TSG-6: a pluripotent inflammatory mediator?

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Abstract

TSG-6 is a multifunctional protein that is up-regulated in many pathological and physiological contexts, where it plays important roles in inflammation and tissue remodelling. For example, it is a potent inhibitor of neutrophil migration and can modulate the protease network through inhibition of plasmin. TSG-6 binds a wide range of GAGs (glycosaminoglycans) [i.e. HA (hyaluronan), chondroitin 4-sulphate, dermatan sulphate, heparin and heparan sulphate] as well as a variety of protein ligands, where these interactions can influence the activities of TSG-6. For example, through its association with HA, TSG-6 can mediate HA cross-linking via several different mechanisms, some of which promote leukocyte adhesion. Binding to heparin, however, enhances the ability of TSG-6 to potentiate the anti-plasmin activity of inter-α-inhibitor, which binds non-covalently to TSG-6 via its bikunin chain. Furthermore, although HA and heparin interact with distinct sites on the Link module, the binding of heparin can inhibit subsequent interaction with HA. In addition, the interactions of TSG-6 with HA, heparin and at least some of its protein ligands are sensitive to pH. Therefore it seems that in different tissue micro-environments (characterized, for example, by pH and GAG content), TSG-6 could be partitioned into functional pools with distinct activities.

Introduction

TSG-6 [the product of TNF (tumour necrosis factor)-stimulated gene-6; also known as TNFIP6] was first described in the early 1990s as a cDNA derived from TNF-treated fibroblasts [1,2]. This protein has since been shown to be produced by many different cell and tissue types in response to a wide variety of stimuli (reviewed in [3]). In particular, TSG-6 expression has been observed in physiological and pathological contexts that are associated with inflammation and tissue remodelling, for example in the sera and joints of arthritis patients [4,5] and in preovulatory ovarian follicles (reviewed in [6]). A diversity of functions have been described for TSG-6 that include inhibition of neutrophil migration [7–9] and modulation of the protease network [7,8,10]. This protein also interacts with a broad spectrum of GAGs (glycosaminoglycan) and protein ligands, including HA (hyaluronan) [11–13], C4S (chondroitin 4-sulphate) [14], heparin [10], inter-α-inhibitor [7,8,10], versican (A.J. Day, unpublished work), aggrecan [15], TSP1 (thrombospondin-1) [16] and PTX3 (pentraxin-3) [17] (see Figure 1). As discussed below, recent work has revealed that TSG-6 is subject to functional partitioning in different tissue micro-environments, where it is likely to be regulated by factors such as pH and its association with different ligands.

Key words: glycosaminoglycan, hyaluronan cross-linking, inflammation, inter-α-inhibitor, protease network, TSG-6.

Abbreviations used: C4S, chondroitin 4-sulphate; GCIC, tumour necrosis factor, COX-2, cyclooxygenase-2; CUB, complement protein subcomponents C1r/C1s, uterine embryonic growth factor and bone morphogenetic protein-1; DS, dermatan sulphate; ECM, extracellular matrix; EGF, epidermal growth factor; GAG, glycosaminoglycan; HA, hyaluronan; H, heavy chain; HS, heparan sulphate; iα1, inter-α-inhibitor; IL, interleukin; LH, luteinizing hormone; PGE2, prostaglandin E2; PTX3, pentraxin-3; TNS, tumour necrosis factor; TSP1, thrombospondin-1.

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Expression of TSG-6

The profile of TSG-6 expression was reviewed in detail in [3]. Recent developments in this area, some of which relate to intracellular signalling pathways, will be discussed here. TSG-6 is produced by cumulus and granulosa cells in the ovarian follicle in response to stimulators of cAMP [e.g. PGE2 (prostaglandin E2) and follicle-stimulating hormone] and this is dependent on the activation of specific kinase cascades [18]. Recent work has revealed that EGF (epidermal growth factor) family members are important in mediating the ovarioly response to LH (luteinizing hormone) [19], where TSG-6 mRNA expression in preovulatory follicles is inhibited by an EGF receptor kinase inhibitor [20]. Also in the context of ovulation, mice null for the nuclear receptor co-repressor RIP140 (receptor-interacting protein 140) are female infertile and this can be attributed to failure of the LH-induced expression of genes that include TSG-6 [21].

