Coregulation in human leukocytes of the long pentraxin PTX3 and TSG-6

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ABSTRACT

The prototypic long PTX3 is a multifunctional protein involved in innate resistance to pathogens and in controlling inflammation. TSG-6 is a hyaluronan-binding protein that is involved in ECM remodeling and has anti-inflammatory and chondroprotective functions. PTX3 and TSG-6 are coregulated by growth differentiation factor-9 in granulosa cells, where they are produced during the periovulatory period and play essential roles in the incorporation of hyaluronan into the ECM during cumulus expansion. The present study was designed to assess whether PTX3 and TSG-6 are coregulated in leukocytes, in particular, in phagocytes and DC. Monocytes, macrophages, and myeloid DC were found to produce high levels of TSG-6 and PTX3 in response to proinflammatory mediators (LPS or cytokines). Unstimulated neutrophil polymorphonuclear granulocytes expressed high levels of TSG-6 mRNA, but not PTX3 transcript, and stored both proteins in granules. In contrast, endothelial cells expressed substantial amounts of PTX3 mRNA and low levels of TSG-6 transcript under the conditions tested. Anti-inflammatory cytokines, such as IL-10, which synergizes with TLR-mediated PTX3 induction but inhibits LPS-induced TSG-6 transcription. Immuno-histochemical analysis confirms the colocalization of the two proteins in inflammatory infiltrates and in endothelial cells of inflamed tissues. Thus, here we show that myelomonocytic cells and MoDC are a major source of TSG-6 and that PTX3 and TSG-6 are coregulated under most of the conditions tested. The coordinated expression of PTX3 and TSG-6 may play a role in ECM remodeling at sites of inflammation. J. Leukoc. Biol. 86: 123–132; 2009.

Introduction

PTXs are a superfamily of evolutionarily conserved proteins that are characterized by the presence of a structural motif termed the PTX domain. Short PTXs, C-reactive protein, and serum amyloid P component are acute-phase proteins in man and mouse, respectively, produced mainly by the liver in response to inflammatory stimuli, such as IL-6 [1]. PTX3 was the first long PTX described as an IL-1β-inducible gene in endothelial cells. A variety of cell types produces PTX3 in response to proinflammatory stimuli; these include endothelial cells, smooth muscle cells, adipocytes, fibroblasts, mononuclear phagocytes, DC, and granulosa cells [1]. Recently, we found that PTX3 is stored in neutrophil-specific granules and undergoes release in response to microbial recognition and inflammatory signals [2]. PTX3 binds selected ligands, such as C1q, and specific microorganisms and microbial moieties, thereby initiating an innate immune response, possibly through complement activation [3–5]. Studies in vivo with PTX3-deficient mice have revealed that PTX3 plays a number of nonredundant roles in innate resistance to selected pathogens [6] and during inflammation [4, 7]. In humans, increased levels of PTX3 are found in different infectious diseases [8, 9], in autoimmune disorders [10, 11], and in inflammatory conditions, reflecting, in particular, the involvement of the vascular bed [12, 13].

TSG-6, also known as TNFAIP6, is a 35-kDa-secreted protein, comprised almost entirely of a Link module and a CUB domain, where the former binds to a wide range of ECM components including HA, chondroitin-4-sulfate, and aggrecan (reviewed in refs. [14, 15]), and the latter has been shown recently to bind fibronectin [16]. TSG-6 is up-regulated rapidly in response to proinflammatory cytokines (e.g., IL-1 and TNF-α) and various growth factors, and it is produced by a wide range of cell types, including chondrocytes, synoviocytes, and smooth muscle cells. High levels of TSG-6 are found...
in the synovial fluids of patients with RA and osteoarthritis and also in sera of individuals with bacterial sepsis and other inflammatory diseases [17, 18]. PTX3 and TSG-6 are expressed by granulosa cells and cumulus cells following the luteinizing hormone surge and are associated with the ECM surrounding the oocyte during the periovulatory period. Moreover, PTX3 binds to TSG-6 and to Iad and is believed to form a multimolecular complex that might support HA cross-linking [19, 20]. These three proteins have crucial roles in the structural organization of cumulus oophorus ECM, which is essential for in vivo fertilization. Indeed, the deficiency of any one of these three molecules causes defective cumulus oophorus expansion and female subfertility or sterility as a result of defective in vivo fertilization [19, 21–23]. PTX3 and TSG-6 transcripts were also found to be induced in decidual stromal cells by the trophoblast in in vitro systems that mimic the alteration of the local immune environment induced by the trophoblast in the process of embryo implantation [24, 25]; however, their role in this context has not been elucidated yet.

