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Transcriptional Regulation of the Endogenous Danger Signal Tenascin-C: A Novel Autocrine Loop in Inflammation

Fu G. Goh,1 Anna M. Piccinini,1 Thomas Krausgruber, Irina A. Udalova, and Kim S. Midwood

Inappropriate expression of proinflammatory mediators underpins the pathogenesis of autoimmune disease and tumor metastasis. The extracellular matrix glycoprotein tenascin-C is an endogenous activator of innate immunity that promotes the synthesis of inflammatory cytokines via activation of TLR4. Little tenascin-C is observed in most healthy adult tissues, but expression is specifically upregulated at sites of inflammation. Moreover, high levels of tenascin-C are associated with chronic inflammation and found in the tumor stroma. In this study, we show that the expression of tenascin-C is induced in immune myeloid cells activated by a variety of inflammatory stimuli, including specific TLR ligands. Its synthesis is transcriptionally regulated and requires the specific activation of AKT/P13K and NF-κB signaling pathways. Using a bioinformatic approach, we identified a large number of conserved noncoding regions throughout the tenascin-C genomic locus that may contribute to its transcriptional regulation during inflammation. We also demonstrate that tenascin-C expression is transient during acute inflammation. In contrast, persistently high levels of expression occur in the inflamed synovium of joints from rheumatoid arthritis patients. Thus, misregulated expression of this endogenous danger signal may promote an autocrine loop of inflammation and contribute to the persistence of inflammation in autoimmune diseases or to tumor egress and invasion during metastasis. The Journal of Immunology, 2010, 184: 2655–2662.
addition, tenascin-C expression is upregulated at both the mRNA and protein levels in synovial tissues in mouse models of joint inflammation (3, 15), and tenasin-C is also arthrogenic in vivo when injected intra-articularly into mice (3). Furthermore, its activation of TLR4 is essential for driving persistent joint inflammation in vivo (3). These data implicate tenasin-C as a key proinflammatory mediator in the RA joint. However, the origin of the high levels of tenascin-C in the joint is not known, and it is not clear how synovial tissue levels of this DAMP are regulated during inflammation.

In this study, we show that tenascin-C expression correlates with disease activity in human rheumatoid joints. We also demonstrate that the high level of tenascin-C synthesis observed in diseased joints is driven by specific subsets of synovial cells, including immune cells of the myeloid lineage. We show that although tenascin-C expression is persistently activated in chronic inflammation, it is subject to tight transcriptional control during acute inflammation; expression is induced upon immune cell activation and then is effectively and rapidly downregulated. Finally, we provide the first insights into the molecular machinery that exerts this control over endogenous tenascin-C expression in human myeloid cells during inflammation.

Understanding how tenascin-C expression is regulated during an acute immune response will reveal how this process can be subverted during chronic inflammation and may provide the means to modulate tissue levels of this danger signal.

Materials and Methods

Patient specimens

Synovial membranes were obtained from patients with RA or OA undergoing joint replacement surgery as described previously (24). The study was approved by the local trust ethics committee, and waste tissue (synovium from joint replacement surgery) was obtained only after receiving signed informed consent from the patient and ensuring tissue anonymity to protect patient identity. Synovia were either fixed in 10% (v/v) buffered formalin for immunohistochemical analysis or processed for cell isolation.

Immunohistochemical analysis

Fixed synovial membranes were processed to paraffin, and tissue sections (4 μm) were cut and stained with rabbit anti-human tenascin-C polyclonal Ab (191011; Chemicon International, Temecula, CA).

Cell isolation

RA or OA membrane cells (representing a mixed population of all synovial cell types) were isolated from synovial membranes. Synovial fibroblasts, myeloid cells, and lymphoid cells were isolated from this mixed population of RA membrane cells as described previously (25). Immediately after isolation, total synovial fibroblasts, myeloid cells, and lymphoid cells were cultured at 1 × 10⁶ cells/well in RPMI 1640 containing 10% (v/v) FBS and 100 U/ml penicillin/streptomycin (PAA Laboratories, Pasching, Austria) in 96-well tissue culture plates for 24 h before assessing tenascin-C protein levels by Western blotting and ELISA and mRNA levels by quantitative RT-PCR as described below. Levels of inflammatory cytokines were assessed by ELISA according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN).

