Aberrant proteolytic digestion of biglycan and decorin by saliva and exocrine gland lysates from the NOD mouse model for autoimmune exocrinopathy

S. Yamachika[1], J. Brayer[1], G.E. Oxford[1], A.B. Peck[3,4], M.G. Humphreys-Beher[1,3]

[1]Department of Oral Biology, [2]Department of Pathology and Laboratory Science, and the [3]Center for Orphaned Autoimmune Disease, University of Florida, Gainesville, Florida, USA.

Abstract

Objective

The protein components of the extracellular matrix (ECM) are responsible for driving tissue morphogenesis, the development of differentiated function, and the sequestration of biologically active molecules such as growth factors in close proximity to tissue and organ cells. Recent reports indicate that saliva and exocrine tissue lysates from Sjögren's syndrome patients and the non-obese diabetic (NOD) mouse model for autoimmune exocrinopathy demonstrate elevated levels of specific enzymes that degrade the ECM, especially the matrix metalloproteinases (MMPs). To determine if elevated levels of MMPs could be important in exocrine tissue destruction, we examined proteolytic activity against two ECM proteoglycans, decorin and biglycan.

Methods

Purified recombinant human core protein for decorin or biglycan was incubated with saliva or gland lysates from either control BALB/c or NOD mice. Degraded proteoglycan products were estimated by Western blotting analysis using anti-decorin or anti-biglycan monospecific polyclonal antibodies. The levels of TGFβ protein were measured by ELISA.

Results

Proteolytic activity for decorin and biglycan was not observed in the saliva and salivary gland lysates collected from C57BL/6 or BALB/c mice used as normal controls. In contrast, both proteoglycans were degraded by saliva and exocrine gland lysates from NOD mice and the congenic partner strains NOD-scid and NOD.B10.H2^b. This proteolytic activity for proteoglycans was inhibited by the MMP inhibitors, EDTA, GM6001 and 1,10-phenanthroline. Protein steady state levels for TGFβ were increased in the saliva and gland lysates from 20-week old NOD strains, as compared to BALB/c mice and NOD treated with the MMP inhibitor GM6001. With the inhibition of MMP activity, TGFβ levels declined in saliva and gland lysates.

Conclusion

Proteolytic degradation of the ECM molecules decorin and biglycan is elevated in the exocrine tissues of the NOD mouse model for Sjögren's syndrome. Furthermore, the proteolysis of small leucine-rich proteoglycans correlates with the presence of elevated levels of TGFβ in gland lysates and saliva.

Key words

Extracellular matrix proteins, Sjögren's syndrome, proteases, exocrine glands.

Shigeo Yamachika, Post-doctoral fellow, DMD, PhD; Gregory E. Oxford, Graduate student, DDS, PhD; Jason Brayer, Graduate student, BS; Ammon B. Peck, Professor, PhD; Michael G. Humphreys-Beher, Professor, PhD.

This work was supported by NIH/NIDR grant DE 10515 and by an administrative supplement from the Office for Research in Women's Health Policy to Dr. Humphreys-Beher. Dr. Peck was supported by a Juvenile Diabetes Foundation International grant (I96091). Mr. Brayer and Dr. Oxford were supported by NIDR training grant DE 00072.

Please address correspondence and reprint requests to: Dr. Michael G. Humphreys-Beher, Department of Oral Biology, P.O. Box 100424, University of Florida, Gainesville, FL 32610, USA.
E-mail: h-beher@dental.ufl.edu

Introduction

Interactions between the matrix metalloproteinase enzymes (MMPs) and their substrates, the extracellular matrix (ECM) proteins, are essential elements in the morphological development of glandular structure during fetal development (1-3). Paradoxically, these proteases may also accelerate destruction of tissues through the degradation of ECM proteins. Thus, elevated MMP activity is often observed in malignant tumor metastasis, cystic lesion formation (4,5), as well as in tissue destruction in autoimmune disease (6). Sjögren's syndrome is a chronic autoimmune disease, characterized by the clinical presentation of dry mouth (xerostomia) and dry eyes (keratoconjunctivitis sicca). These sicca symptoms appear to result from exocrine gland adenitis (7, 8), the lesions of which contain activated B- and T-lymphocytes that elaborate pro-inflammatory cytokines. Exocrine tissues obtained from both Sjögren's syndrome patients and mouse models for Sjögren's syndrome contain increased levels of proteolytic enzymes involved with ECM degradation and programmed cell death (9-13).

Destruction of ECM by MMPs is often accompanied by alterations in the concentrations of tissue-associated growth factors (14). This is especially true in patients with Sjögren's syndrome (15-18) for transforming growth factor-beta (TGFβ), which may function to prevent lymphocyte infiltration into the exocrine glands (19). This concept has been supported by studies using a TGFβ-knockout mouse genetically predisposed for developing Sjögren's syndrome-like pathology in which massive lymphocytic infiltration of exocrine tissues occurs in the absence of this growth factor (20, 21). One ECM molecule that has been reported to function as a reservoir for TGFβ is decorin (22, 23). Decorin is a small proteoglycan which, like biglycan, belongs to the leucine-rich family of ECM molecules and plays a role in the regulation of fibrillogenesis and the maintenance of tissue differentiation by binding with collagen, fibronectin and thrombospondin. It is thought that a disulfide loop created in the carboxy portion of the molecule is capable of encircling a collagen molecule in its mechanism of stabilizing fibrils and adding orientation during fibrillogenesis (24). This potential role has been demonstrated with the generation of decorin null mice, which grow normally to adulthood, but subsequently exhibit increased dermal fragility, related to abnormal collagen morphology (24).