TSG-6 has been implicated in the process of HA cross-linking, which might potentially occur through a number of different mechanisms, first, by acting as cofactor and catalyst in the covalent transfer of the HC of Iad onto HA (thereby forming HC•HA complexes, which are more aggregated than free HA [26, 27]), and second, more speculatively, by forming multimolecular complexes with PTX3 that act as foci for the attachment of multiple HA chains via the Link module of TSG-6 [19, 28]. It has been shown recently that HC • HA complexes are likely to associate directly with PTX3, leading to an alternative method of cross-linking [20]. Besides its role in HA cross-linking, TSG-6 has anti-inflammatory and chondroprotective roles in arthritis [29–31], which might potentially occur through a number of different mechanisms: LPS (100, 10, or 1 ng/ml), IFN-γ (5000 or 500 U/ml), IL-13, IL-10, or IL-4 (20 ng/ml for all). LPS was added to relevant cultures 30 min before exposure to other stimuli. Three to four donors were tested for each condition.

Northern blot analysis

Total RNA was extracted using TRizol, according to the manufacturer’s instructions (Invitrogen, Life Technologies, Carlsbad, CA, USA), blotted, and hybridized as described [39]. The probes hPTX3, a full-length hPTX3 cDNA, and hTSG-6, a cDNA corresponding to the Link module of hTSG-6 (Link_TSG6) [44], were labeled with [α-32P] dCTP (3000 Ci/mmol) (Amersham, Buckinghamshire, UK) using the Megaprime DNA labeling system (Amersham, Buckinghamshire, UK).
RESULTS

Expression of TSG-6 and PTX3 mRNA in human cells

To study TSG-6 and PTX3 mRNA expression in human cells, we performed Northern blot analysis of total RNA using radio-labeled hTSG-6 and hPTX3 cDNAs as probes. When comparing TSG-6 and PTX3 expression under identical conditions, blots were first hybridized with the TSG-6 probe and then, after stripping, with the PTX3 probe.

Analysis of material from unstimulated cell lines (Fig. 1, A and B) generally revealed little or no expression of TSG-6 or PTX3 mRNA. TSG-6 was detectable only in the bronchial epithelial cell line BEAS-2B (Fig. 1A), and PTX3 was evident in endothelial cells (HUVEC and HDMEC) and fibroblasts as well as BEAS-2B cells. TNF-α-treated fibroblasts and IL-1β-treated HUVECs showed up-regulation of PTX3 and to a lesser extent, TSG-6 mRNA, and LPS induced the production of PTX3 but not TSG-6 in HUVECs.

To compare TSG-6 and PTX3 expression in leucocyte populations, we isolated fresh human monocytes, T lymphocytes, NK cells, and PMN from the peripheral blood of healthy donors. Macrophages and MoDC were differentiated from monocytes as described above, and B cells were prepared from tonsils. As shown in Figure 1B, under resting conditions, there was no evidence of TSG-6 transcript in monocytes, macrophages, circulating B cells, large B cells from tonsils, or NK cells (freshly isolated or cultured cell lines). In contrast, freshly isolated PMN exhibited a strong signal for TSG-6 mRNA, which almost disappeared after 20 h in culture (see Fig. 2). T lymphocytes and to a lesser extent, immature MoDC also expressed low levels of TSG-6 mRNA (Fig. 1B). None of the cell types tested expressed PTX3 mRNA in resting conditions (Fig. 1B).

The stimulation of lymphocytes with PHA induced TSG-6 expression but had no effect on PTX3 (Fig. 1B). In contrast, TSG-6 and PTX3 transcripts were induced in monocytes, macrophages, and MoDC following the engagement of TLR4 by LPS (see Figs. 1B and 2). In MoDC, which are the major producers of PTX3 upon TLR engagement [39], PTX3 and TSG-6 were induced by LPS in a dose-dependent manner with expression being detectable from a dose of 1 ng/ml (see below). Analysis of the kinetics of TSG-6 and PTX3 expression in monocytes after LPS stimulation (Fig. 2) revealed that the induction of both mRNAs was rapid and transient, peaking at 4 h and being strongly reduced after 20 h of stimulation. LPS also caused up-regulation of TSG-6 expression in PMN after 4 h of incubation, which persisted after 20 h (Fig. 2); PTX3 message was not detected in PMN in response to any of the stimuli tested, as expected [2], and was not studied further.