Following the observation that TSG-6 is induced in cervical smooth-muscle cells by TNF and PGE2 [22], constitutive expression of TSG-6 has been observed in cultured human uterine cervical fibroblasts, where this is up-regulated in response to IL-1 (interleukin-1) and TNF and during pregnancy [23]. In a separate study, TSG-6 was found to be the most highly up-regulated mRNA (12-fold) in vascular smooth-muscle cells in response to the G-proteins Gαq (Q/L) and G11α (Q/L), which is consistent with these being involved in TNF-mediated signalling [24].

In a study of mRNA expression in bronchial epithelial cells from asthma patients, a significant increase in TSG-6 expression was observed following allergen challenge [25]. This is consistent with our own observations of TSG-6 protein expression in asthmatics and smokers (R. Forteza,
Figure 1 | TSG-6 binding partners and functions

TSG-6 binds specifically to GAGs, including HA, CS, DS, heparin and HS, and to the proteoglycans aggrecan and versican (via protein–protein and protein–GAG interactions). It also interacts both covalently and non-covalently with IαI: non-covalent interactions (through the bikunin chain) potentiate the anti-plasmin activity of IαI (this is enhanced by the association of TSG-6 with heparin), whereas covalent TSG-6–HC complexes are intermediates in the modification of HA to HC–HA. This latter process gives rise to HA cross-linking, which can also be mediated by the formation of non-covalent TSG-6–PTX3 and TSG-6–TSP1 complexes. TSG-6 has also been shown to induce or enhance the binding of HA to CD44, where this may be mediated by the formation of cross-linked HA fibrils, resulting in CD44 clustering on the cell surface. The potent inhibitory effect of TSG-6 on neutrophil migration, along with HA cross-linking and its effect on the protease network, are likely to be key factors in the anti-inflammatory and chondroprotective functions of TSG-6. Dotted arrows indicate a binding process or function affected by an interaction with another ligand.


TSG-6 binding partners

TSG-6 is a 35 kDa secreted protein, composed mainly of contiguous Link and CUB (complement protein subcomponents C1r/C1s, urchin embryonic growth factor and bone morphogenetic protein 1) modules that correspond to residues 37–128 and 129–250 in the pre-protein respectively [3,11,26]. Figure 2 shows a prediction of the protein structure (lacking the N- and C-terminal segments), which was generated using the NMR-derived structure of the Link module [12] together with a molecular model of the CUB module, based on the known structures of spermadhesins and the CUB1 module of complement components C1s [3,26,27] (V.A. Higman, C.D. Blundell, A. Almond and A.J. Day, unpublished work). Link modules are a common feature of proteins that interact with HA [28]; however, this domain of TSG-6 contains the binding sites not only for HA, but also for most (if not all) of the other ligands illustrated in Figure 1 [10,14–17,29–31]. The structure of the Link module (Link_TSG6) has been determined by NMR spectroscopy in both its free and HA-bound forms, revealing that, upon interaction with HA, conformational changes and side chain rearrangements result in the opening of a shallow groove on the protein surface [12]. The groove contains the five key HA-binding residues Lys46, Tyr47, Tyr50, Phe105 and Tyr113 (numbered as in the full-length protein; shown in red/orange in Figure 2) defined previously [32] together with Arg116, which is believed to form a salt bridge with bound HA [12]. These and other results have been used to build a high-resolution model of the Link_TSG6–HA complex [13].

