Recombinant soluble betaglycan is a potent and isoform-selective transforming growth factor-β neutralizing agent

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INTRODUCTION

Transforming growth factor-β (TGF-β) is the prototype of a superfamily of autocrine and paracrine factors that are involved in development, cell differentiation and proliferation [1,2]. TGF-β controls many physiological processes and, thus, disturbances in its regulation or signalling pathway can lead to disease [3,4]. TGF-β is central to the control of extracellular-matrix production and, thereby, in wound repair [5]. TGF-β promotes the accumulation of extracellular matrix by increasing its synthesis and decreasing its degradation [1]. TGF-β is necessary for proper wound healing; however, excess TGF-β leads to the fibrosis that is observed in various diseases; a deleterious effect that has been called the ‘dark side’ of TGF-β. Because of its potential use as an anti-TGF-β therapeutic agent, we have purified and characterized baculoviral recombinant soluble betaglycan. Baculoviral soluble betaglycan is a homodimer formed by two 110 kDa monomers associated by non-covalent interactions. This protein is devoid of glycosaminoglycan chains, although it contains the serine residues, which, in vertebrate cells, are modified by these carbohydrates. On the other hand, mannose-rich carbohydrates account for approximately 20 kDa of the mass of the monomer. End-terminal sequence analysis of the soluble betaglycan showed that Gly1 is the first residue of the mature protein. Similarly to the natural soluble betaglycan, baculoviral soluble betaglycan has an equilibrium dissociation constant ($K_d$) of 3.5 nM for TGF-β1. Ligand competition assays indicate that the relative affinities of recombinant soluble betaglycan for the TGF-β isoforms are TGF-β2 > TGF-β3 > TGF-β1. The anti-TGF-β potency of recombinant soluble betaglycan in vitro is 10-fold higher for TGF-β2 than for TGF-β1. Compared with a commercial pan-specific anti-TGF-β neutralizing antibody, recombinant soluble betaglycan is more potent against TGF-β2 and similar against TGF-β1. These results indicate that baculoviral soluble betaglycan has the biochemical and functional properties that would make it a suitable agent for the treatment of the diseases in which excess TGF-β plays a central physiological role.

Key words: insect cell protein processing, soluble receptors, transforming growth factor-β antagonists.
TGF-β signalling function for betaglycan has not been rejected [19].

A naturally occurring soluble form of betaglycan is found in serum and extracellular matrices [20]. This form is generated by a still uncharacterized proteolytic cleavage of the extracellular portion of the membrane betaglycan [21]. In contrast to its membrane counterpart, soluble betaglycan produced in insect cells by cDNA recombinant methods, is a TGF-β antagonist [22]. As the first step to determine if this recombinant soluble betaglycan could be a useful therapeutic anti-TGF-β agent, we have purified it and characterized its biochemical and functional properties. Our data indicate that recombinant soluble betaglycan is a dimeric glycoprotein that binds TGF-β with high affinity and isoform selectivity. In addition, we found that in the in vitro assays tested, baculoviral soluble betaglycan neutralizes TGF-β with high potency, opening up the possibility of its use as a therapeutic anti-TGF-β agent.

**EXPERIMENTAL**

**Reagents and cell cultures**

COS-1 and MvILu (CCL-64; American Type Culture Collection) cells were grown in Dulbecco's modified Eagle medium supplemented with 10% fetal-bovine serum (Gibco/BRL, Grand Island, NY, U.S.A.). SF9 cells were grown in Grace's medium supplemented with 10% fetal-calf serum, yeastolate and lactalbumin hydrolysate. High five cells (H5; Invitrogen, Carlsbad, CA, U.S.A.) were grown in spinner flasks at 27 °C at 100 rev/min, using Express Five (Gibco/BRL) serum-free medium supplemented with 4 mM glutamine. TGF-β1 and pan-specific TGF-β neutralizing antibody (AB-100-NA) (R & D Systems, Minneapolis, MN, U.S.A.). TGF-β2 and TGF-β3 were gifts from Dr N. Cerletti (Ciba Geigy, Basel, Switzerland). The anti c-myc monoclonal antibody (9E10) has been described previously [23]. Unless otherwise specified, all other biochemicals were from Sigma-Aldrich Co.