Regulation of TSG-6 and PTX3 expression by pro- and anti-inflammatory stimuli

We next analyzed whether LPS-induced TSG-6 and PTX3 mRNA levels in leucocytes were comodulated by a series of pro- and anti-inflammatory cytokines.

Treatment with IL-4 alone did not modify TSG-6 or PTX3 expression in monocytes (not shown), MoDC, or PMN.

Detection of TSG-6 and PTX3 by confocal microscopy

Neutrophils were freshly isolated or cultured in the presence of 100 ng/ml LPS for 30 min or 4 h and seeded on precoated (poly-L-lysine) glass coverslips or cytosusp, fixed with 4% (v/v) formaldehyde in PBS, pH 7.4, for 5 min with 0.3% (v/v) Triton (Sigma-Aldrich) and 0.1% (v/v) SDS in PBS, pH 7.4, for 1 h at 4°C with 10% (v/v) normal goat serum (Sigma-Aldrich) and glycine 0.1% (v/v) in PBS. Specimens were incubated with 1 μg/ml affinity-purified polyclonal RAH-1 [47] or purified preimmune rabbit serum as control, followed by incubation with Alexa Fluor 488-conjugated goat anti-rabbit as secondary antibody (Molecular Probes, Eugene, OR, USA), washes with 0.2% (v/v) BSA and 0.05% (v/v) Tween-20 in PBS (pH 7.4). Sections were mounted with FluorSaveTM reagent (Calbiochem, San Diego, CA, USA) and analyzed with an Olympus Fluoview FV1000 laser-scanning confocal microscope. Images (1024×1024 pixels) were acquired with a 10×/1.4 NA Plan-Apochromat oil immersion objective (Olympus, Hamburg, Germany).

Immunohistochemistry

The expression patterns of TSG-6 and PTX3 were studied by immunohistochemistry in colon tissues from two patients with inflammatory bowel disease. Samples were collected and frozen in liquid nitrogen. Sections (9 μm) were cut and mounted on poly-L-lysine-coated slides. After fixation with acetone/chloroform for 3 min, sections were incubated for 2 h with the following antibodies: affinity-purified rabbit IgG against hPTX3 (50 μg/ml) [2], mouse anti-lactoferrin mAb (1 mg/ml; HyCult Biotechnology, Uden, The Netherlands), or IgG isotype control mAb for 1 h at 4°C. After each step, cells were washed with 0.2% (v/v) BSA and 0.05% (v/v) Tween-20 in PBS (pH 7.4). Sections were mounted with FluorSaveTM reagent (Calbiochem, San Diego, CA, USA) and analyzed with an Olympus Fluoview FV1000 laser-scanning confocal microscope. Images (1024×1024 pixels) were acquired with a 10×/1.4 NA Plan-Apochromat oil immersion objective (Olympus, Hamburg, Germany).
However, IL-4 was seen to inhibit LPS-induced expression of TSG-6 and PTX3 in MoDC (Fig. 3A) and less efficiently, in monocytes (not shown). In MoDC, this inhibitory effect of IL-4 was particularly strong in the case of PTX3, which was barely detectable when the high or low doses of LPS (10 and 1 ng/ml, respectively) were used. TSG-6 was strongly induced in MoDC even with the lower dose of LPS, and the inhibitory effect of IL-4 was substantially less than that seen for PTX3 (Fig. 3A). In PMN incubated for 4 h with LPS and IL-4, the TSG-6 mRNA level was only reduced slightly compared with that in cells treated with LPS alone (Fig. 3B). However, after 20 h incubation, IL-4-mediated inhibition of this LPS activity was pronounced. PMN were also treated with IL-13, which inhibited LPS-induced TSG-6 message expression, although to a lesser extent than IL-4 (Fig. 3B). These, along with previous results for PTX3 [40], suggest that TSG-6 and PTX3 are coregulated by the anti-inflammatory cytokines IL-4 and IL-13 in leukocytes.

It has been demonstrated that in apparent contrast to its general anti-inflammatory role, IL-10 alone slightly induces PTX3 mRNA expression in monocytes and DC; furthermore, it synergizes with LPS in up-regulating PTX3 transcript and protein levels [40, 48]. IL-10 was not able to induce TSG-6 expression in monocytes (not shown) or MoDC (Fig. 3A) or PMN (Fig. 3B) and actually exerted a slight negative effect on LPS-induced TSG-6 transcription in monocytes (not shown), MoDC, and PMN (Fig. 3, A and B).