Our characterization of the TSG-6 Link module has identified not only the HA-binding site described above, but also a distinct surface (defined as residues Lys55, Lys69, Lys76 and Lys89; shown in blue in Figure 2) that can accommodate heparin and various forms of HS (heparan sulphate), where the binding affinity for a heparin 8-mer is approx. 6-fold greater than that for an 8-mer of HA [10]. We have also observed that other sulphated GAGs, i.e. CS and DS (dermatan sulphate), interact with TSG-6 at the same site as heparin (D.J. Mahoney, C.M. Milner and A.J. Day, unpublished work). Wisniewski et al. [29] have reported binding of C6S to the TSG-6 Link module, but this is likely to be due to contamination by C4S of the C6S preparation used. Although the HA- and heparin-binding sites on TSG-6 are non-overlapping, the association of Link_TSG6 with heparin can prevent subsequent HA binding [10]. This has been hypothesized to be due to an allosteric mechanism, whereby heparin may lock the HA-binding groove in its ‘closed’ conformation.

The association of the Link module with heparin also has a profound effect on another property of TSG-6, namely its potentiation of the anti-plasmin activity of IαI [10]. IαI is an unusual proteoglycan consisting of bikunin (a Kunitz-type protease inhibitor) and two HCs (heavy chains) (HC1 and HC2, ~85 kDa) that are covalently linked by a C4S chain; the latter is attached to Ser12 of bikunin by a standard GAG linkage, while the HCs are attached to C4S via ester bonds [33]. Although IαI alone has only weak serine protease inhibitory activity (causing ~5–8% inhibition of plasmin activity) in the presence of TSG-6, this is elevated to approx. 40% inhibition [7,8]. We have shown recently that this effect is mediated through the non-covalent association of the TSG-6 Link module with bikunin [10]. Analysis of mutants of Link_TSG6 has revealed substantial overlap with the HA-binding site, where mutation of any one of four out of the five key residues (i.e. Lys56, Lys57, Tyr58 or Tyr63, shown in red in Figure 2) reduces bikunin binding considerably [10]. The effect of TSG-6 on IαI’s anti-plasmin activity is significantly enhanced when TSG-6 is bound to heparin [10]. Since heparin binding results in dimerization of Link_TSG6, we have hypothesized that such dimers could interact with the two Kunitz-type domains of bikunin, thereby changing their relative orientation and making the protease-binding sites more accessible.

In addition to its non-covalent association with bikunin, TSG-6 has been shown to form covalent complexes with each of the HCs of IαI, i.e. TSG-6–HC1 and TSG-6–HC2 (see Figure 1). These approx. 115 kDa species were first isolated and characterized from the ECM (extracellular matrix) of...
The structural organization of the Link (upper) and CUB (lower) modules of TSG-6 is shown, where these were modelled using the NMR-derived structure of the Link module [12] and a prediction of the CUB module structure based on the co-ordinates of three spermadhesins (PDB accession codes 1SPP and 1SFP) and (for the metal ion-binding site) the complement component C1s (PDB accession code 1NZI). The image on the right is rotated through 90° about the vertical axis compared with that on the left. The TSG-6 backbone is shown with β-sheets in yellow and α-helices in pink. Within the Link module, those residues that bind to HA, or to both HA and bikunin, are highlighted by orange and red space-filling representations respectively. Heparin-binding residues are shown in dark blue [10]. The predicted position and conformation of bound HA 8-mer [13] are indicated by magenta sticks with space-filling green dots. Residues His39 and His64 (cyan) do not contribute to HA binding, but have been shown to influence the TSG-6-mediated induction/enhancement of the HA–CD44 interaction, perhaps being involved in the cross-linking of HA to form fibrils via self-association of TSG-6 [39]. The predicted metal ion-binding site in the CUB module is indicated in purple, with an Mg2+ ion shown in green [30].