The ability of decorin to act as a reservoir of TGFβ is achieved by sequestration of growth factors in the ECM from their respective cell surface receptors. Because both decorin and biglycan work to maintain the structure of the ECM, it is not surprising that the local destruction of proteoglycans in various connective tissue autoimmune diseases leads to altered concentrations of biologically active TGFβ (25-28). Degradation of small proteoglycans is associated with the enzymatic activity of MMP-2, MMP-3 and MMP-7 (28). The expression of decorin and biglycan is regulated by TGFβ and a number of inflammatory cytokines. Both IL-1 and IL-4 up-regulate decorin expression, while TNFα and TGFβ inhibit transcription. In contrast, IL-6 and TGFβ inhibit biglycan transcription which is counteracted by the presence of TNFα (24).

Reports of elevated levels of MMP activity in the saliva and exocrine tissues of Sjögren's syndrome patients, as well as animal models exhibiting Sjögren's syndrome-like pathology (9, 18, 29-31), led us to examine the exocrine tissues of NOD mice with active sialoadenitis for their proteolytic action on the ECM-associated proteoglycans, decorin and biglycan. We report that the exocrine tissue and saliva of NOD mice contain increased levels of proteolytically degraded decorin and biglycan. The degradation most likely is the result of MMP activity in the exocrine tissues. The proteolytic cleavage of decorin and biglycan is accompanied by elevated levels of TGFβ.

Materials and methods

Materials

Groups of n=6 female C57BL/6, BALB/c, NOD/Lt, NOD-*scid* and NOD.B10.$H2^b$ mice, 8 and 20 weeks of age, were obtained from the Department of Pathology Mouse Facility, University of Flori-

da (32, 33). Pilocarpine, d,l-Isoproterenol, EDTA and 1,10-phenanthroline were purchased from Sigma Chemical Co. (St. Louis, MO). Reagents for SDS-PAGE were purchased from Fisher Scientific (Pittsburgh, PA). PVDF membrane used for Western blotting analysis was purchased from Millipore Corporation (Bedford, MA). Purified core proteins of decorin and biglycan, and monospecific polyclonal antibodies for these proteoglycans were the generous gifts of Dr. David J. McQuillan, Center for Extracellular Matrix Biology, Institute of Biosciences and Technology, Texas A & M University (34, 35).

Saliva collection and preparation of gland lysates.

Saliva was collected from each strain of mice (n = 6) after stimulation of secretion by 0.2 mg of isoproterenol and 0.5 mg pilocarpine per 100 g body weight dissolved in phosphate-buffered saline (PBS) as previously described (32, 33). The secretagogue cocktail was administered (0.1 ml volume) through an intraabdominal injection. Saliva was collected for 10 min using a micropipette and placed into chilled 1.5 ml microfuge tubes. Saliva samples were immediately frozen and kept in a -70°C freezer until analysis.

The parotid, submandibular and lacrimal glands were collected after the mice had been anesthetized following inhalation of metaphane and killed by cervical dislocation. Each gland was homogenized in 50 mM Tris buffer (pH 7.5) after elimination of the surrounding connective tissue, fat and lymph nodes, and kept frozen at -70°C until final analysis. The effect of the MMP inhibitors, 10 μM EDTA or 10 μM 1,10-phenanthroline on proteolytic activity was investigated by their addition to the incubation buffer. For preparation of soluble fractions of gland lysates, tissues (n = 5 mice) from each strain were pooled. Proteins from lysates were extracted with 1.0 N HCl and centrifuged at 10,000 xg at 4°C to remove insoluble matrix and membrane proteins (36). Protein concentrations of both saliva and gland lysate were measured by the methods of Bradford (37) using a Bio-Rad protein assay kit (Hercules, CA), with bovine serum albumin as a standard.

For the *in vivo* MMP inhibitor studies, sets of female BALB/c, NOD/Lt, NOD-*scid* and NOD.B10.$H2^b$ mice (n = 3) received every other day intraperitoneal injections of 100 mg/kg N-[2(R)-2-(hydroxamido carbonylmethyl)4-methylpentanoyl]-L-tryptophane methylamide (GM6001®) a broad spectrum MMP inhibitor, for a period of 13 weeks beginning at 7 weeks of age (26). Animals were given food and water *ad libitum*, with food removed 18 hrs prior to euthanization by cervical dislocation. Gland lysates and saliva were prepared as above.

Proteoglycan degradation by saliva or gland lysate.

Proteoglycan degrading activities of saliva (10 μl), and exocrine gland lysates (10 μg) from BALB/c and congenic NOD mice were investigated by the following methods. Purified core protein of decorin or biglycan (0.2 μg) were incubated with each saliva or gland lysate in PBS for 2 hr at 37°C, in a total volume of 25 μl. To detect the digestion of endogenous murine proteoglycans, 50 μg of total gland lysate was separated by polyacrylamide electrophoresis. To investigate the influence for proteolytic activities by MMPs, EDTA or 1,10-phenanthroline was added to the PBS. These reactions were stopped by the addition of tracking dye containing 1% 2-mercaptoethanol and 10% sodium dodecyl sulfate (SDS), the samples were subsequently heated for 5 min at 100°C, prior to loading into the well of polyacrylamide gels.

SDS-PAGE and Western blotting analysis

Detection of degraded proteoglycan peptides in tissue and saliva samples were determined through separation on 10% SDS-polyacrylamide gels (38). The protein fragments were then transferred to PVDF membranes (39). After transferring, the membranes were blocked with 5% non-fat dry milk for 2 hr and immunoblotted overnight with a rabbit polyclonal antibody to decorin or biglycan at a 1:500 dilution. After 3 times washing with 10 mM Tris buffer (pH7.4) containing 200 mM NaCl, the membrane was incubated an additional 2 hr with 1:5000 diluted alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin (40). The blots were washed and exposed by chromogenic substrate, NTB (32, 33).