IFN-γ is a negative regulator of TLR-mediated induction of PTX3 transcription and protein release [49]. Therefore, it is not surprising that IFN-γ alone has no effect on PTX3 mRNA production and that it down-regulates LPS-induced transcription in MoDC (Fig. 3A). Interestingly, LPS-induced TSG-6 expression was variably modulated by IFN-γ, depending on the cell type analyzed; we observed some induction of TSG-6 message by IFN-γ alone in monocytes (not shown) and to a greater extent in PMN after 20 h of incubation and some enhancement of LPS-induced transcription in PMN (Fig. 3B). In macrophages (not shown) and MoDC (Fig. 3A), IFN-γ had no effect on TSG-6 transcription, alone or in combination with LPS.

Figure 1. TSG-6 and PTX3 expression in different human cell lines and leukocyte subpopulations. (A) Total RNA was extracted using TRIzol from bronchial epithelial cells (A549), transformed bronchial epithelial cells (BEAS-2B), fibroblasts (W138), and endothelial cells (HUVEC, HDMEC), cultured in the presence of LPS, TNF-α, or IL-1β where specified and analyzed by Northern blotting. Total RNA (10 μg) was run in each lane, and the lower panels show the ethidium bromide-stained membranes to confirm mRNA transfer. Blots were first hybridized with a human Link_TSG6 cDNA probe (hTSG-6) and then, after stripping, with hPTX3. (B) Total RNA was extracted from freshly isolated human monocytes, cultured macrophages, MoDC, PMN, B and T lymphocytes, and NK cells. Where indicated, cells were incubated with LPS (10 ng/ml) for 4 h or in the case of lymphocytes, with PHA (100 U/ml) for 48 h. Northern blot analysis with hTSG-6 and hPTX3 probes was performed as described for A.
Release of TSG-6 by MoDC and PMN
MoDC have been studied extensively in relation to the production of PTX3 upon TLR engagement and the regulation of this effect by pro- and anti-inflammatory cytokines [39, 40]. As illustrated in Figure 4A, which shows one out of three experiments performed, we measured TSG-6 levels in MoDC supernatants, cultured for 24 h with LPS as an inflammatory stimulus in the absence or presence of IL-4, IL-10, or IFN-γ as modulators. The secretion of TSG-6 into the supernatants of LPS-treated MoDC was confirmed by Western blot analysis (Fig. 4B); in addition to free TSG-6 protein (~35 kDa), a species of ~120 kDa, which is likely to correspond with a covalent complex of IgI HC with TSG-6 (i.e., TSG-6•HC), was also detected as a result of the presence of bovine serum in the culture medium [27, 50, 51]. In agreement with the mRNA data (Fig. 3A), LPS-induced TSG-6 release was not modified by treatment with IFN-γ. IL-4 and IL-10 had small but significant inhibitory effects on LPS-induced TSG-6 expression. IFN-γ, IL-4, or IL-10 alone did not induce protein secretion.

As shown in Figure 4, C and D, which illustrates the results for one out of five donors tested, TSG-6 as well as PTX3 are released by PMN upon engagement of TLR4 by LPS. The kinetics of TSG-6 release is in agreement with transcription data and with experiments performed in the presence of actinomycin D (not shown), which suggest degranulation followed by de novo transcription. As reported previously [2], the time scale of the PTX3 increase in the supernatant indicates rapid release and absence of transcription.

Localization of TSG-6 in neutrophils
PTX3 is stored in neutrophil-specific granules [2]. Here, confocal microscopy was used to identify the localization of TSG-6 within freshly isolated and cultured neutrophils. The images shown in Figure 5A point to the presence of TSG-6 in PMN granules of resting PMN, where it colocalizes partially with PTX3 in lactoferrin-positive PMN granules, i.e., specific granules. Thirty minutes after LPS treatment, TSG-6 and PTX3

Figure 2. Kinetics of PTX3 and TSG-6 expression in human monocytes and PMN. Monocytes and PMN were isolated from buffy coats and incubated with LPS (100 ng/ml) for 4 h or 20 h. RNA was extracted, and the expression of TSG-6 was analyzed by Northern blot analysis. Membranes were subsequently stripped and hybridized with the PTX3 probe. The lower panels show ethidium bromide-stained membranes.