ovulated COCs (cumulus oocyte complexes) from mice [34] and were also shown to form rapidly (30 s at 37°C) in vitro from recombinant human TSG-6 and serum-derived IzI [26,30]. The observations that TSG-6 and IzI co-localize in the cumulus matrix and that mice with functional deletions for either IzI [35,36] or TSG-6 [18,37] are female infertile indicated that both proteins have essential roles in ovulation. We have shown that TSG-6–HC complexes are intermediates in the covalent modification of HA with HCs [30]. These HC–HA complexes (also referred to as SHAP-HA) occur in the cumulus ECM during ovulation [37], in the bronchial secretions of asthmatics (R. Forteza, S. Casalino-Matsuda, M.E. Monzon-Medina, M.S. Rugg, C.M. Milner and A.J. Day, unpublished work) and in arthritic synovial fluids [38]. HC–HA complexes have been implicated in HA cross-linking via HC–HC interactions (see [39]). Although it is known that the ester linkage in TSG-6–HC2 involves the C-terminal Asp residue of the HC [31], the attachment site on TSG-6 has not yet been determined. It seems likely that this is within the Link module, since a monoclonal antibody (A38) that has its epitope in this domain can block TSG-6–HC formation in COCs ex vivo [18].

The modification of HA by association with HCs is only one of the mechanisms by which TSG-6 is now known to mediate HA cross-linking [39]. Aside from tsg-6−/− and bikunin−/− mice [35–37], animals null for the glycoprotein PTX3 are also female infertile and this has been attributed to an essential role for this long pentraxin in cumulus matrix expansion [17,40]. PTX3 is up-regulated by inflammatory stimuli [40] and shares similar temporal and spatial distributions
to those of TSG-6 and HA in the periovulatory follicle [17,34,41]. However, we have shown that PTX3 does not bind HA directly, but interacts with the Link module of TSG-6 at a site distinct from the HA-binding surface [6,17]. PTX3 occurs as disulphide-linked multimers of approximately ten protermer subunits (~45 kDa) and each of these could potentially interact with a TSG-6 molecule, thereby forming a node to which multiple HA chains could bind [6,17]. The infertility of ptx3−/− mice suggests that this mechanism of HA cross-linking is essential for cumulus matrix stability in addition to the formation of HC–HA complexes; i.e. TSG-6 plays at least two essential and non-redundant roles in the stabilization of the cumulus matrix.

It has recently been shown that the Link module of TSG-6 binds to TSP1, an ECM protein that is induced by inflammatory signals [16]. This interaction is likely to occur via the N-module of TSP1 at a site on the Link module distinct from both the HA and heparin-binding surfaces. Interestingly, heparin does inhibit the interaction of TSG-6 and TSP1; however, this has been attributed to the association of heparin with the N-module of TSP1 [16]. TSP1 is a trimeric protein and could act in a manner analogous to PTX3, forming foci for the cross-linking of HA chains via its binding with multiple TSG-6 molecules [39]. Although this may not be essential in the context of ovulation (tsp1−/− mice have only mildly impaired fertility of unknown cause [16]), it may be relevant in other inflammatory situations where TSG-6 and TSP1 are co-expressed (e.g. in rheumatoid synovium). Furthermore, we have shown that TSP1 promotes the formation of TSG-6–HC complexes, thus enhancing the covalent transfer of HCs to HA [16]; i.e. TSP1 could modulate HA cross-linking through more than one TSG-6-mediated mechanism.

### Sensitivity to pH

The interaction of TSG-6 with HA is sensitive to pH, being close to maximal at pH 6 and decreasing dramatically as the pH is raised or lowered [14]; however, the structural basis for this effect remains to be determined. In addition, the associations of aggrecan G1-domain [14] and TSP1 [15] with TSG-6 are pH-dependent, where binding increases as the pH is lowered towards 6. While binding to aggrecan is inhibited by HA [15], this is not the case for TSP1 [16], indicating that the interaction surfaces involved are distinct from each other.

As described above, heparin augments the potentiation of the anti-plasmin activity of IozI by TSG-6, where this enhancement is significant between pH 6 and 6.75, but does not occur at higher pH values [10]. This suggests that in inflammatory environments, where there is acidosis, the co-localization of TSG-6, IozI and heparin (or activating proteoglycans) might result in down-regulation of the protease network and thereby regulate matrix remodelling. For example, plasmin has been implicated as an essential component in the early phases of rheumatoid arthritis [42].