TGFβ$_1$ protein detection

The concentration of TGFβ$_1$ in saliva and gland lysates was measured by an antibody sandwich method of the enzyme-linked immunoadsorbant assay (ELISA), using the TGFβ$_1$ E_{max}™ ImmunoAssay System (Promega, Madison, WI). The kit assay included the purified TBFβ$_1$ protein for generating the standard concentration curve. Saliva and gland lysates from BALB/c and NOD mice were diluted in PBS to 500 μg/ml for the assay. Following acid extraction as described above, test material and a TGFβ$_1$ standard were added in a 1:2 serial dilution to monoclonal antibody-coated 96-well microtiter plates. The plates were incubated at room temperature for 90 min with shaking, followed by washing and the addition of a second rabbit polyclonal TGFβ$_1$ antibody. Subsequent to a 2 hr incubation, the plates were again washed and incubated for 2 hr with an anti-rabbit horseradish peroxidase-conjugated antibody.

After removal of unbound antibody, peroxidase substrate was added for color development. The reaction was terminated by the addition of 1 M phosphoric acid and the color change was measured at an optical density of 450 nm, using a Bio-Rad automated plate reader (Emoryville, CA). The TGFβ$_1$ concentration in saliva and gland lysates was calculated by linear regression against the TGFβ$_1$ standard curve.

Statistical analysis

All measures of variance are given as standard errors of the mean. Using the Shapiro and Wilk's test (41), the distributions for saliva and gland lysates TGFβ concentration were found to be normal ($p > 0.05$) and were analyzed by a parametric analysis of variance (ANOVA). Test of ANOVA between independent means were performed using SAS computer software programs (SAS Institute, Cary NC). Values were considered significant when $p < 0.05$.

Results

Detection of endogenous murine decorin and biglycan in salivary gland lysates

The presence of endogenous decorin and biglycan in the submandibular gland was investigated by Western blot analysis using polyclonal antibodies against core proteins for these proteoglycans as primary antibodies. As shown in Figure 1, immunoreactive peptides for biglycan of M_r = 65-70, 33 and 21 kDa were detected in submandibular gland lysates from normal control BALB/c and NOD/Lt mice. Similar peptides were observed in the congenic immunodeficient NOD-*scid* mice, and the model for primary Sjögren's syndrome-like pathophysiology, NOD.B10.*H2*^{b}. Two additional peptide fragments of M_r = 29-32, kDa were present in the lysates prepared from the three NOD strains, but were not major peptides detected by the antibody in control BALB/c mice (Fig.1). An additional unique band was detected in NOD strains at 25 kDa, while a peptide band at 23 kDa was present in low concentrations in the control mice. Pre-incubation of the primary antibody with recombinant biglycan or decorin eliminated the detection of any of the peptide bands presented in the figure.

A faint doublet band with an M_r of approximately 55-60 kDa, potentially intact murine decorin, was detected in the submandibular gland lysates of control BALB/c mice (Fig. 1). In contrast, a number of degradation products from decorin were observed in the submandibular gland lysates from congenic NOD and parental NOD/Lt strain at M_r = 32 kDa. NOD.B10.*H2*^{b} and NOD-*scid* mice appear to have additional bands detectable at M_r = 40 kDa, while NOD-*scid* and NOD/Lt have an additional peptide band reactive at 25 kDa. The reason for the variability of detected peptide products for decorin as compared to biglycan in the gland lysates from the various NOD strains is not clear.

Degradation of exogenous human recombinant decorin and biglycan

The specific degradation of decorin and biglycan was followed by Western blot using the same antibodies as above and the addition of exogenous proteoglycan substrates to the incubation cocktail containing saliva or gland lysate samples. Figure 2 shows a typical degradation profile of decorin (right panel) and biglycan (left panel) after incubation with 20-week old C57BL/6, BALB/c or 8 and 20 weeks of age NOD/Lt saliva. Proteolytic activity towards decorin and biglycan was not observed by the saliva collected from BALB/c or C57BL/6 mice used as a normal control, whereas decorin and biglycan were degraded by saliva from 20 weeks of age NOD/Lt mice. Submandibular gland lysates from 8-week old NOD/Lt mice showed a mild amount of degradation of biglycan as indicated by the decreased staining of the upper band of the doublet, but no such pattern of degradation was evident towards the