**Cell transfection and luciferase assay**

Transient transfections were performed by the diethylaminoethyl-dextran method [24]. For the anti-c-myc Western blot, 72-h conditioned media from the COS-1 cells transfected with the soluble betaglycan or the glycosaminoglycan chain (GAG)-less soluble betaglycan vectors (the LS or LS gag constructs reported in [22]) were transferred to nitrocellulose and probed with the 9E10 monoclonal antibody. For the luciferase assay, MvILu cells transfected with the p3TP-lux reporter plasmid [25], were seeded in 24-well multicluster wells (5 × 10⁴ per well) for assay. On the following day, the transfected cells were supplemented with 20 pM TGF-β [dissolved in Dulbecco's modified Eagle medium containing 0.2% (v/v) fetal-bovine serum] and 0–400 nM soluble betaglycan (or neutralizing antibody) (see the legend to Figure 6) and incubated for 18 h. After incubation, cells were washed twice with PBS and lysed in 25 mM Tris/phosphate (pH 7.8), 2 mM dithiothreitol (DTT), 2 mM 1,2-diaminocyclohexane-N,N,N’,N’-tetra-acetic acid, 10% (v/v) glycerol and 1% (v/v) Triton X-100, for 15 min at room temperature. Luciferase activity was measured in the clear lysates using the Luciferase Reporter Assay System (Promega, Madison, WI, U.S.A.) in a Berthold luminometer (Lumat LB9501).

**Purification of recombinant soluble betaglycan**

High five cells at a density of 2 × 10⁴/ml were infected, at a multiplicity of infection of 10, with a high titre stock of the soluble betaglycan baculovirus. The conditioned media were harvested 48 h post-infection and immediately processed. Conditioned media were supplemented with 1 mM PMSF, centrifuged at 18000 g for 20 min at 4 °C to remove debris, concentrated × 10 using the Minitan Ultrafiltration System (Millipore, Bedford, MA, U.S.A.), and subjected to immobilized metal-ion affinity chromatography (IMAC) with fast-flow chelating Sepharose (Amersham Pharmacia Biotech, Little Chalfont, Bucks., U.K.). Chelating Sepharose (a 60 ml bead-column) was loaded with 50 mM NiCl₂ and then equilibrated in washing buffer (25 mM Hepes, pH 7.5/1 M KCl/20 mM imidazole/1 mM PMSF). The concentrated conditioned media were loaded and the column was washed with 6 volumes of washing buffer and eluted with a 20–250 mM linear gradient of imidazole. Fractions (5 ml) were collected and analysed for the presence of soluble betaglycan by SDS/PAGE and detected with silver staining. Fractions containing soluble betaglycan were pooled, in an Amicon Ultrafiltration chamber (Millipore), dialysed against PBS containing 1% (v/v) glycerol and 1 mM PMSF and stored at −70 °C.

**Gel filtration and native/denaturing gels**

For gel filtration, 30–50 μg of purified baculoviral soluble betaglycan were subjected to HPLC (Beckman Gold 126) on a BioSep Sec-S3000 (300 mm × 7.8 mm) column. The column buffer was 25 mM NaH₂PO₄/150 mM NaCl (pH 6.8) and the flow rate was 1 ml/min. Protein in the eluate was monitored at A₂₈₀; fractions were collected and analysed by SDS/PAGE and silver staining. Native PAGE was as described previously [26,27] and proteins bands were revealed by silver staining. For titration of denaturing and reducing reagents (urea, guanidine HCl, DTT, 2-mercaptoethanol, SDS, Triton X-100 and Nonidet P40), the protein samples were incubated for 30 min at 37 °C in 59 mM Tris/HCl (pH 6.9)/8% (v/v) glycerol/0.025% Bromophenol Blue and SDS or DTT at the concentrations indicated in Figure 3B. The samples were then loaded on to the gel and subjected to PAGE. Calculation of protein mass was according to the Ferguson method, using molecular mass standards of catalase and a set of monomers, dimers, trimers and tetramers of BSA [28].