Figure 3. Comparison of TSG-6 and PTX3 expression in human leukocytes stimulated with pro- and anti-inflammatory stimuli. (A) MoDC were differentiated from monocytes and incubated for 4 h with the stimuli indicated; two different concentrations of LPS were used (1 and 10 ng/ml), where this was added to the cultures after 30 min preincubation with IL-10, IL-4 (20 ng/ml), or IFN-γ (5000 U/ml). After total mRNA extraction, TSG-6 and PTX3 expression was determined by Northern blot analysis. The lower panels show the ethidium bromide-stained membranes. The figure shows PTX3 and TSG-6 expression in MoDC in one donor out of the three tested, where these all gave similar results. (B) hPMN were incubated for 4 h or 20 h with LPS (100 ng/ml), alone or in combination with IFN-γ (5000 U/ml), IL-10, IL-4, or IL-13 (20 ng/ml), where the latter was added to cultures 30 min prior to LPS. Ctrl, Control.
staining in granules was reduced, suggesting release by degranulation (Fig. 5B). After 4 h, immunoreactivity for TSG-6 in granules had disappeared but was now present in the cytoplasm, localized in vesicular structures (Fig. 5B). In contrast, no immunoreactivity for PTX3 was observed at this time-point after LPS treatment. These results are in agreement with the mRNA studies described above, which indicated LPS-induced TSG-6 transcription; on the other hand, PTX3 mRNA is expressed only at the promyelocytic stage, where the protein is stored in granules and released upon TLR engagement [2].

Immunohistochemistry
To assess PTX3 and TSG-6 localization in inflamed tissues in vivo, their expression was analyzed by immunohistochemistry in biopsies of colon mucosa from patients with inflammatory bowel disease. As shown in Figure 6, a huge inflammatory infiltrate, involving the mucosa and the superficial layers of the submucosa, is associated with detachment and distortion of the crypts. PTX3 expression (Fig. 6A) is observed in the inflammatory cells of the lamina propria and also beneath the surface mucosa and in the perivascular spaces, with an interstitial and extracellular pattern of distribution. TSG-6 staining (Fig. 6E) is diffusely localized in the inflammatory infiltrate in the mucosa and submucosa. PTX3 and TSG-6 were expressed in the cytoplasm of macrophages (Fig. 6, B and F), granulocytes (Fig. 6, C and G), and in some endothelial cells of the lamina propria (Fig. 6, D and H). For both proteins, no immunostaining was observed in the absence of primary antibodies. Double immunofluorescence analysis indicated that there was TSG-6 protein expression in CD15+/H11001 neutrophils (Fig. 7A), CD68+/H11001 macrophages (Fig. 7B), and CD31+/H11001 endothelial cells (Fig. 7C).

These results suggest that PTX3 and TSG-6 are expressed in inflammatory conditions in vivo by leukocytes and endothelial cells.

DISCUSSION
PTX3 is a multifunctional soluble pattern recognition receptor involved in inflammation and innate immunity [1]. Similarly, TSG-6, which has anti-inflammatory and chondroprotective effects, is produced in the context of inflammatory processes and diseases (reviewed in refs. [14, 15]). Both of these proteins are up-regulated specifically during cumulus oophorus expansion in response to hormonal ovulatory stimuli and oocyte soluble factors, such as the TGF-β family member growth differentiation factor-9 [21]. PTX3 and TSG-6 colocalize in the cumulus matrix and play a crucial role in cumulus expansion, where deficiency of either protein causes instability of the cumulus ECM as a result of defective hyaluronan incorporation and female infertility [19, 22]. The results reported here provide new insights into the expression of the TSG-6 and PTX3 genes in various cell types, including different leukocyte populations, in response to pro- and anti-inflammatory mediators and demonstrate the expression of both proteins in leukocytes and endothelial cells in inflammatory conditions.

In this study, we generally observed little or no basal expression of TSG-6 or PTX3 mRNA in any of the cell types analyzed,
with the exception of TSG-6 in neutrophils. However, both mRNAs were induced rapidly by proinflammatory stimuli, consistent with their involvement in inflammation and innate immune responses.

Our attention has been focused particularly on leukocytes, as although these cells are known to be a major source of PTX3, TSG-6 has not been studied extensively in this context [14]. TSG-6 has emerged, in expression-profiling analyses, among the inflammation-inducible genes in leukocytes [52, 53], but to our knowledge, after the original description by Lee and colleagues [54], who observed TSG-6 mRNA in PBMCs after treatment with TNF, Con A, or PHA, this is the first systematic analysis of TSG-6 expression and regulation in leukocyte subpopulations.