Primary human myeloid cell culture and activation

Human monocytes isolated from peripheral blood (London Blood Bank, London, U.K.) were differentiated into monocyte-derived macrophages (MDMs) or monocyte-derived dendritic cells (MDDCs) (26, 27) and cells plated (1 × 10⁵ cells/ml) in RPMI 1640 containing 5% (v/v) FBS and 100 U/ml penicillin/streptomycin for 24 h before stimulation with 10 ng/ml LPS (ultrapure LPS from Escherichia coli 0111:B4; InvivoGen, Wiltshire, U.K.) for 1, 4, 8, or 24 h at 37°C before assessment of tenascin-C protein and mRNA levels as described below. MDDCs were also stimulated with 100 ng/ml 5,6-oxido-7,9,11,14-eicosatetraenoic acid (LTA), 1 μg/ml PAM3, 25 μg/ml polynosine-polycytidylic acid (poly(C)), 1 μg/ml lipopolysaccharide (LPS), respectively, in the presence or absence of DMSO, actinomycin D (0.5 μg/ml), LY294002 (10 μM), wortmannin (100 μM), SH5 (1 μM), SB203580 (10 μM), SP600125 (10 μM), U0126 (10 μM) (Calbiochem, San Diego, CA), or TPCA1 (5 μM) (a gift from GlaxoSmithKline, Research Triangle Park, NC). Tenascin-C mRNA and protein levels were assessed at 4 or 24 h, respectively. Viability of the cells was not significantly affected throughout each experiment as examined by MTT assay (Sigma-Aldrich). Kinase inhibitors were used at the lowest dose that gave maximal inhibition of phosphorylation in human MDDCs assessed by Western blotting using phospho-specific Abs to each target.

Plasmids, transfections, and infections

The sequences and restriction maps of the NF-κB expression constructs in the pENTR vector (Invitrogen) modified to contain the CMV promoter and IRES-linked GFP are available upon request. The constructs were recombined into pAD/PL DEST vector (Invitrogen) for adenosine production and delivery into MDDCs. The construct expressing mutant IκBα was cloned into the pENTR vector (Invitrogen) modified to contain the CMV promoter and IRES-linked GFP. The plasmid was subsequently introduced into MDDCs using Lipofectamine 2000 (Invitrogen). MDDCs were infected with adenosine expression vector (pENTR) or RelA or mutant IκBα using Lipofectamine 2000 (Invitrogen). MDDCs were infected with adenosine expression vector (pENTR) or RelA or mutant IκBα using Lipofectamine 2000 (Invitrogen). MDDCs were infected with adenosine expression vector (pENTR) or RelA or mutant IκBα using Lipofectamine 2000 (Invitrogen). MDDCs were infected with adenosine expression vector (pENTR) or RelA or mutant IκBα using Lipofectamine 2000 (Invitrogen).

Bioinformatic analysis

Genomic sequences were obtained using the publicly available UCSC hg18 human genome assembly (http://genome.ucsc.edu/). The ECR Browser (http://ecrbrowser.dcode.org/) was used to analyze sequence conservation in the tenasin-C genomic locus. The nucleotide sequences were inspected with JASPAR transcription factor binding sites searching software.
Tenascin-C has been reported to be specifically but widely distributed; it localizes to the invading pannus, superficial and deep cells of the synovial layer, subsynovial tissue, blood vessels, lymphoid aggregates, and areas of fibrosis (15, 18–20). We confirmed this pattern of localization by immunohistochemical staining of synovial membranes isolated from RA patients undergoing joint replacement. Tenascin-C was observed in the synovial and subsynovial layers around blood vessels and in areas of cellular infiltration (Fig. 1A). No staining was observed in negative control sections stained with nonspecific rabbit serum or where the primary Ab was omitted (data not shown). We also quantified the amount of tenascin-C synthesized by RA synovial membrane cells. Total cells isolated from the synovia obtained from RA patients exhibited significantly higher levels of tenascin-C protein than cells isolated from noninflamed synovia obtained from OA patients (Fig. 1B, 1C). The predominant form of tenascin-C upregulated in RA synovia was 320 kDa in size (Fig. 1C). During the progression of RA, inflammatory cytokines, such as IL-6, IL-8, and TNF drive, prolonged inflammation and joint destruction (31). High levels of tenascin-C protein in RA synovia significantly correlated with high levels of IL-6 (Fig. 1D), IL-8, and TNF (data not shown).