**Deglycosylation experiments**

Chemical deglycosylation of soluble betaglycan was performed as described by Sojar and Bahl [29]. Briefly, 50 μg of alkylated, lyophilized protein was incubated at 4 °C for 2 h, in the dark, with 100 μl of trifluoromethanesulfonic acid (TFMS) in glass tubing saturated with N₂. The reaction was stopped by the addition of 60% (v/v) pyridine at −20 °C. The protein was extensively dialysed against water, and concentrated in a Speed Vac (Savant Instruments). For analysis, the deglycosylated protein was subjected to IMAC using chelating Sepharose, as described above for the purification of recombinant soluble betaglycan. The proteins eluted were separated by SDS/PAGE and bands were detected by Western blot using the anti-c-myc antibody and concanavalin A.

**N-terminal sequencing**

For N-terminal sequencing, baculoviral soluble betaglycan was first resolved by SDS/PAGE and electroblotted on to PVDF membranes (Millipore). The transferred protein was revealed by Coomassie-blue staining and subjected to automated Edman degradation on a gas-phase protein sequencer (LF 3000;
Beckman Instruments) equipped with an on-line HPLC system (Beckman Gold). The HPLC equipment included a model 126 pump and a 168 diode-array detector set at 268 nm and 293 nm for signal and reference respectively.

**Affinity labelling in solution**

TGF-β1 was labelled with \(^{125}\)I by the chloramine T method, as described previously [30]. For affinity labelling in solution, 10 ng of soluble betaglycan and 100 pM of \(^{125}\)I-labelled TGF-β1 (in the absence or presence of 0–4 nM of non-radioactive TGF-β1) were incubated for 3 h at 4 °C in PBS supplemented with 0.05%, (v/v) Triton-X 100. Cross-linking was started by the addition of 0.1 mg/ml disuccinimidyl suberate (Pierce, Rockford, IL, U.S.A.) and stopped after 15 min by the addition of Tris/HCl (pH 7.5) to a final concentration of 10 mM. The reaction mixture was immunoprecipitated with the anti-c-myc antibody 9E10, separated by SDS/PAGE and bands were revealed by Phosphor-Imager (Molecular Dynamics). Quantitative densitometry of radiolabelled soluble betaglycan was carried out using the ImageQuant software; data were analysed using the Prism software.

**RESULTS**

**Expression and purification of baculoviral recombinant soluble betaglycan**

We have described the preparation of recombinant soluble betaglycan by mutagenesis of the rat wild-type betaglycan cDNA (the LS mutant described in [22]). Briefly, the modified cDNA encoded a secretory protein with engineered epitopes designed to facilitate its immunodetection and purification. A human c-myc epitope was placed at the N-terminus, with a stop codon, preceded by a His\(_6\) sequence, inserted immediately before the code for the transmembrane region of the receptor (Figure 1A). Expression of this modified cDNA in COS-1 cells resulted in the secretion of proteins, detected by the anti-c-myc antibody, that exhibited the migration pattern of a ‘part-time’ proteoglycan; that is, the smeared, high molecular mass (approx. 200 kDa), GAG-containing proteoglycan along with its core protein (Figure 1B, lane 1). However, the expression of the modified cDNA in SF9 insect cells, using the baculovirus system, yielded only a core protein with a molecular mass of 110 kDa (Figure 1B, lane 3). Baculoviral soluble betaglycan migrated slightly faster than the GAG-less soluble betaglycan expressed in mammalian cells (Figure 1B, compare lanes 2 and 3). The mobility of the baculoviral protein indicated the inability of the insect cells to add GAG to the core protein, and also a decreased capacity to add other types of oligosaccharides (see below). The bands observed around and below 69 kDa most likely correspond to degradation products derived from the secreted proteins. High five (H5) cells were used to prepare larger amounts of baculoviral soluble betaglycan because this cell line produced at least five times more of this protein than the SF9 cells (results not shown).