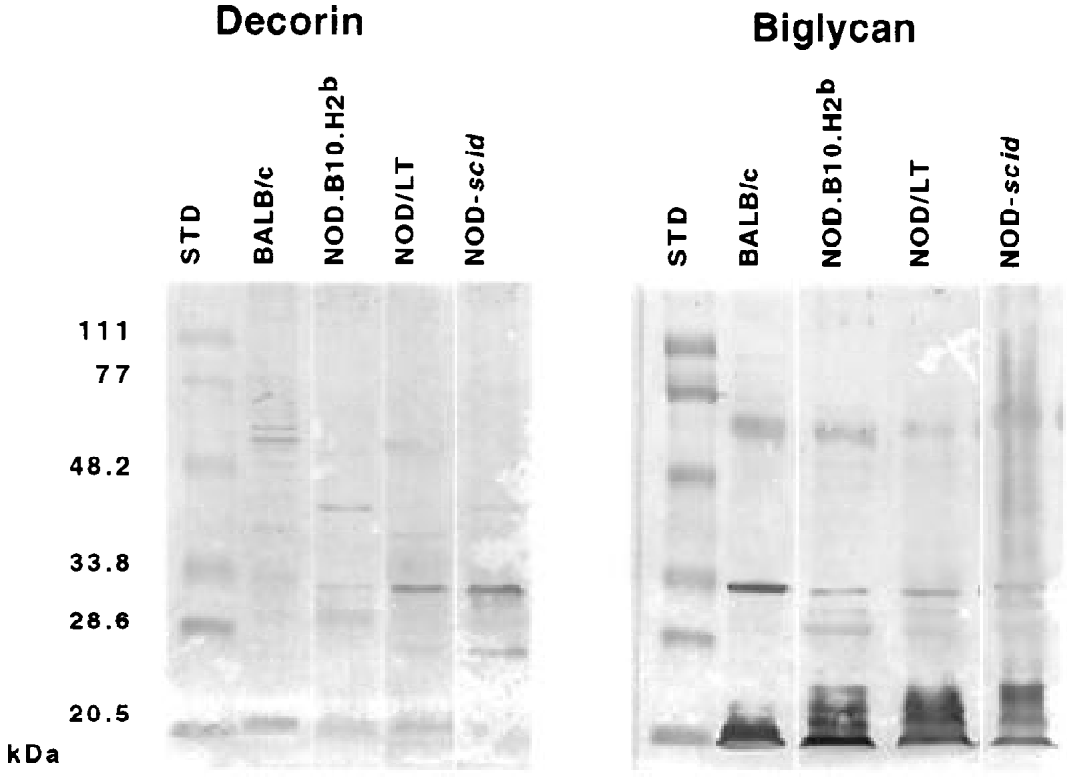

Fig. 1. Western blot detection of decorin and biglycan in the submandibular gland lysates from normal control BALB/c mice and congenic NOD mice. Fifty µg of protein was separated on 10% SDS-PAGE gels followed by detection with monospecific polyclonal antibodies to decorin or biglycan. Murine biglycan and decorin show a molecular mass of $M_r \approx$ 65-70 kDa. The blot presented is representative of the detection pattern identified in n = 6 mice/strain (33). Each sample was analyzed on two separate occasions for reproducibility. Pre-stained molecular weight standards are; 110,000 Da, phosphorylase B; 77,000 Da, bovine serum albumin; 48,200 Da, ovalbumin; 33,800 Da, carbonic anhydrase; 28,600 soy bean trypsin inhibitor; 20,500 Da, lysozyme.

exogenous decorin substrate. Similar degradation patterns were obtained with NOD-*scid* and NOD.B10. *H2*^{b} saliva (data not shown). Decorin and biglycan appear to be especially sensitive to proteolytic digestion by saliva of older NOD mice as the intact core peptide was nearly completely degraded. The enzymatic degradation of both proteoglycans was inhibited by the addition of the divalent cation chelator, EDTA, or the specific MMP inhibitor, 1,10-phenanthroline (Fig. 2). An inability to degrade exogenously added proteoglycan was observed using saliva from 20 week old NOD mice treated by chronic infusion of the MMP inhibitor GM6001.

Figure 3 shows the degradation of decorin and biglycan after incubation with exocrine gland lysates. When decorin or biglycan was incubated with subman-

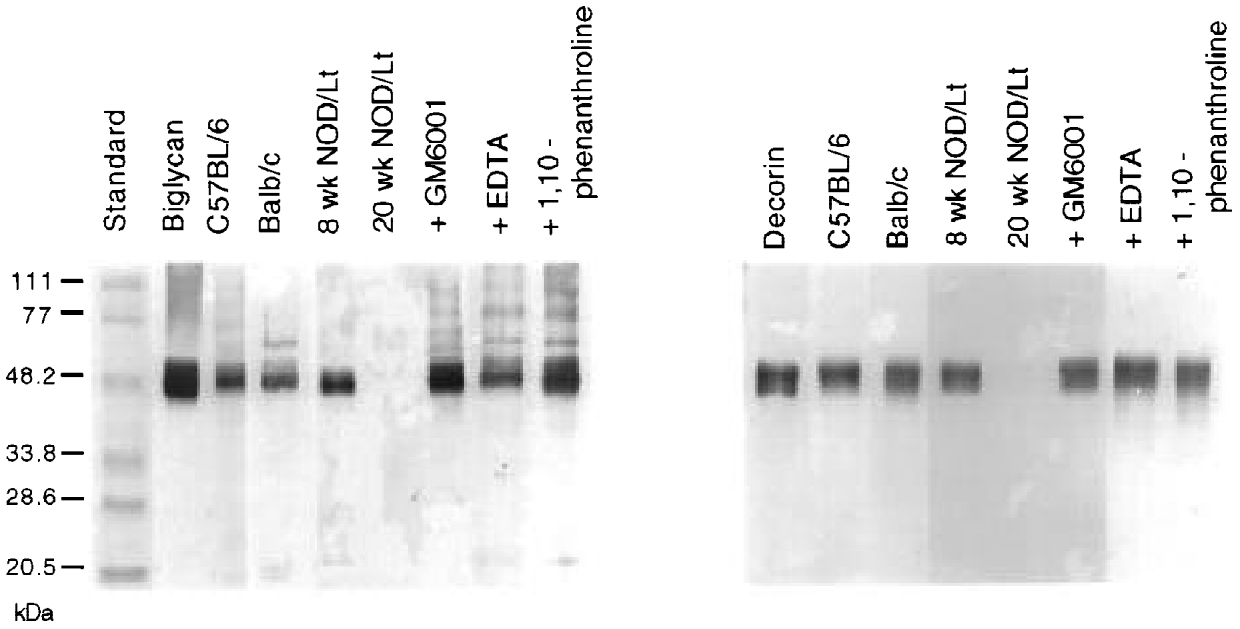

Fig. 2. Western blot detection of decorin and biglycan following incubation with saliva from C57BL/6, BALB/c or 8- and 20-week old NOD/Lt mice. Purified recombinant human decorin or biglycan core protein (0.2 µg) was incubated with the saliva (10 µl) from NOD/Lt, C57BL/6 or BALB/c mice. The proteins were separated on 10% SDS-PAGE gels followed by detection with a monospecific polyclonal antibody to decorin or biglycan. Inhibition of proteoglycan digestion was provided by the inclusion of EDTA, 1,10-phenanthroline or saliva from mice treated with GM6001. Molecular weight standards are as in Figure 1.