These data are consistent with published reports that show widespread localization of tenascin-C at specific loci throughout inflamed synovia and further demonstrate quantitatively higher levels of tenascin-C in inflamed versus noninflamed synovia. These data also provide evidence that high levels of tenascin-C protein in the RA joint correlate with high levels of markers of disease activity. Taken together, these results confirm that tenascin-C is an important pathological factor in RA.

Identification of tenascin-C–producing cells within RA synovia

To determine whether cells resident in the synovium act as a source of tenascin-C, we quantified tenascin-C mRNA in mixed populations of cells extracted from inflamed synovial membranes of RA patients. High levels of tenascin-C mRNA comparable to levels of the largely abundant ribosomal gene RLPO were consistently observed in these cells, and tenascin-C mRNA was significantly more abundant than TNF mRNA relative to levels of RLPO (Fig. 2A).

The RA synovium comprises a mixture of cells of many different lineages, including fibroblast-like cells, myeloid cells, and lymphocytes. To determine which cell types among these are responsible for synthesizing tenascin-C, we purified fibroblasts, myeloid cells, and lymphocytes from the total population of cells isolated from RA membranes and analyzed levels of tenascin-C mRNA in each. Both myeloid cells and fibroblasts were major contributors of tenascin-C synthesis, whereas mRNA levels in lymphoid cells were relatively low (Fig. 2B). These data are consistent with published data demonstrating tenascin-C expression in synovial fibroblasts (19) but also show for the first time that myeloid cells synthesize significant levels of tenascin-C mRNA within the RA synovium.

Transient induction of tenascin-C expression in acute inflammation

Tenascin-C expression specifically colocalizes to areas of immune myeloid cell activation during the acute innate immune response upon infection and injury, in addition to the tumor stroma and in autoimmune diseases, such as RA (17). As such, these cells may be a major source of tenascin-C at global sites of inflammation, not just in the RA synovium. However, nothing is currently known about how tenascin-C expression is induced and regulated in cells of the myeloid lineage.

To further examine tenascin-C expression in immune myeloid cells during inflammation, we used a model of acute inflammation. Primary human monocytes, MDMs, and MDDCs were activated by the bacterial cell wall component LPS. Nonactivated cells expressed little tenascin-C, but expression significantly increased in response to stimulation with LPS. Levels of mRNA peaked between 4 and 8 h, returning to basal levels by 24 h (Fig. 3A). Although the absolute levels of mRNA induction varied between different donors...
FIGURE 3. Tenascin-C expression is induced in activated myeloid cells. A, Tenascin-C mRNA in myeloid cells from a single donor stimulated with LPS (10 ng/ml) for 0, 4, 8, or 24 h, normalized to ribosomal protein PO levels and to the level of gene expression in nonactivated monocytes, which were assigned the value of 1 (ΔΔCT method). B, Tenascin-C mRNA in MDDCs from eight different donors without (−) or with LPS stimulation (LPS) for 4 h. C, Tenascin-C protein in MDDCs after stimulation with LPS for 0, 1, 4, 8 or 24 h. Data shown are the mean values from four independent donors (± SEM; ***p < 0.001). D, Representative Western blot of tenascin-C in supernatants of MDDCs (c, purified human tenascin-C).