Soluble betaglycan was purified from conditioned media by affinity chromatography on His\(_6\)-agarose, with yields in the range 6–10 mg/litre of conditioned media. As shown in Figure 1C, this single affinity-chromatography step allowed the purification to homogeneity of baculoviral soluble betaglycan.

**N-terminal sequence and glycosylation of baculoviral soluble betaglycan**

Post-translational processing of mammalian membrane and secretory proteins in insect cells often is defective, and results in proteins with abnormal properties [31]. Thus the N-terminal end sequence and degree of glycosylation of our preparation was determined. The first 16 cycles of the Edman degradation of purified baculoviral soluble betaglycan revealed the amino acid sequence shown in Figure 2(A). This sequence confirmed the identity of our recombinant protein and indicated that its signal peptide was properly processed in H5 cells. The signal peptide cleavage occurred between Ala\(^{782}\)-Gly\(^{783}\) of the pro-peptide (Figure 2A), exactly as predicted by the von Heijne rules [32]. Based on the conceptual translation of its cDNA, the predicted mass of recombinant soluble betaglycan was 87252 Da. Thus, from the molecular mass deduced in SDS/PAGE, we predicted a sub-

![Image of recombinant baculoviral soluble betaglycan](http://example.com/betaglycan.png)
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Figure 2 N-terminal sequence and glycosylation state of baculoviral soluble betaglycan

(A) N-terminal sequence of baculoviral soluble betaglycan was determined. The residues identified are indicated and aligned with the conceptual translation deduced from the open reading frame (ORF) of its encoding cDNA. The first residue of the mature protein corresponds to Gly24 (bold). The predicted signal peptide is underlined and the engineered c-myc epitope is shown in underlined italics. (B) Baculoviral soluble betaglycan was chemically deglycosylated with TFMS (\(+\)) as described in the Experimental section and then purified by IMAC. The TFMS-treated sample was divided into equal volumes, and separated by SDS/PAGE (lanes 2 and 4), and blotted for concanavalin A (Con A) or anti-c-myc (Anti-c-myc). As a control (\(-\)), an equivalent sample of baculoviral soluble betaglycan was not treated with TFMS but was subjected to same purification and detection procedure as the TFMS-treated sample (lanes 1 and 3).

deglycosylated the protein, removing approx. 20 kDa of its mass, as determined by SDS/PAGE.

Baculoviral soluble betaglycan is a non-covalent dimer

Gel-filtration chromatography and gel electrophoresis, under native conditions of baculoviral soluble betaglycan, indicated that it is a dimeric protein. Purified baculoviral soluble betaglycan, subjected to HPLC with a BioSep Sec-S3000 column, eluted as a protein with a calculated mass of 220 kDa, which migrated as a 110 kDa protein under reducing conditions in SDS/PAGE (Figure 3A). Gel electrophoresis under native conditions, which confirmed the mass estimated by gel-filtration chromatography, was also used to titrate the stability of the dimer against several denaturing and reducing reagents. Figure 3(B) shows that the lowest concentration of SDS and DTT required to completely dissociate the dimer was 350 mM and 10 mM respectively. Similar experiments demonstrated that treatment with 5 M urea or 2 M guanidinium hydrochloride caused dissociation of the dimer (results not shown). When chaotropic and reducing agents were combined, their individual concentrations required to dissociate the dimer were significantly reduced. For example, when urea and DTT were used together, the lowest concentrations needed for dissociation was 2 M and 1 mM respectively. These results suggest that, despite the odd number of cysteine residues per monomer (see Figure 1A), the baculoviral soluble betaglycan dimer is sustained by non-covalent interactions.