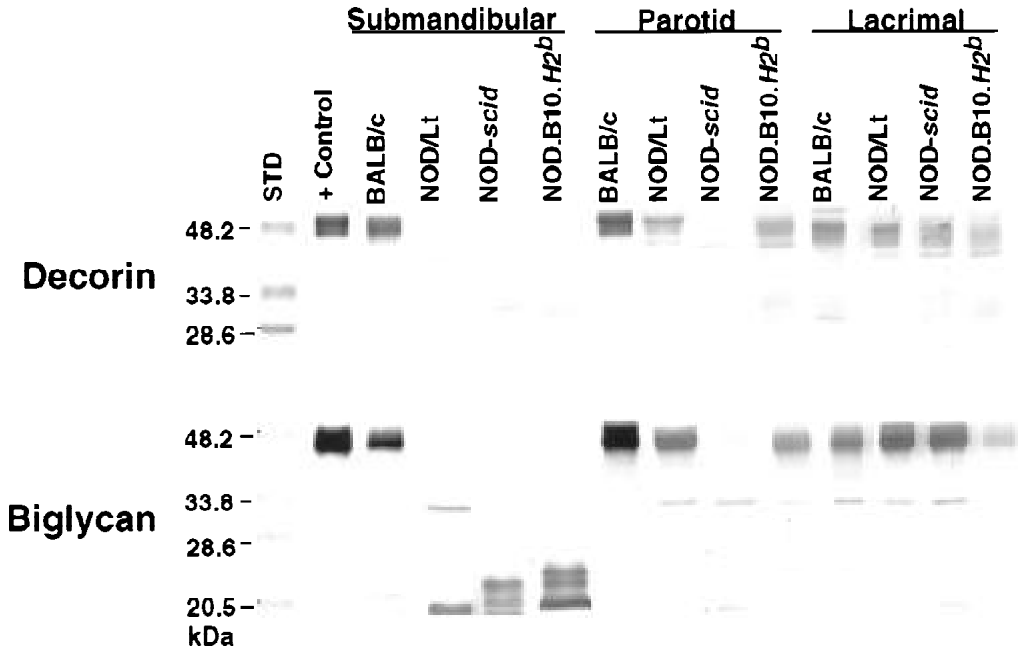

Fig. 3. Western blot detection of recombinant decorin and biglycan degradation products in the gland lysates from BALB/c or NOD strain mice. Decorin or biglycan core protein (0.02 µg) was incubated with each gland lysate (10 µg), followed by detection with a monospecific polyclonal antibody. Gland lysates represent a pool of exocrine tissues from n = 6 animals per group. The gel was run on 3 separate occasions for reproducibility. Control lanes represent recombinant proteoglycans without incubation with gland lysates.

dibular gland lysates, the proteoglycan band was detected in a degraded form in NOD/Lt, NOD-*scid* and NOD.B10.*H2*^{b} mice, but not in BALB/c control mice. Furthermore, similar low molecular weight peptide fragments were generated to those previously observed in the submandibular gland lysates (see Figure 1) when examining endogenous mouse proteoglycan proteins. The proteoglycan bands were reduced in both size and intensity by parotid and lacrimal gland lysates from NOD/Lt, NOD-*scid* and NOD.B10.*H2*^{b} mice. The greatest degree of proteolytic digestions appeared in the parotid gland lysates from NOD-*scid* mice. The least degradation of exogenous decorin and biglycan was observed in lacrimal gland lysates, although decorin was more sensitive to proteolysis than biglycan (Fig. 3). All lacrimal gland preparations from the mice, including the BALB/c control had a detectable digestion fragment at $M_r \approx 30$ kDa. Submandibular gland lysates prepared from mice receiving chronic infusion of the MMP inhibitor GM6001, were no longer capable of degrading the exogenously added proteoglycan decorin (Fig. 4). Lysates prepared from young NOD mice as additional controls, showed mild proteolytic activity to decorin when compared to GM6001-treated 8- and 20-week old NOD/Lt mice or BALB/c controls.

*Detection of TGF*β *in saliva and exocrine tissues*

The concentration of biologically active TGFβ_1 was determined for saliva and gland lysates from congenic NOD strains and BALB/c control mice. As presented in Table I, saliva levels of acid extractable TGFβ_1 of 20-week old NOD strains ranged from 123- to 246-fold higher than that detected in saliva from BALB/c mice ($p < 0.001$). The concentration of growth factor isolated from younger age NOD-*scid* mice (8 weeks of age) was lower than in older mice, 352 ± 189 pg/ml, when compared to older NOD-*scid*, but 80-fold greater than observed in BALB/c ($p < 0.01$). The level of biologically active TGFβ_1 released from gland lysates from NOD mice was also greater than that obtained from control mice. The parotid, submandibular and lacrimal gland levels of growth factor were approximately 2-fold higher in NOD/Lt mice than in BALB/c (Table I; $p < 0.02$). In contrast, the concentration of TGFβ_1 in gland lysates from NOD-scid and NOD.B10.*H2*^{b} were almost 3-fold greater compared to BALB/c gland lysates ($p < 0.01$). Gland lysates from NOD/Lt and congenic strains of mice given GM6001 had reduced levels of TGFβ_1. The concentrations were similar to those detected in control mice (Table I; $p > 0.05$).

Discussion

Decorin and biglycan are small proteoglycans, classified as belonging to the leucine-rich repeat family of proteoglycans, which are found primarily in connective tissues as a component of the ECM (22). This family of proteoglycans contain a leucine-rich peptide domain that is capable of interaction with collagen fibrils and specifically binding mol-

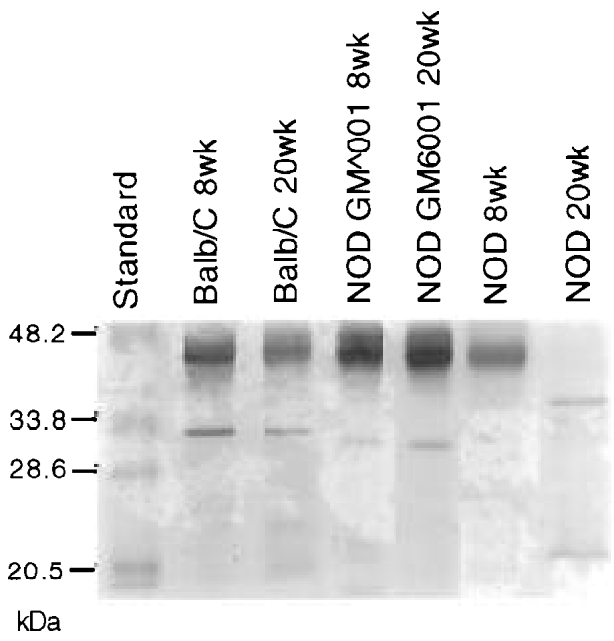

Fig. 4. Western blot detection of exogenous decorin proteolytic digestion by submandibular gland lysates from BALB/c and NOD/Lt mice treated with GM6001. Decorin core protein was detected as described in Figure 3. Gland lysates were collected from 20 week old control mice and 8 and 20 week old NOD/Lt treated for one and 13 weeks, respectively, with GM6001. The gel was run on 3 separate occasions for reproducibility.