Mononuclear phagocytes responded to LPS engagement of TLR4 by rapidly producing TSG-6 and PTX3 in a dose-dependent manner. Thus, similarly to PTX3, TSG-6 behaves as an immediate early gene in leukocytes. In particular, we observed high levels of TSG-6 mRNA in monocytes, macrophages, and MoDC, which was rapidly detectable even upon stimulation with low levels of LPS, and this result was confirmed at the protein level, in vitro and in inflamed tissues. Freshly isolated PMN were the only cell type analyzed that had high basal levels of TSG-6 mRNA expression; this was elevated following treatment with LPS but was lost after 20 h in culture without stimulation. In contrast, PMN incubated with LPS and/or IFN-γ maintained high levels of TSG-6 mRNA after 20 h of culture.

PTX3 mRNA is not detectable in mature PMN under any of the conditions analyzed, whereas it is produced by pro-myelocytes [2]; the protein is stored in neutrophil-specific granules and undergoes release in response to microbial recognition and inflammatory stimuli [2], resulting in pathogen recognition and clearance. Confocal analysis suggested that TSG-6 is also present in specific granules, where it partially colocalizes with PTX3, and both proteins are released upon LPS stimulation. Finally, immunohistochemistry demonstrated the coexpression of TSG-6 and PTX3 in leukocytes at inflammatory sites in vivo.

TSG-6 is an anti-inflammatory mediator, which inhibits neutrophil recruitment in different models of inflammation [36–38], an activity that is mediated by the Link module and might be a result of modulation of neutrophil adhesion to the endothelium. Interestingly, PTX3 has also been shown to have regulatory effects on inflammatory reactions [7, 55]. The rapid availability of PTX3 at the onset of inflammation could be important for the development of an appropriate innate immune response to invading pathogens, whereas the local expression of TSG-6 could serve to control neutrophil migration, thus preventing host tissue damage [35]. Furthermore, the combined actions of PTX3 and TSG-6 might support wound healing through the deposition of HA-rich ECM; this would be in line with the role suggested for neutrophils in tissue remodeling based on the transcriptional profiles seen in innate responses [56]. The secretion of TSG-6 protein by neutrophils is also of interest in relation to its recently reported role as an

Figure 5. Localization of TSG-6 and PTX3 within neutrophil granules. Neutrophils were freshly isolated (A) or cultured in the presence of 100 ng/ml LPS for 30 min or 4 h (B). Cells were fixed and stained for hTSG-6 (green), PTX3 (red), or lactoferrin (red; see Materials and Methods). Nuclei were stained with Hoechst 33258. A representative cell is shown in each panel. Specific granules were identified as lactoferrin positive.
inhibitor of RANKL-mediated osteoclastic bone resorption [57]. RANKL is expressed by normal and inflammatory neutrophils, and its receptor (RANK) and also osteoprotegrin (a soluble inhibitor of RANKL) are expressed only by neutrophils exposed to inflammatory stimuli, e.g., in synovial fluids from patients with RA [58]. Therefore, TSG-6 can be added to the list of molecules expressed by neutrophils that modulate bone remodeling, where these cells may have an important role in the regulation of bone turnover at inflammatory sites [59].

In phagocytes, we observed that preincubation of cells with anti-inflammatory cytokines (IL-4 and IL-13) resulted in marked reductions in LPS-induced expression of TSG-6 and PTX3 mRNA. In contrast, pretreatment with IL-10 resulted in differential regulation of the two genes, where IL-10 acted syn-
mononuclear phagocytes, DC, and neutrophils may be involved in the assembly of HA-rich matrices, the divergent effects of IL-10 in modulating their expression suggest that these two proteins might have distinct roles in tissue remodeling during chronic inflammation.

The in vitro and ex vivo results reported here suggest that mononuclear phagocytes, DC, and neutrophils may be important sources of TSG-6 at sites of inflammation, where this protein is understood to participate in the regulation of leukocyte recruitment and in tissue repair and remodeling.

ACKNOWLEDGMENTS

This work was supported by Associazione Italiana per la Ricerca sul Cancro (IRC), Ministero Istruzione Università e Ricerca (MIUR), European Commission (MUGEN, EMBC), the Arthritis Research Campaign (grant 16539), and the Medical Research Council.

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**KEY WORDS:** extracellular matrix, inflammation, acute-phase reactants, dendritic cells, neutrophils