Baculoviral soluble betaglycan binds TGF-β with high affinity and isoform selectivity

Soluble betaglycan, found in natural sources, exhibits high affinity for TGF-β1 [20]; therefore, it was important to evaluate the affinities of recombinant soluble betaglycan for the three mammalian TGF-β isoforms. Scatchard plots of saturation binding experiments indicated that purified baculoviral soluble betaglycan binds one molecule of 125I-labelled TGF-β1 per
Recombinant baculoviral soluble betaglycan

Figure 3 Determination of the oligomeric state of baculoviral soluble betaglycan

(A) Purified soluble betaglycan was subjected to gel filtration as described in the Experimental section. Aliquots of the chromatography eluate were analysed by SDS/PAGE and stained with silver. The fractions corresponding to the major peak (7.4 min) are shown. The inset is a plot of the elution:void volume ratios versus the log of the mass of soluble betaglycan and the standards used to calibrate the column. (B) Purified soluble betaglycan, subjected to denaturation (SDS) and reduction (DTT), was analysed by native gel electrophoresis as described in the Experimental section. The migration of an intact sample of soluble betaglycan (C) and the molecular mass standards (M, kDa) are shown. The dimers and monomers of baculoviral soluble betaglycan are indicated with arrows.

Figure 4 TGF-β1 saturation binding of baculoviral soluble betaglycan

(A) Baculoviral soluble betaglycan (10 ng) was subjected to affinity labelling in solution (as described in the Experimental section) with the indicated concentrations of 125I-labelled TGF-β1. Anti-c-myc immunoprecipitates of the reaction mixtures were resolved by SDS/PAGE and revealed by exposure to a phosphor screen (Storm; Molecular Dynamics). The location of the labelled soluble betaglycan (sol BG) is indicated with an arrow. Molecular masses are indicated on the left. (B) The level of binding was estimated from densitometric analysis of the labelled soluble betaglycan (sol BG from (A)) using the ImageQuant Software, and was plotted against the 125I-labelled TGF-β1 concentration. (C) Scatchard transformation of the data shown in (B) using the Prism software package.

molecule of dimer, with a $K_d$ of 3.5 nM (Figure 4). This $K_d$ value is in good agreement with the value of 1.9 nM reported by Andres et al. [20] for soluble betaglycan produced by 3T3-L1 fibroblasts.

In order to establish the relative affinity of recombinant soluble betaglycan for the three mammalian TGF-β isoforms, we performed ligand binding competition experiments. For this purpose, affinity labelling in solution was carried out with a constant amount of 125I-labelled TGF-β1 and increasing amounts (from 0–4 nM) of competing non-radioactively labelled TGF-β1, TGF-β2 or TGF-β3 (Figure 5). The amount of 125I-labelled TGF-β1 cross-linked to soluble betaglycan was quantified from the PhosphorImager scans of SDS/PAGE gels (Figures 5A, 5B and 5C) and plotted as the percentage of labelled TGF-β bound against the concentration of the unlabelled competitor. Similar to the results observed for natural soluble betaglycan [20], the half-maximal homologous competition for TGF-β1 labelling was approximately 0.5 nM. However, the other TGF-β isoforms exhibited a higher degree of competition. In the presence of 0.5 nM TGF-β3, the 125I-TGF-β1 affinity labelling of baculoviral soluble betaglycan was only $\sim 25\%$, whereas, with the same concentration of TGF-β2, 125I-TGF-β1 affinity labelling of baculoviral soluble betaglycan was $\sim 90\%$. Taken together, the data suggested that the relative affinities of baculoviral soluble betaglycan for the three mammalian TGF-β isoforms were, TGF-β2 > TGF-β3 > TGF-β1.
Figure 5  TGF-β isoform-specific competition for the TGF-β1 binding of baculoviral soluble betaglycan