(Fig. 3B), the kinetics of tenascin-C expression in all three cell types from all donors was identical. The highest level of induction was observed in MDDCs, and this was consistently ~10-fold higher than in monocytes and MDMs. Expression of the ECM glycoprotein fibronectin was not induced by LPS in any cell type (data not shown), consistent with published data (32). Increased tenascin-C mRNA correlated with tenascin-C protein synthesis. Nonactivated MDDCs secreted low levels of tenascin-C, which significantly increased between 8 and 24 h after stimulation (Fig. 3C). Tenascin-C was secreted as a single band of molecular mass 320 kDa (Fig. 3D), similar to the predominant form observed in RA samples.

These data demonstrate that tenascin-C expression in immune myeloid cells is transiently induced in acute inflammation. This is in stark contrast to the sustained expression we observed in chronically inflamed tissues, such as the RA synovium, indicating that abnormal regulation of the tenasin-C gene occurs in pathological inflammation. We therefore further investigated how the expression of tenascin-C is regulated in immune myeloid cells to begin to understand how this may be compromised in RA.

Tenascin-C expression is transcriptionally regulated during inflammation

To examine whether expression of tenascin-C in myeloid cells occurred at the level of gene transcription, we examined recruitment of RNA polymerase II (Pol II) to the transcription start site of tenasin-C in MDDCs stimulated with LPS. Pol II recruitment was observed as early as 30 min and steadily increased up to 4 h post-LPS stimulation (Fig. 4A). Furthermore, tenasin-C mRNA expression was significantly inhibited by actinomycin D (data not shown), confirming the role of transcriptional regulation in modulating the levels of tenasin-C expression. We also detected a significant reduction of tenasin-C expression. We also detected a significant reduction of tenasin-C expression in MDDCs stimulated with LPS for 4 h with no additional treatment (c) and are the mean values from three independent donors (± SEM; ***p < 0.001).

Activation of PI3K/AKT and NF-κB–mediated signaling pathways drives tenasin-C expression

Distinct patterns of gene expression are induced upon TLR activation. This is mediated by selective activation of specific signaling pathways and transcription factors (1). Among the major pathways activated during inflammation and shared by TLR1/2, TLR2/6, TLR4 and TLR5, are those driven by PI3K/AKT, MAPK, or NF-κB signaling (35). We used inhibitors of each of these three pathways to determine which, if any, are required for transcription of the tenasin-C gene upon activation of MDDCs.

The induction of tenasin-C expression by LPS was significantly inhibited by the PI3K-specific inhibitors LY294002 (Fig. 6A) and wortmannin (data not shown) and by the AKT inhibitor (SH5) at

FIGURE 4. Tenascin-C expression is transcriptionally regulated by LPS. A, Chromatin immunoprecipitation analysis of Pol II recruitment in MDDCs following stimulation with LPS. Data shown are the mean of three independent donors (± SEM; ***p < 0.001). B, Tenasin-C mRNA in MDDCs stimulated with LPS for 4 h with no additional treatment (c) or with addition of DMSO or cyclohexamide (CHX). Data are expressed as the percentage of stimulated control cells (c) and are the mean values from three different donors (± SEM; *p < 0.05). c, control cell; CHX, cyclohexamide.
both the protein (Fig. 6A) and mRNA level (data not shown). DMSO had no significant effect on either tenasin-C mRNA or protein. Inhibition of MAPKs using the p38 inhibitor SB203580, the JNK inhibitor SP600125 and downstream inhibition of ERK1 and 2 using the MEK inhibitor UO126 did not significantly affect tenasin-C expression (Fig. 6A). However, TPCA1, an inhibitor of IκB kinase 2 (36) significantly inhibited tenasin-C protein (Fig. 6A) and mRNA (data not shown) synthesis. Taken together, these data suggest that activation of PI3K/AKT and NF-κB signaling pathways contribute to the induction of tenasin-C expression in activated MDDCs but that signaling by MAPKs is not required. Moreover, overexpression of the NF-κB subunit RelA in nonactivated primary human MDDCs enhanced tenasin-C mRNA (Fig. 6B) and protein (data not shown) synthesis, whereas expression of a dominant-negative (superrepressor) mutant of IκB inhibited LPS-induced tenasin-C expression by ~75% (Fig. 6C).

