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Autoreactivity of Serum Immunoglobulin to Periodontal Tissue Components: A Pilot Study

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Abstract

Background—Periodontal diseases are inflammatory diseases resulting in the destruction of tissues of the periodontium. Although bacteria must be present for periodontal disease to occur, a susceptible host is also required, which is determined by genetic, environmental, and acquired factors. One such factor, autoimmunity, may play a role in the tissue destruction. Data indicate that some antibodies that occur in the gingival lesion are directed to host tissue components, such as type I collagen, although investigations of other periodontal autoimmune targets are limited.

Methods—Histologic sections and extracts from periodontally healthy teeth and the associated soft tissues were probed with serum from localized aggressive periodontitis (LAgP), chronic periodontitis (CP), and periodontally healthy subjects to determine autoreactivity to components of the periodontium. Any autoreactivity observed was characterized further by mass spectrometry and enzyme-linked immunosorbent assay.

Results—Autoreactivity to components of the periodontium was observed in CP and LAgP. Known autoimmune targets, such as collagen and heat shock protein, were identified along with multiple potential autoimmune targets, including members of the extracellular matrix, such as vimentin, spectrin, filamin, actin, lamin, keratin, and tubulin. Finally, it was determined that the autoreactivity observed in LAgP was more severe and diverse than that observed in CP.

Conclusion—These data demonstrated that autoimmune reactivity can play a role in the tissue destruction of periodontal disease but that the nature of the autoreactivity may differ based on the type and/or stage of periodontal disease.

Keywords

Antibody; autoimmunity; collagen; periodontal diseases

Periodontal disease is an all-encompassing term relating to the destructive inflammatory disorders of the hard and soft tissues surrounding teeth. Although all forms of inflammatory periodontal disease are associated with a constellation of bacteria, they have heterogeneous characteristics. For instance, generalized chronic periodontitis (CP) is a slowly progressive disease of tissue destruction affecting a wide variety of sites, whereas localized aggressive periodontitis (LAgP) involves rapid tissue destruction of fewer sites. If left untreated, both lead to the destruction of soft tissues, such as the gingiva and periodontal ligament, and the calcified tissues, cementum, and alveolar bone.¹ Immune cells are numerous and occupy a large portion of the periodontal lesion. Here, T and B cells are found in similar

proportions.^{2,3} Although T cells exhibit immunoregulatory features, including T-helper 1 and T-helper 2 mechanisms, B cells transform into antibody-producing plasma cells upon activation. Data have demonstrated that the immunoglobulins produced by plasma cells are mainly directed to antigens present in the sub-gingival biofilm.⁴ However, there are data to indicate that antibodies may also occur in the gingival lesion, which are directed to host tissue components.⁵

An autoimmune basis for the pathogenesis of periodontal disease was first postulated in 1965.⁶ Since then, a multitude of studies have focused on detecting autoantibodies directed toward various self-antigens, such as type I, II, IV, V, and VI collagens,⁷⁻¹⁰ antidesmosomal antibodies,¹¹ antibodies against aggregated immunoglobulin G (IgG),⁷ host DNA,¹² and antineutrophil cytoplasmic autoantibodies.¹³ Many of these antibodies have been analyzed in peripheral blood samples, gingival biopsies, and gingival crevicular fluid (GCF) from patients with all classifications of periodontal disease as well as healthy controls. Although most of the research has focused on anticollagen antibodies, many of the reports are contradictory. For instance, although Ftis et al.¹⁴ reported that the level of antibodies to collagen type I in peripheral blood was significantly higher in patients with periodontitis than in healthy controls, Hirsch et al.¹⁰ reported that anticollagen-producing cells were rarely detected in peripheral blood and levels of anticollagen antibodies in serum were low in patients with periodontitis. At the same time, Sugawara et al.¹⁵ compared anticollagen IgG levels in the GCF and serum of patients with periodontitis; they reported that the IgG levels in GCF were slightly higher than those in autologous sera compared to healthy control subjects. A more recently identified target for autoimmune reactions in periodontitis is heat shock protein 60 (HSP60). A study¹⁶ of 23 patients with periodontitis and 18 controls reported a higher frequency of seropositivity to HSP60 in the periodontitis group. Another group¹⁷ reported that HSP60 antibodies remained unchanged during the treatment of CP, suggesting that the synthesis of these antibodies is regulated independently during the course of periodontal infection. Taken together, these data suggest that several components related to autoimmune reaction can occur in periodontal lesions and involve antibodies to tissue or cell products. The contradictory findings also indicate that autoreactivity may be specific for disease classification and/or the stage of different periodontal diseases. The aim of this pilot study was to compare and characterize the autoreactivity of two distinct classifications of periodontal disease, LAgP and CP, to periodontally healthy controls.

MATERIALS AND METHODS

The trial was designed as a controlled pilot study conducted from January 23, 2007 until January 22, 2008. Three groups of subjects were included: CP, LAgP, and periodontally healthy subjects (PDH). The diagnosis of periodontal disease was made according to the American Academy of Periodontology Classification of 1999.¹⁸ Additionally, six periodontally healthy subjects undergoing orthodontic treatment and requiring premolar teeth extraction as part of their routine treatment were recruited to use the extracted teeth and surrounding tissues for targets of autoreactivity. Twelve teeth and associated tissues were extracted. Subjects in the CP and PDH groups were recruited from the Periodontology Clinic, College of Dentistry, University of Florida. Subjects in the LAgP group were recruited from the Leon County Health Department, Tallahassee, Florida. Subjects undergoing orthodontic treatment were recruited from the Orthodontics Clinic, College of Dentistry, University of Florida. The Institutional Review Board for the protection of human subjects at the University of Florida approved this protocol. All data and samples were obtained under written and oral informed consent.

