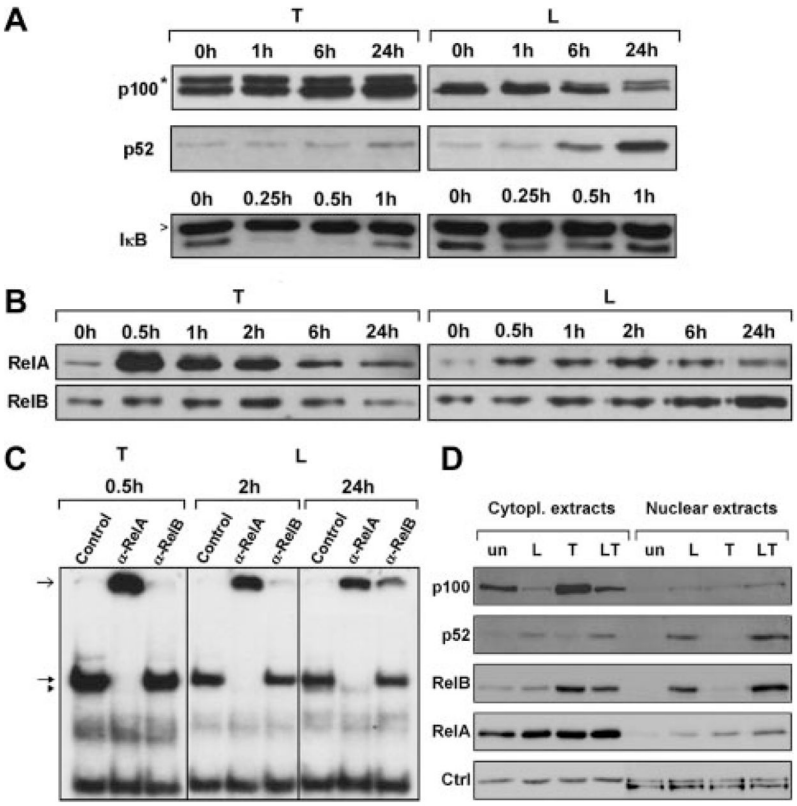

Figure 1.

SMC TNFR-1 and LTβR signaling differentially activates classical and alternative NF-κB pathways. SMC were stimulated with 1 ng/mL TNF or 10 μg/mL α-LTβR or buffer for the indicated time points. A, Immunoblot analysis of TNF-induced p100 accumulation and IκBα degradation (left) and α-LTβR–induced p100 to p52 processing (right) in total cell homogenates. *,>Unspecific bands. B, Immunoblot analysis of TNF-induced and α-LTβR–induced nuclear translocation of RelA and RelB in SMC. C, Electrophoretic mobility shift assay/super-shift analysis of TNF-induced RelA complexes and α-LTβR–induced RelA and RelB complexes. Open arrow, shifted complexes; closed arrow, RelA complexes; arrowhead, RelB complexes; control, pre-immune serum. D, Combined stimulation of TNFR-1 and LTβR in SMC hyperinduces p52 and RelB nuclear translocation. Immunoblot analysis of cytoplasmic and nuclear p100, p52, RelB, and RelA levels in untreated (un) and in 24-hour α-LTβR–stimulated (L), TNF-stimulated (T), or α-LTβR+TNF–stimulated (LT) SMC. Actin and polymerase II Western blotting for cytoplasmic and nuclear extracts, respectively (Ctrl in D), or by Coomassie staining of immunoblots (A–C; not shown) served as loading controls.