To further study the transcriptional regulation of tenasin-C, HEK293 cells overexpressing TLR4/CD14/MD-2 were stimulated with LPS. Upon activation this cell line upregulates the synthesis of proinflammatory mediators (37) and tenasin-C (Supplemental Fig. 1). Induction of tenasin-C expression in HEK293s was inhibited by LY294002 (LY), wortmannin, SH5, and TPCA1 and was unaffected by p38, JNK, and MEK inhibitors (data not shown) suggesting that the same transcriptional machinery drives tenasin-C expression in both MDDCs and HEKs in response to LPS stimulation. In addition, expression of the dominant-negative (superrepressor) mutant of IκB inhibited LPS-induced tenasin-C expression (Supplemental Fig. 1B) as effectively as the known NF-κB-dependent gene TNF (data not shown). Knocking the expression of the NF-κB subunit RelA down to ~15% using short interfering RNAs also inhibited TNF (data not shown) and tenasin-C expression (Supplemental Fig. 1C), whereas over expression of RelA stimulated TNF (data not shown) and tenasin-C expression (Supplemental Fig. 1D) in nonstimulated cells.

These data confirm that specific activation of both PI3K/AKT and NF-κB signaling pathways, but not MAPKs, are important for the induction of tenasin-C expression by LPS.

**Identification of putative NF-κB binding sites in conserved noncoding regions of the tenasin-C gene locus**

We demonstrated that tenasin-C expression in MDDCs is regulated on the transcriptional level. The human tenasin-C gene is located on chromosome 9q32-q34 (116954270–116708662) (38) where it is flanked by the DEC1 and TNFSF8 genes. It comprises 30 exons separated by 29 introns (39–41). The entire coding region (exons 2–30) is restricted to a ~7.5-kbp segment of this locus; this constitutes only 3.6% of the overall gene, which spans ~211 kbp. The exons range in size from 90 to 1410 bp and the introns from 578 to 26,827 bp (42). The proximal 220 bp upstream of the transcription start site in exon 1 is sufficient for the basal expression of tenasin-C gene reporter constructs indiscriminately in cell lines producing (SK-MEL-28 human melanoma) or not producing (hamster R1-G9 glucagonoma and human FL ovary carcinoma) endogenous tenasin-C (39). However, nothing is known about the cis-regulatory elements responsible for inducible transcription of the tenasin-C gene in primary human myeloid cells during inflammation.

One feature of functional regulatory elements is their strong evolutionary conservation (43). We inspected the organization of the entire ~211 kbp of the tenasin-C genomic locus and identified multiple conserved noncoding sequences (CNS) throughout this region (Fig. 7A). Next, we examined in more detail the region 5’ upstream of the tenasin-C transcriptional start site as well as the first intron, a large 26,827-bp region that separates the untranslated

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**FIGURE 5.** Induction of tenasin-C expression by specific inflammatory stimuli in MDDCs. Tenasin-C protein in MDDCs either left unstimulated (−) or after stimulation with LTA, PAM3, LPS, poly(I:C) (PIC), flagellin (fla), or R848 for 24 h. Data are shown as the average of triplicate values from a single representative experiment from a total of three independent donors (± SD), fla, flagellin; PIC, poly(I:C).