Table 1. TGFβ_1 concentration in saliva and exocrine gland lysates*.

Tissue/Source	BALB/c	NOD/Lt	NOD-*scid*	NOD.B10.*H2*^{b}
Submandibular	17.2 ± 0.5/ 15.5 ± 4.6	28.3 ± 4.3/ 13.9 ± 6.2	51.8 ± 10.5/ 14.1 ± 4.4	57.7 ± 14.7/ 12.9 ± 2.4
Parotid	34.4 ± 4.1/ 37.0 ± 7.8	89.7 ± 26.0/ 33.0 ± 5.6	103.2 ± 19.8/ 42.7 ± 9.6	94.7 ± 3.0/ 35.8 ± 4.2
Lacrimal	48.5 ± 2.4/ 35.1 ± 5.7	97.5 ± 10.4/ 37.4 ± 6.3	123.0 ± 3.4/ 30.3 ± 8.3	93.0 ± 2.8/ 36.7 ± 5.1
Saliva†	4.4 ± 3.1/ 6.3 ± 2.9	757.0 ± 244.4/ 5.6 ±3.8	545.5 ± 81.6/ 4.5 ± 4.7	1085 ± 255.7/ 5.3 ± 3.9

*The concentration of TGFβ_1 was determined from the soluble fraction of gland lysates (n = 5 animals per pool). The concentration of TGFβ_1 is expressed as the mean pg/100 µg protein ± standard error. All assays were performed in duplicate on two separate occasions for reproducibility.
†Values expressed as mean pg/ml saliva ± standard error.

ecules of TGFβ (42-44). The presence of these proteoglycans have been demonstrated in rat submandibular glands using biochemical and immunohistochemical techniques (45). It was suggested that decorin is expressed in the fibrous connective tissues surrounding excretory ducts and plays an important role in the regulation of basement membrane-connective tissue interactions (45). Western blot analysis of submandibular gland lysates from NOD and BALB/c, using polyclonal antibodies against core proteins of decorin or biglycan, suggest that these proteoglycans are also present in the submandibular glands of adult mice.

The results of the present study further demonstrate that these proteoglycans are susceptible to digestion by ectopically expressed MMPs of NOD mice exhibiting Sjögren's syndrome-like pathology. The ability to inhibit the digestion of these proteins by EDTA and 1,10-phenanthroline, along with the pattern of digestion peptides resembling the products generated by purified MMPs (28), suggest that the presence of MMP-2 and MMP-3 in NOD mice (29) could be responsible for the aberrant degradation of decorin and biglycan. It is interesting to note that aberrant proteolytic degradation of leucine-rich proteoglycans has been observed in autoimmune arthritis (25, 26).

The ECM proteins often contain sequences of growth factor domains or are capable of sequestering growth factors in close proximity to cells (43, 46). The release of TGFβ from decorin has previously been detailed following digestion with MMP-2, MMP-3 and MMP-7 (28). Several of these MMPs have been shown to be elevated in the fluids of patients and animal models for Sjögren's syndrome (9, 18, 29, 31). These MMPs were capable of degrading decorin and biglycan into numerous small peptides similar in size to those observed in our Western blots of human recombinant proteoglycans (28).

Sequestration of TGFβ by ECM proteoglycans does not lead to a neutralization of biological activity. Both soluble and ECM-TGFβ complexes retain biological activity (28, 46). Reports on the levels of TGFβ mRNA from exocrine tissue biopsy of Sjögren's syndrome patients have described both increased and decreased steady state expression for this growth factor (14-17). It must be noted, however, that mRNA levels do not equate to the protein levels present in the tissues, although at least one report provides evidence from labial salivary gland biopsies for increased levels of $TGF\beta_2$ protein (47). The tissues isolated from such patients are end-stage in nature as compared to the active process of disease occurring in NOD mice; thus, it is difficult to make a comparison between the changes in the animal model for autoimmune exocrinopathy and human pathology. Interestingly, an animal model for autoimmune exocrinopathy, the TGF-β knockout mouse, demonstrates a pathophysiology similar to Sjögren's syndrome (18, 20). It is unclear as to the role of $TGF\beta_1$ in the disease process since treatment of these mice with exogenous growth factor fail to suppress the serology of Sjögren's syndrome-like histopathology. For proper epithelial cell proliferation and differentiation, TGFβ may require localization within the ECM milieu (48). Therefore, without proper confinement within the ECM, $TGF\beta_1$ may have a reduced interaction with glandular cell surface receptors.

The detection of elevated levels of proteases in Sjögren's syndrome patients and the NOD mouse appear to influence the composition of the ECM surrounding the epithelial cells of the exocrine glands. Besides activation of transcription for genes regulating ECM proteins, TGFβ has been shown to down-regulate MMP synthesis (44). Long term inhibition of MMP activity by *in vivo* treatment with GM6001 (29) resulted in drops in the level of TGFβ in saliva and gland lysates of NOD mice to the concentrations observed in control C57BL/6 or BALB/c mice. However, under these conditions disease pathogenesis was not altered (29).