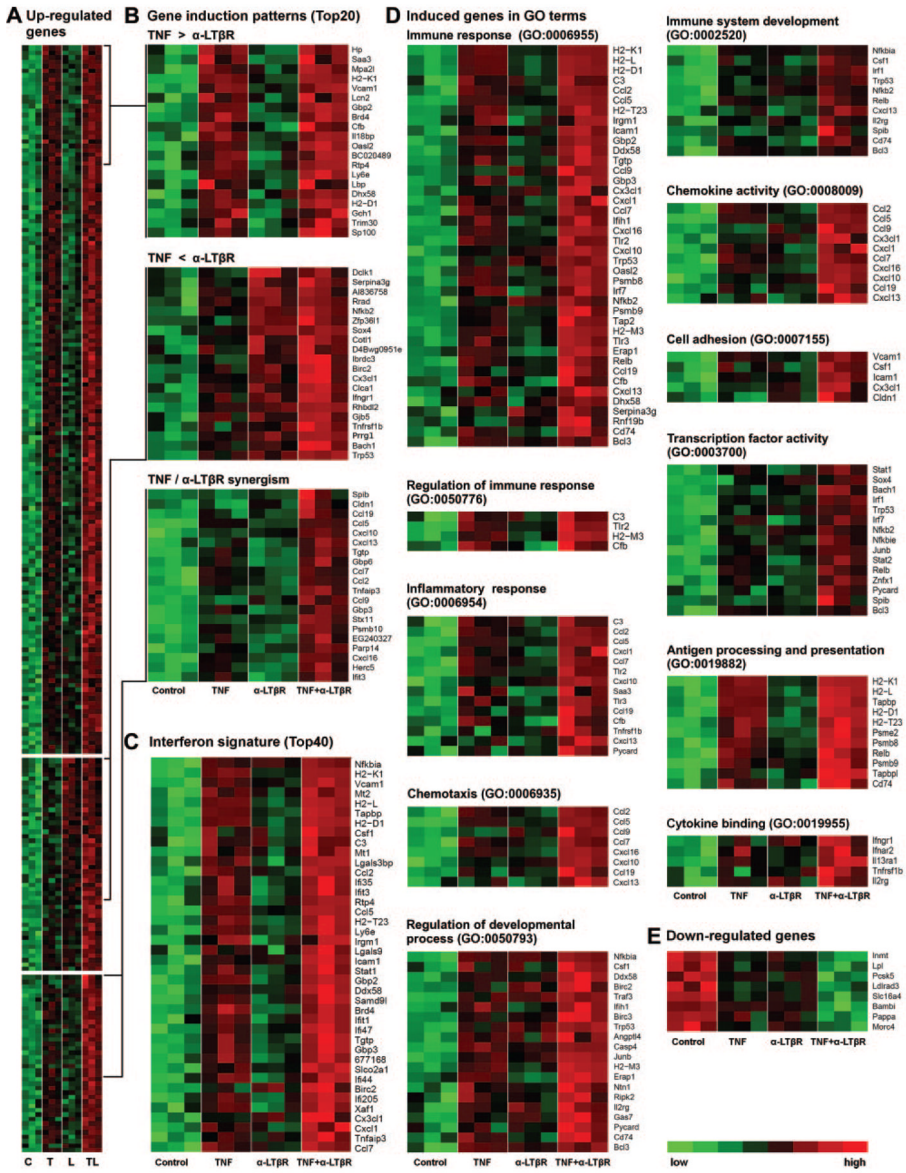

Figure 2.

TNFR-1 and LTβR activation induces mRNAs encoding lymphorganogenic molecules. A, SMC were stimulated with TNF (T) or α-LTβR (L) or both (LT) as described in Figure 1, and microarrays were prepared after 24 hours. Heat maps (A) represent upregulated genes (left heat map) and (B) the top 20 induced genes of 1 of 3 induction patterns. C, Top 40 of interferon signature derived from www.interferome.com data bank are displayed for ease of reading. D, Induced genes in gene ontology terms. E, Downregulated genes.