**FIGURE 6.** Blocking PI3K/AKT and NF-κB signaling pathways inhibits tenasin-C induction in MDDCs. A, Tenasin-C protein in MDDCs stimulated with LPS for 24 h in the presence of DMSO, LY294002 (LY), SH5, SB203580 (SB), SP600125 (SP), UO126 (UO), or TPCA1 (TP). Data are the mean values from three different donors (± SEM; *p < 0.01). B, Tenasin-C mRNA in LPS-stimulated MDDCs infected with adenovirus expressing control vector (c) (○) or RelA (□) at a multiplicity of infection of 50. Data shown are the mean fold induction of mRNA compared with control at 0 h (± SD) of a representative of independent experiments from three different donors. C, Tenasin-C mRNA in unstimulated MDDCs (0 h) or LPS-stimulated MDDCs (4 h) infected with adenovirus expressing control vector (c) or dominant-negative IκB (IκBdn) at a multiplicity of infection of 50. Data shown are the mean fold induction of mRNA compared with control without LPS (± SD) of a representative of independent experiments from three different donors, c, control vector, IκBdn, dominant-negative IκB; LY, LY294002; SB, SB203580; SP, SP600125; TP, TPCA1; UO, UO126.
first exon from the translational start site in exon 2. This intron has previously shown to contribute to the cell-specific expression of tenascin-C in human melanoma and carcinoma cell lines (39). We identified 15 conserved noncoding sequences in the 5′ upstream region (Fig. 7B, pink boxes), whereas 20 conserved noncoding sequences were located in the first intron of the gene (Fig. 7C, pink boxes). To elucidate which conserved noncoding sequences may be involved in NF-κB regulation of tenascin-C expression during inflammation, we computationally mapped putative NF-κB binding sites within each conserved noncoding sequence: 15 were identified in the 5′ upstream region (Table I) and 18 in the first intron (Table II). These data indicate that a large number of putative cis-regulatory elements exist within the tenascin-C gene.

**Discussion**

Exerting tight control over tissue levels of inflammatory mediators is key to an effectively regulated immune response. ECM molecules that are specifically upregulated in response to tissue damage act as danger signals that drive inflammation to mediate tissue repair. Although many studies support a role for the inappropriate synthesis of these DAMPs in driving chronic inflammation (12, 13), little is known about how their expression is induced or regulated. This study has identified a novel autocrine mode of action for the proinflammatory ECM glycoprotein tenascin-C that is tightly controlled during acute inflammation but that proceeds unchecked during destructive joint inflammation. In particular, we show that primary human immune myeloid cells are a major source of tenascin-C during inflammation. Furthermore, the high levels of tenascin-C expression in MDDCs imply that, in addition to its established function in stimulating innate immunity, it may also play a role in mediating adaptive immunity.

We have demonstrated that tenascin-C expression in MDDCs is regulated at the level of gene transcription. Little is known about the mechanisms of tenascin-C transcription. Gene reporter studies have demonstrated that the proximal 220 bp upstream of the transcription start site plays a role in basal transcription (39). The activity of this basal promoter can be both positively and negatively modulated by noncoding sequences; the untranslated first exon contains two discrete regions that repress or enhance transcription of the reporter in human glioblastoma cells lines (44). In addition, as with many ECM genes, including collagen type I (45) and aggrecan (46), cell-specific transcription of tenascin-C is directed by the cooperation of intronic

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### Table I. Putative NF-κB binding sites in the 5′ region of the tenascin-C gene

<table>
<thead>
<tr>
<th>CNS No.</th>
<th>Locus</th>
<th>Relative Score</th>
<th>Sequence</th>
<th>Strand</th>
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**CNS**, conserved noncoding sequence.
enhancer elements with the basal promoter; the first intron contributes to cell-type specific inducible expression in human melanoma cell lines (39). Our inspection of the tenasin-C genomic locus revealed that this gene spans a large area of chromosome 9, although the coding region made up a relatively low proportion of this area. We identified many highly conserved regions within the noncoding areas of the tenasin-C gene. These spanned the entire gene locus from the 5' to the 3' flanking genes, indicating that expression of tenasin-C may be subject to complex regulatory mechanisms by multiple sequences throughout the whole locus. This may explain how the specific temporal and tissue-specific pattern of expression reported for tenasin-C during development and in the adult (reviewed in Refs. 16 and 17) is mediated. Determining how cell-specific transcription is orchestrated within this large locus will require further systematic analysis of functional noncoding sequences.