In summary, the detection of ectopic degradation of decorin and biglycan appears to take place through the aberrant MMP activity associated with Sjögren's syndrome-like disease in NOD mice. The degradation of ECM proteins may provide the signal for increased growth factor synthesis in an attempt to re-establish eptithelial cell anchorage-dependent differentiated function. These events in disease pathogenesis may require glandular cells to interact with an ECM-growth factor complex rather than free growth factor. The inability of GM-6001 to delay or prevent autoimmune exocrinopathy in NOD mice does not rule out the possibility that ECM molecules other than decorin and biglycan, which are susceptible to MMPs and not inhibited by GM6001, may be involved in the pathogenesis. Alternatively, the age of initiation of GM6001 infusion, beginning at 7 weeks of age, may be too late to prevent disease progression in spite of the presence of normal glandular histology and biochemical markers established for the disease.

Acknowledgements

The authors would like acknowledge the technical assistance of Ms. Amy Schawley. We would also like to thank Dr. Elisabeth Peen, Division of Rheumatology, Department of Medicine, University of Florida for helpful discussions and assistance in the preparation of this manuscript.

References

1. FITCH J, LINSENMAYER T: Interstitial basement membrane component in development. *In* YURCHENCO P, BIRK O and MECHAM R (Eds.): *Extracellular Matrix Assembly and Structure.* New York, Academic Press 1994: 441.
2. BERNFIELD MR, BANERJEE SD, KODA J, RAPRAGER AC: Remodeling of the basement membrane: Morphogenesis and maturation. *In* PORTER R and WHELAN J (Eds.): *Basement Membranes and Cell Movement.* UK, Pittman Press 1984: 172.
3. BIRKEDAL-HANSEN H, MOORE WGI, BODDEN MK *et al.*: Matrix metalloproteinases: A review. *Crit Rev Oral Biol Med* 1993;4:197-251.
4. AZUMA M, TAMATANI T, YUKI T *et al.*: Increased matrix metalloproteinase-2 activity induced by TGF-β_1 in duct cells of human salivary gland is associated with the development of cyst formation *in vivo*. *J Oral Pathol Med* 1996; 25: 467-73.
5. LIN S, CHIANG C, HONG C *et al.*: Immunolocalization of interstitial collagenase (MMP-1) and tissue inhibitor of metalloproteinase-1 (TIMP-1) in radicular cysts. *J Oral Pathol Med* 1997; 26: 458-63.
6. CHANDLER S, MILLER KM, CLEMENTS JM *et al.*: Matrix metalloproteinases, tumor necrosis factor and multiple sclerosis: An overview. *J Neuroimmunol* 1997; 72: 155-61.
7. TALAL N: Sjögren's syndrome: historical overview and clinical spectrum of disease. *Rheum Dis Clin North Am* 1992; 18: 507-16.