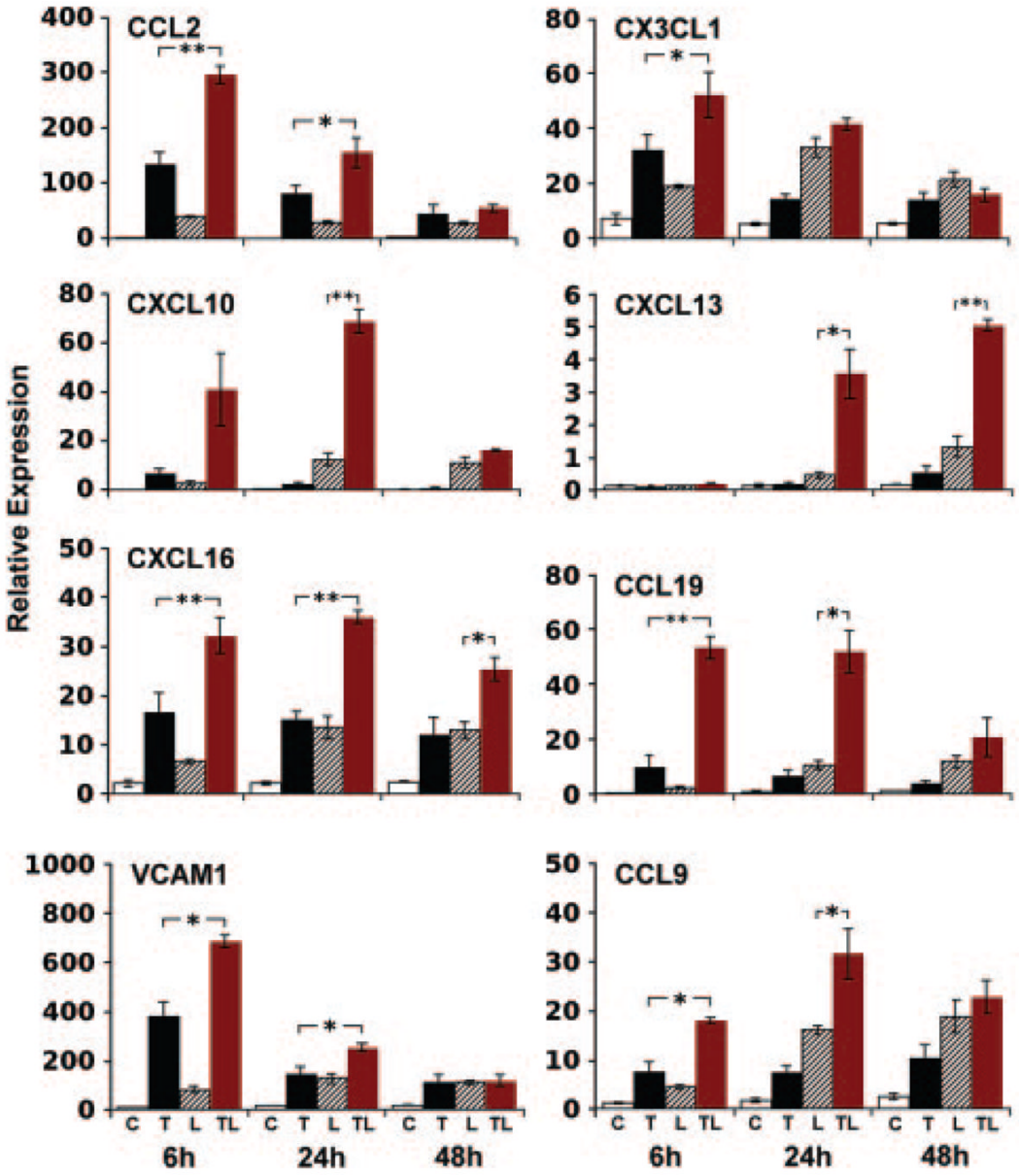

Figure 3.

Induction kinetics of inflammatory and lymphorganogenic chemokine and vascular cell adhesion molecule-1 mRNA. SMC were stimulated with agonists as described in Figure 1. At the indicated time points, quantitative reverse-transcription polymerase chain reaction analyses were performed using β-actin as internal control. Data represent means of 3 independent experiments±SEM. Unpaired Student *t* test. **P*<0.05. ***P*<0.01.