Baculoviral soluble betaglycan was subjected to affinity labelling in solution with 100 pM ¹²⁵I-labelled TGF-β1 and the indicated concentrations of competing unlabelled (A) TGF-β1, (B) TGF-β2 or (C) TGF-β3. The location of soluble betaglycan (Sol BG) is indicated with arrows. Molecular mass markers are shown on the left of the panels. (D) The percentage competition was estimated from densitometric analysis of the labelled soluble betaglycan [Sol BG from (A), (B) and (C)], using the ImageQuant Software, and was plotted against the competitor TGF-β concentration.

Baculoviral soluble betaglycan inhibition of TGF-β responses is also isoform selective

We have previously demonstrated that conditioned media containing baculoviral soluble betaglycan blocks the binding of TGF-β to type II and I receptors and inhibits the TGF-β anti-proliferative effect in Mv1Lu cells [22]. To determine the precise potency of the purified preparation, we assayed its effect on the TGF-β-dependent luciferase expression exhibited by Mv1Lu cells transfected with p3TP-lux, a TGF-β-responsive reporter plasmid [25]. Purified baculoviral soluble betaglycan inhibited the luciferase response in Mv1Lu cells treated with 20 pM TGF-β (a concentration that induces the maximal luciferase activity; results not shown). This inhibition depended on the concentration of the added soluble betaglycan (Figure 6). In several experiments, the estimated EC₅₀ of baculoviral soluble betaglycan for TGF-β1 was ~ 20 nM (Figure 6A). On the other hand, as expected from its higher affinity for TGF-β1, the EC₅₀ for this isoform was one order of magnitude lower (~ 2 nM) than that of TGF-β2 (Figure 6B). We also compared this effect with that of a widely used, commercially available, neutralizing pan-specific TGF-β antibody (AB-100-NA; R & D Systems). The antibody exhibited EC₅₀ of 20 nM and 11 nM for TGF-β1 and TGF-β2 respectively (results not shown). In this assay, low concentrations of baculoviral soluble betaglycan (25 nM) inhibited the response to TGF-β2 by 100% (Figure 6B). However, at 10-fold greater concentrations (for example 400 nM), the maximal inhibition of the TGF-β1 response was about 80% (Figure 6A), further emphasizing the notable higher affinity of betaglycan for the TGF-β2 isoform.

DISCUSSION

In many diseases, such as diabetes mellitus, TGF-β has been identified as the major physiopathological agent leading to renal failure, a complication with serious medical and socioeconomic consequences [8]. However, this is just one of the many examples in which agents capable of neutralizing TGF-β would be of great pharmacological value [3,5,33]. In the present work, we demonstrate that a recombinant soluble form of betaglycan, produced in insect cells using the baculoviral expression system, has the biochemical and functional properties needed to become such a pharmacological anti-TGF-β agent.

Baculoviral soluble betaglycan is efficiently expressed as a secretory glycoprotein in which the signal peptide is cleaved with the specificity expected for mammalian cells. However, in contrast to the soluble betaglycan found in natural sources [20], it lacks...
GAG chains and exhibits a lesser degree of glycosylation. This occurs, despite the fact that baculoviral soluble betaglycan contains the serine residues that are modified with GAG chains in the rat and mouse wild-type receptors [22,34]. This deficiency resides in the insect cells, since the same cDNA, when expressed in COS-1 cells, produces a GAG-containing soluble receptor. On the other hand, similar to results reported for other extracellular proteins expressed in insect cells [35], baculoviral soluble betaglycan has a lesser amount (when compared with the mammalian counterpart) of mannose-containing oligosaccharides. Thus baculoviral soluble betaglycan has a lower degree of glycosylation, which nonetheless, could be advantageous for its wider systemic distribution when administered into the intact animal.