8. FOX RI, KANG H-I: Pathogenesis of Sjögren's syndrome. *Rheum Dis Clin North Am* 1992; 18: 517-38.
9. KONTTINEN YT, KANGASPUNTA P, LINDY O *et al.*: Collagenase in Sjögren's syndrome. *Ann Rheum Dis* 1994; 53: 836-9.
10. AZUMA M, MOTEGI K, AOTA K *et al.*: Role of cytokines in the destruction of acinar structure in Sjögren's syndrome salivary glands. *Lab Invest* 1997; 77: 269-80.
11. FOX P, SPEIGHT P: Current concepts of autoimmune exocrinopathy: Immunologic mechanisms in the salivary pathology of Sjögren's syndrome. *Crit Rev Oral Biol Med* 1996; 7: 144-58.
12. KONG L, OGAWA N, NAKABAYASHI T *et al*.: Fas and Fas ligand expression in the salivary glands of patients with primary Sjögren's syndrome. *Arthritis Rheum* 1997; 40: 87-97.
13. WU AJ, LAFRENIE RM, PARK C *et al.*: Modulation of MMP-2 (gelatinase A) and MMP-9 (gelatinase B) by interferon-gamma in a human salivary gland cell line. *J Cell Physiol* 1997; 171: 117-24.
14. BLACK RA, RAUCH CT, KOZLOSKY CJ *et al*.: A metalloproteinase disintegrin that releases tumour necrosis factor-alpha from cells. *Nature* 1997; 385: 729-31.
15. BOUMBA D, SKOPOULI FN, MOUTSOPOULOS HM: Cytokine mRNA expression in the labial salivary tissues from patients with Sjögren's syndrome. *Br J Rheumatol* 1995; 34: 326-33.
16. KIZU Y, SAKURAI H, KATAGIRI S *et al*.: Immunohistological analysis of tumor growth factor beta 1 expression in normal and inflamed salivary glands. *J Clin Pathol* 1996; 49: 728-32.
17. OHYAMA Y, NAKAMURA S, MATSUZAKI G *et al.*: Cytokine messenger RNA expression in the labial salivary glands of patients with Sjögren's syndrome. *Arthritis Rheum* 1996; 39: 1376-84.
18. OGAWA N, DANG H, LAZARIDIS K *et al*.: Analysis of transforming growth factor beta and other cytokines in autoimmune exocrinopathy (Sjögren's syndrome). *J Interferon Cytokine Res* 1995; 15: 759-67.
19. CHANDRASEKAR B, MCGUFF HS, AUFDERMORTE TB *et al.*: Effects of calorie restriction on transforming growth factor β_1 and proinflammatory cytokines in murine Sjögren's syndrome. *Clin Immunol Immunopathol* 1995; 76: 291-6.
20. DANG H, GEISER AG, LETTERIO JJ *et al.*: SLE-like autoantibodies and Sjögren's syndrome-like lymphoproliferationin TGF-beta knockout mice. *J Immunol* 1995; 155: 3205-12.
21. NAKABAYASHI T, LETTERIO JJ, GEISER AG *et al.*: Up-regulation of cytokine mRNA, adhesion molecule proteins, and MHC class II proteins in salivary glands of TGF-beta knockout mice: MHC class II is a factor in the pathogenesis of TGF-beta knockout mice. *J Immunol* 1997; 158: 5527-35.
22. GOETINCK PF: Proteoglycans in development. *In* BODE HR (Ed.): *Current Topics in Developmental Biology*, Vol. 25. San Diego, Academic Press 1991: 111.
23. YAMAGUCHI Y, MANN DM, RUOSLAHTI E: Negative regulation of transforming growth factor-β by the proteoglycan decorin. *Nature* 1990; 346: 281-4.
24. IOZZO RV: Matrix proteoglycans: From molecular design to cellular function. *Annu Rev Biochem* 1998; 67: 609-52.
25. CS-SZABÓ G, ROUGHLEY PJ, PLAAS AHK *et al.*: Large and small proteoglycans of osteoarthritic and rheumatoid articular cartilage. *Arthritis Rheum* 1995; 38: 660-8.
26. WHISCH-PREHM P, MIEHLKE R, KRESSE H: Presence of small proteoglycan fragments in normal and arthritic human cartilage. *Arthritis Rheum* 1992; 35: 1042-52.
27. KURODA K, SHINKAI H: Gene expression in types I and III collagen, decorin, matrix metalloproteinases and tissue inhibitors of metalloproteinases in skin fibroblasts from patients with systemic sclerosis. *Arch Dermatol Res* 1997; 289: 567-72.
28. IMAI K, HIRAMATSU A, FUKUSHIMA D *et al.*: Degradation of decorin by matrix metalloproteinases: Indication of the cleavage sites, kinetic analyses and transforming growth factor-β_1 release. *Biochem J* 1997; 322: 809-14.
29. YAMACHIKA S, NANNI JM, NGUYEN H-KT *et al.*: Excessive synthesis of matrix metalloproteinases in the exocrine tissues of the NOD mouse models for Sjögren's syndrome. *J Rheumatol* 1998; 25: 2371-80.
30. ROBINSON CP, YAMACHIKA S, ALFORD CE *et al.*: Elevated levels of cysteine protease activity in saliva and salivary glands of the nonobese diabetic (NOD) mouse model for Sjögren syndrome. *Proc Natl Acad Sci USA* 1997; 94: 5767-71.
31. KONTTENIN YT, HALINEN S, HANEMAAIJER R *et al.*: Matrix metalloproteinase (MMP)-9 type IV collagenase/gelatinase implicated in the pathogenesis of Sjögren's syndrome. *Matrix Biol* 1998; 17: 335-47.
32. ROBINSON CP, YAMAMOTO H, PECK AB *et al.*: Genetically programmed development of salivary gland abnormalities in the NOD (nonobese diabetic)-scid mouse in the absence of detectable lymphocytic infiltration: A potential trigger for sialoadenitis in NOD mice. *Clin Immunol Immunopathol* 1996; 79: 50-9.
33. ROBINSON CP, YAMACHIKA S, BOUNOUS DI *et al.*: A novel NOD-derived murine model of primary Sjögren's syndrome. *Arthritis Rheum* 1998; 41: 150-6.
34. MOSCATELLO DK, SANTRA M, MANN DM *et al.*: Decorin suppresses tumor cell growth by activating the epidermal growth factor receptor. *J Clin Invest* 1998; 101: 406-12.
35. HOCKING AM, STRUGNELL RA, RAMAMURTHY P *et al.*: Eukaryotic expression of recombinant biglycan. Post-translational processing and the importance of secondary structure for biological activity. *J Biol Chem* 1996; 271: 19571-7.
36. HU Y, NAKAGAWA Y, PURUSHOTHAM KR, HUMPHREYS-BEHER MG: Functional changes in salivary glands of autoimmune disease-prone NOD mice. *Am J Physiol* 1992; 263: E607-14.
37. BRADFORD MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; 72: 248-54.
38. LAEMMLI UK: Cleavage of structual proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; 227: 680-5.
39. TOWBIN H, STAEHLIN T, GORDON J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc Natl Acad Sci USA* 1979; 78: 4350-4.
40. ROBINSON CP, CORNELIUS J, BOUNOUS DI *et al.*: Characterization of the changing lymphocyte populations and cytokine expression in the exocrine tissues of autoimmune NOD mice. *Autoimmunity* 1998; 27: 29-44
41. DEIORIO FC: Descriptive statistics. In: *SASS Applications Programming: A Gentle Introduction.* Boston, PWS-Kent Publishing Co. 1991: 87.
42. GIRI SN, HYDE DM, BRAUN RK *et al.*: Antifibrotic effect of decorin in a bleomycin hamster model of lung fibrosis. *Biochem Pharmacol* 1997; 54: 1205-16.
43. HILDEBRAND A, ROMARIS M, RASMUSSEN LM *et al.*: Interaction of the small interstitial proteoglycans biglycan, decorin and fibromodulin with transforming growth factor beta. *Biochem J* 1994; 302: 527-34.
44. SVENSSON L, HEINEGARD D, OLDBERG A: Decorin-binding sites for collagen type I are mainly located in leucine-rich repeats 4-5. *J Biol Chem* 1995; 270: 20712-6.
45. KAMADA A, TAMURA I, OKAZAKI J *et al.*: Characteristics and localization of rat submandibular gland proteoglycans. *Archs Oral Biol* 1996; 41: 951-8.
46. MARTI HP, LEE L, KASHGARIAN M, LOVETT DH: Transforming growth factor-β_1 stimulates glomerular mesangial cell synthesis of the 72-kD type IV collagenase. *Am J Pathol* 1994; 144: 82-94.
47. KOSKI H, KONTTINEN YT, GU X-H, HIETANEN J, MALMSTRÖM M: Transforming growth factor β_2 in labial salivary glands in Sjögren's syndrome. *Ann Rheum Dis* 1995; 54: 744-7.
48. SCHÖNHERR E, HAUSSER H, BEAVAN L *et al.*: Decorin-type I collagen interaction; presence of separate core protein binding domains. *J Biol Chem* 1995; 270: 8877-83.