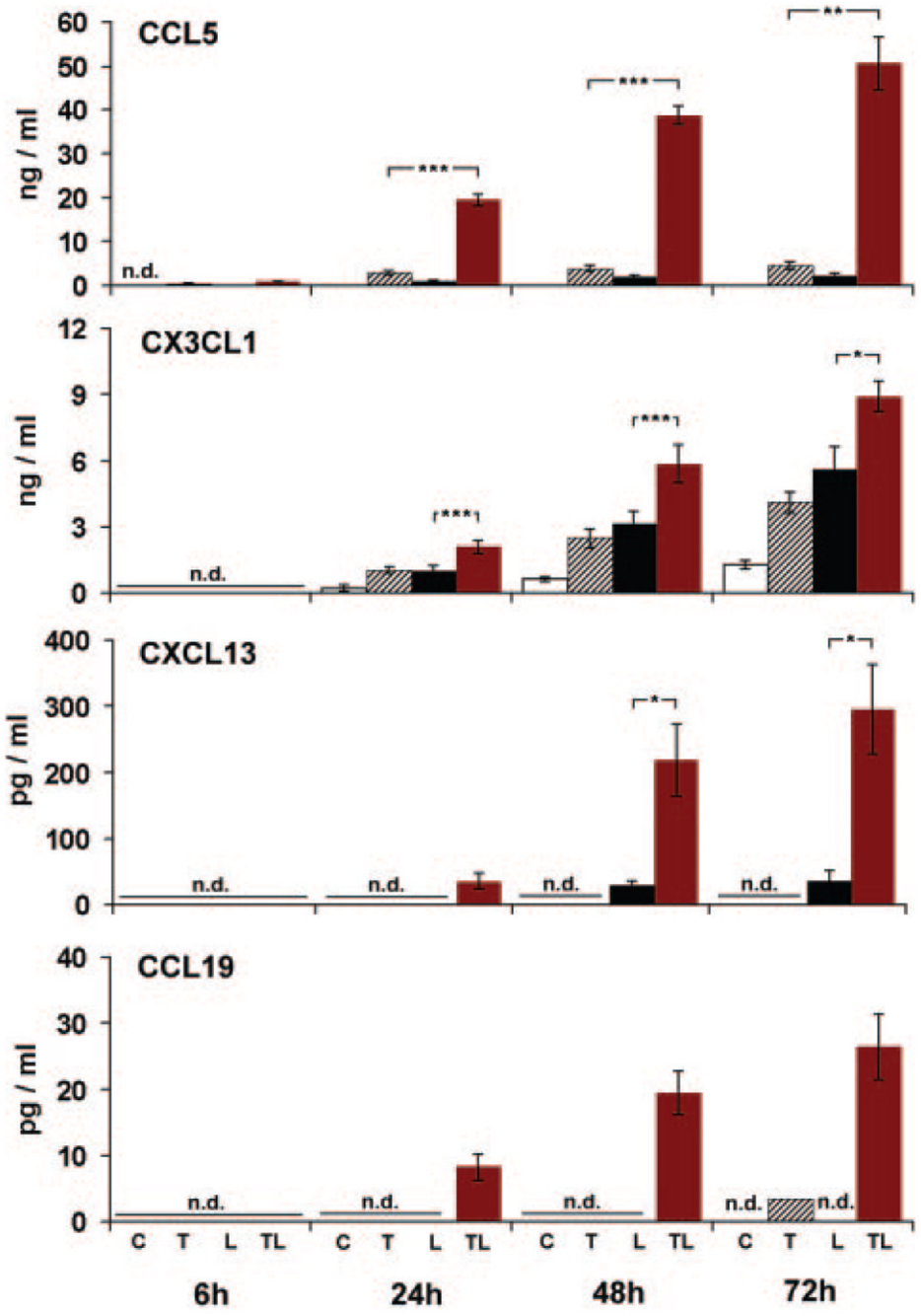

Figure 4.

Hyperinduction of de novo synthesis and prolonged secretion of CCL5, CX3CL1, CXCL13, and CCL19 by TNF/α-LTβR activation. SMC were stimulated with TNF, α-LTβR, or both, as described in Figure 1. At the indicated time points, chemokine levels in supernatants were determined by enzyme-linked immunosorbent assays. Data represent means of 3 or 5 (6 hours, n=3; 24 hours, n=5; 48 hours, n=5; 72 hours, n=3) independent SMC cultures±SEM. Student unpaired *t* test. *P<0.05. **P<0.01. ***P<0.001. Only probability values for chemokine levels with the highest values are shown for reasons of clarity. At 72 hours, in 2 experiments, SMC that received TNF CCL19 levels were below the detection limit and the CCL19 level within the detection limit is displayed for the remaining data point.

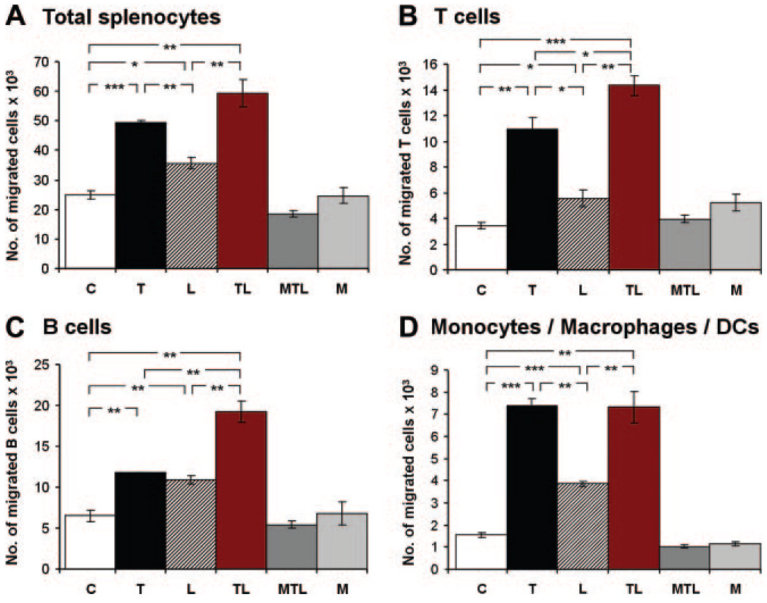

Figure 5.