The quaternary structure and the TGF-β affinities of baculoviral soluble betaglycan resemble those of the wild-type receptor. It has been reported that the membrane-bound beta-baculoviral soluble betaglycan resemble those of the wild-type receptor that native baculoviral soluble betaglycan associates as dimers composed of 110 kDa monomers. Treatment with diverse non-reducing chaotropic agents dissociates the soluble betaglycan dimer, indicating that its association occurs through non-covalent interactions. This is unexpected, because the odd number of cysteine residues in each monomer of soluble betaglycan suggested that dimerization would require an interchain disulphide bond between the monomers.

Soluble betaglycan binds TGF-β1 with an affinity (K_d ~ 3.5 nM) that is comparable to the soluble betaglycan found in natural sources [20]. It also binds the other TGF-β isoforms with even higher relative affinities, showing at least a 5-fold higher affinity for TGF-β2 than for TGF-β1. The higher TGF-β2 affinity of the recombinant soluble receptor is equal to that of the wild-type membrane-bound receptor [38–40]. 125I-labelled TGF-β1-saturation binding experiments indicated a stoichiometry of one molecule of TGF-β1 per molecule of soluble betaglycan dimer. This finding is difficult to explain, since previous mutagenesis experiments show that there are two ligand binding sites in the betaglycan ectodomain [22,41,42]. This could indicate that binding of TGF-β1 requires the two ligand binding domains to be present in one monomer and that the first binding prevents further binding to the other monomer. Further studies are required to test the validity of this hypothesis.

Crude preparations of baculoviral soluble betaglycan have been used to block the growth-inhibition effect of TGF-β2 in the highly TGF-β-sensitive Mv1Lu cells [22]. In the present study, we assayed the homogeneously purified soluble betaglycan and found that it blocked TGF-β2 and TGF-β1 in a TGF-β assay in vitro, that driven by the p3TP-lux reporter [25]. These studies also showed the same TGF-β isoform selectivity in the ligand binding competition assays, which were at least one order of magnitude more effective against TGF-β2. Similar isoform selectivity was observed also in the TGF-β binding assay using fetal-bovine heart endothelial cells (results not shown). It is relevant that the potency of baculoviral soluble betaglycan is comparable with that of a widely used anti-TGF-β neutralizing antibody that has been successfully used in vivo [43]. The fact that the baculoviral recombinant soluble betaglycan prepared by us had a consistent TGF-β neutralizing activity is in sharp contrast with the enhancement of the TGF-β bioactivity observed with a bacterially expressed, His-tagged, full-length betaglycan ectodomain protein, the bg1,2,3 fusion protein [42]. Despite inhibiting TGF-β1 binding to Mv1Lu cells, the bg1,2,3 protein was able to enhance the growth inhibitory effect of TGF-β1 in this cell line. The most likely explanation for this discrepancy is that the renaturing protocol employed to prepare the bg1,2,3 protein resulted in a folding that differs from that of baculoviral soluble betaglycan. Further experimentation is required to establish and compare the tri-dimensional structures of baculoviral and bacterial soluble betaglycans. These studies will provide useful information on what determines that the same polypeptide behaves as a TGF-β neutralizing or a TGF-β promoting agent.

The affinity of baculoviral soluble betaglycan for the diverse TGF-β isoforms is similar or higher than that of other agents that neutralize TGF-β in vivo [44–48]. The TGF-β1 K_i of a recombinant form of the TGF-β1 latency-associated peptide and of a recombinant soluble type II TGF-β receptor were 8 nM and 120 nM respectively [49]. The estimated affinities of the small interstitial proteoglycans that bind TGF-β1, biglycan, decorin and fibromodulin are in the range 1–20 nM [50]. These values predict that baculoviral soluble betaglycan will have a potent TGF-β neutralizing action in vivo. Furthermore, being a soluble receptor that is found in natural sources, it would be expected that administration systemically would not elicit an immune response in experimental animal models.

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