### TSG-6 in inflammation

As noted above, a major site of TSG-6 expression is in the inflamed synovial tissue of arthritis patients [5] and studies using murine models of arthritis have revealed that TSG-6 has potent anti-inflammatory and chondroprotective effects in this context [43–46]. A number of mechanisms might contribute to these activities of TSG-6, for example, the TSG-6-mediated down-regulation of the protease network described above [7,10]. This is supported by evidence that matrix metalloproteinase activity is inhibited by the treatment of arthritic mice with recombinant TSG-6 [45,46], since plasmin is a key activator of these enzymes. Furthermore, there is increased plasmin activity in the inflamed paw joints of tsg-6−/− mice compared with wild-type controls in a model of arthritis [47]. TSG-6 has also been shown to be a potent inhibitor of neutrophil migration in vivo, causing >50% inhibition (at a 3 μg systemic dose) in air pouch models of acute inflammation [8]. This effect is mediated entirely by the Link module and characterization of Link,TSG6 mutants indicates that it correlates with neither HA binding nor potentiation of IozI’s anti-plasmin activity. Further analysis, using intravital microscopy, suggests that TSG-6 might influence multiple aspects of neutrophil extravasation, in particular firm adhesion, with endothelial cells possibly playing a role in the ‘presentation’ of TSG-6 [9]. Recently, it has been shown that the progression and severity of proteoglycan-induced arthritis are significantly increased in tsg-6−/− mice compared with wild-type controls and this is associated with early and more extensive infiltration of neutrophils into the synovia of the knockout mice [47].

Although our previous results indicate that the TSG-6–HA interaction may not be involved in the inhibition of neutrophil migration [8], there is evidence that TSG-6 might influence leucocyte extravasation through its modulation of CD44/HA-dependent rolling on the endothelium [48]. We have shown that preformed complexes of TSG-6 (or Link,TSG6) and HA can enhance/induce the binding of HA to CD44 on constitutive/inducible cell backgrounds (i.e. those expressing CD44 in active or inactive HA-binding states) [48]. Furthermore, cell rolling is increased on substrates comprising TSG-6–HA complexes as compared with HA alone. It seems likely that TSG-6 can cross-link HA to form stable fibres where residues outside the HA-interaction surface (in addition to those that mediate HA binding) have been implicated in this process, most probably through TSG-6 self-association (shown in cyan in Figure 2). The formation of such fibrils could promote the activation of CD44 through receptor clustering [49]. HA cross-linking, through the various TSG-6–mediated mechanisms outlined above, might also be relevant to the anti-inflammatory and chondroprotective effects of TSG-6. For example, HA networks at the surface of synovium or cartilage could form a protective barrier, potentially preventing matrix degradation, and/or acting as a scaffold for matrix regeneration [39].

Finally, there is evidence that TSG-6 can regulate the expression of various molecules that have important roles in the control of inflammation. One such example is the up-regulation of the enzyme COX-2 (cyclo-oxygenase-2) in a macrophage cell line [50], where COX-2 can mediate the production of both pro- and anti-inflammatory prostaglandins.
In this study, TSG-6 appeared to induce synthesis of PGD2 (prostaglandin D2), which promotes the resolution of inflammation [50]. Furthermore, in tsg-6−/− mice, the induction of proteoglycan-induced arthritis results in elevated levels of the pro-inflammatory mediators serum amyloid A and IL-6, compared with wild-type controls [47], while TSG-6 treatment significantly reduced the levels of TNF, PGE2 and KC (a murine homologue of IL-8) in the mouse air pouch model of inflammation [8].

Concluding remarks

Molecular characterization of TSG-6 over recent years has identified a growing number of ligands for this multifunctional protein and has revealed that the activities of TSG-6 can be differentially modulated through these interactions. The use of mutants has defined a number of interaction surfaces on the Link module and similar analyses are ongoing, depending on its micro-environment. In particular, GAG composition may fine-tune the functional properties of this pluriotopic protein.

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References


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