The induction of tenasin-C in MDDCs is NF-κB dependent; this is the first transcription factor identified that is able to drive endogenous tenasin-C expression. Transfection of c-Jun into rat embryonic fibroblasts activated a construct comprising the −220 to +79-bp fragment of the basal promoter, and this required synergistic binding of NF-κB and c-Jun (47), supporting the importance of NF-κB in controlling tenasin-C transcription. We found 33 putative NF-κB binding sites in conserved noncoding sequences within the 5’ region of the tenasin-C gene and in the first intron (known to be important for cell-specific induction of the gene); putative binding sites in other conserved noncoding sequences may also exist. In addition, the requirement for de novo protein synthesis for tenasin-C mRNA expression and relatively delayed kinetics of Pol II recruitment to its transcription start site suggests a possible role for chromatin remodeling in the regulation of tenasin-C in MDDCs. Future work will focus on systematically dissecting any epigenetic mechanisms that regulate tenasin-C gene expression during acute inflammation in human myeloid cells.

In MDDCs, tenasin-C expression was induced by specific components of the bacterial cell wall, including LTA, PAM3, and LPS, as well as bacterial flagellin, indicating that tenasin-C expression can be directly induced in response to pathogenic invasion. Synthesis of tenasin-C may also be induced as a direct consequence of tissue damage or mechanical strain. Loading the anterior latissimus dorsi muscle in the chick wing (48) or the soleus muscle in the rat hind limb (49) specifically, rapidly, and reversibly induced high levels of tenasin-C mRNA. Cyclical stretch of isolated chicken fibroblasts and neonatal rat ventricular cardiac myocytes also induces tenasin-C transcription (50, 51). In addition, denatured, but not native, type I collagen has been shown to induce tenasin-C protein in fetal rat smooth muscle cells (52). Taken together, these data suggest that tenasin-C expression may be triggered as an immediate and direct result of tissue injury or infection.

Our data suggest that signaling molecules that are spatially restricted to the plasma membrane may be involved in driving tenasin-C expression. Tenasin-C synthesis was specifically induced by TLRs that can be located at the cell surface; TLR2, 4, and 5, but not to the endosome; and TLR3 or TLR8. Compartmentalization of TLRs is key to defining the stimulation of specific downstream pathways by recruitment of distinct adaptor molecules and associated factors (reviewed in Ref. 53). We also showed that only a specific subset of pathways that are activated by stimulation of TLR4 by LPS are needed to drive tenasin-C synthesis; activation of AKT/Pi3K was essential, but activation of MAPK was not required. Our results highlight the fact that synthesis of individual proinflammatory mediators is induced by activation of specific signaling pathways downstream of TLR4. For example, TLR4-induced production of TNF, but not IL-6, requires the activity of p38 MAPK (54) and suppressor of cytokine signaling-1 selectively inhibits LPS-induced IL-6 production but not the production of TNF, G-CSF, or IFN (55).

Unraveling the specificity of these pathways may lead to the ability to selectively modulate tissue levels of different proinflammatory mediators.

Taken together, data from this study have demonstrated that tenasin-C expression is rapidly and transiently induced in immune myeloid cells upon activation in response to tissue injury and infection. Synthesis of this proinflammatory mediator contributes to orchestrating an immune response designed to eliminate the pathogen and/or repair damaged tissue. This proceeds via an autocrine loop whereby expression of tenasin-C is induced upon activation of cell surface TLRs, and subsequent activation of TLR4 by tenasin-C drives the synthesis of proinflammatory cytokines. Under normal circumstances, tenasin-C expression is downregulated before repair is complete, consistent with controlled resolution of inflammation. However, in RA, persistent gene expression leads to high tissue levels of tenasin-C that would then drive a nonresolving loop leading to chronic inflammation. Further information about how tissue levels of DAMPs, such as tenasin-C, are controlled may yield strategies to block inappropriate inflammation in autoimmune diseases and cancer.

Disclosures

The authors have no financial interests of conflict.

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