Activated—but not quiescent—SMC promote chemotaxis of splenic T cells, B cells, and monocytes/macrophages/DC. SMC were cultured and stimulated with TNF, α-LTβR, or both, as described in Figure 1. After 48 hours, supernatants were collected and examined for migration activity as described in Materials and Methods. C, control SMC; T, TNF-stimulated SMC; L, α-LTβR-stimulated SMC; TL, TNF/α-LTβR-stimulated SMC; MTL, medium+ TNF/α-LTβR; M, medium. Numbers represent means of triplicate migration chambers±SEM of a representative experiment (3 independent experiments of 2 independent SMC preparations). Student *t* test. *$P \leq 0.05$. **$P \leq 0.01$. ***$P \leq 0.001$.

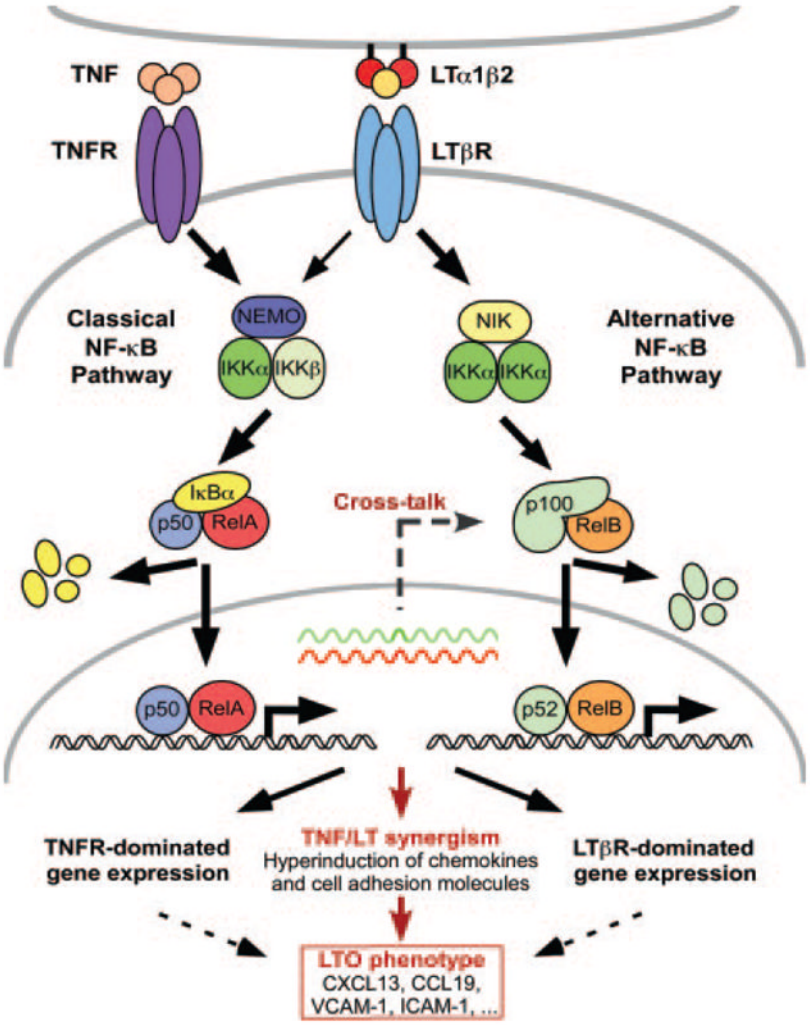

Figure 6.

Cross-talk of NF-*κ*B pathways in mouse aorta SMC stimulated with TNF and *α*-LT*β*R generates a phenotype resembling LTO. Cross-talk between TNFR-1–induced and LT*β*R-induced NF-*κ*B target gene activation in SMC is likely to be attributable to p100 and RelB accumulation in response to TNF. Subsequent LT*β*R-mediated processing of the p100 inhibitor-to-p52 and nuclear translocation of p52-RelB complexes activates expression of molecules involved in adaptive immune responses, lymphorganogenic chemokines, and adhesion molecules in SMC.