Clinical Evaluation

Subjects in the CP, LAgP, and PDH groups were examined with respect to probing depth (PD), location of the gingival margin in relation to the cemento-enamel junction (GM), clinical attachment level (CAL), and bleeding on probing (BOP). PD was measured to the closest lower half millimeter with a manual periodontal probe. CAL was calculated as PD + GM. BOP was recorded as the presence/absence of bleeding within 15 seconds following pocket probing. The probing assessments were recorded at six sites for all teeth, except third molars. For the patients undergoing orthodontic treatment, periodontal health was confirmed from data available from periodontal examination as part of their patient care program (data not shown).

Serum, Premolar, and Gingival Tissue Sample Collections

Approximately 10 ml peripheral blood was collected by venipuncture from five subjects in each experimental group, after which serum was separated by centrifugation and stored at -80°C until assays were performed. Two to four premolar teeth were extracted from six additional periodontally healthy patients. Briefly, patients received local anesthesia followed by surgical extraction of the tooth and associated marginal gingival tissue (~1 mm). Four specimens were placed in formalin for immunohistochemical analysis, whereas eight were placed in cell-extraction buffer for protein extract analysis.

Immunohistochemical Preparation and Examination

Formalin-preserved specimens were paraffin embedded, and 5- μm sections were mounted on microscope slides. Mounted sections were deparaffinized in xylene (25 minutes), followed by stepwise rehydration in 100%, 95%, and 70% ethanol. Any endogenous peroxidase activity of the equilibrated sections was quenched by incubation in hydrogen peroxide (H_2O_2). Quenched sections were probed with 1:500 dilution pooled serum ($n = 5$) of the indicated experimental groups overnight at 4°C . Probed sections were rinsed three times in phosphate buffered saline (PBS), followed by a 2-hour incubation with 1:5,000 dilution of peroxidase-conjugated goat anti-human Ig and developed with 3,3'-diaminobenzidine. Developed sections were counterstained with hematoxylin, and the cover slip was attached with mounting medium.

Gingival and Cementum Extract Preparation

Cementum scrapings and the associated gingival tissue from eight premolar teeth were homogenized, and proteins were extracted using cell-extraction buffer. Briefly, specimens were placed in 1 ml cell-extraction buffer supplemented with a protease inhibitor cocktail and 1 mM phenylmethanesulphonyl fluoride and allowed to incubate on ice for 30 minutes. Insoluble fractions of the extracts were removed by centrifugation, and the final protein concentration was determined using a bicinchoninic acid assay.

Western Blot Analysis

Twenty micrograms of total protein extract were denatured by boiling in the presence of 4 $\times$ sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) loading dye and beta 2 mercaptoethanol, after which it was loaded onto a 2% to 15% SDS-PAGE gradient gel and allowed to migrate. Proteins were transferred onto nitrocellulose membranes and probed with 1:500 dilution of a pool ($n = 5$) or 1:500 dilution of individual participant serum. Probed blots were reacted with horseradish peroxidase-conjugated goat anti-human immunoglobulin and developed using a peroxide and luminal reagent along with x-ray film. A densitometer was used to capture the developed image and semiquantitate band density.

Protein Purification and Identification

Database searching—Tandem mass spectra (MS) were extracted, charge-state deconvoluted, and deisotoped.[‡] All MS/MS samples were analyzed using databases[§] set up to search a subset of the International Protein Index human database¹⁹ also assuming trypsin. The databases were searched with a fragment ion mass tolerance of 0.30 Da and a parent ion tolerance of 0.30 Da. Lodoacetamide derivative of cysteine was specified as a fixed modification. S-carbamoylmethylcysteine cyclization (N-terminus) of the n-terminus, deamidation of asparagine and glutamine, and oxidation of methionine were specified as variable modifications.

Criteria for Identification

A scaffold was used to validate MS/MS-based peptide and protein identifications. Peptide identifications were accepted if they could be established at >95.0% probability, as specified by a validation algorithm.^{20||} Protein identifications were accepted if they could be established at >99.0% probability and contained at least two identified peptides. Protein probabilities were assigned by the algorithm.^{21¶} Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony.

Enzyme-Linked Immunosorbent Assay (ELISA)

Twenty micrograms per well of collagen type I were coated on a 96-well plate in a carbonate coating buffer. Following blocking with PBS/Tween (0.05%)/bovine serum albumin (0.5%) for 1 hour and washing, 25 µl serially diluted (1:500–1:256,000) individual patient sera were used for probing. Probed wells were reacted with 25 µl 1:5,000 dilution of goat anti-human Ig conjugated with alkaline phosphatase, after which 100 µl p-nitro phenyl substrate reagent was used for development. The reaction was stopped using 100 µl 3M NaOH, and development was read at an optical density of 405 nm.

Statistical Analysis

Means $\pm$ SD were calculated to describe clinical data. For the semiquantitative band densities and antibody titers to collagen type I, *P* values were calculated using analysis of variance and the Student *t* test with Welch's correction making the following comparisons: LAgP versus CP, LAgP versus PDH, and CP versus PDH. The unpaired *t* test assumes that the two populations have the same variances (same standard deviations). A modification of the *t* test (developed by Welch) can be used when one is unwilling to make that assumption. With the Welch *t* test, the degrees of freedom are calculated from a complicated equation, and the number is not obviously related to sample size.²² *P* < 0.05 was considered significant.

RESULTS

Clinical Characteristics of Participants

Because chronic and aggressive periodontal diseases have different rates and timing with regard to their onset and disease progression, it was of interest to determine whether autoreactivity to periodontal lesions plays a similar role in both disease processes. The characteristics of the experimental groups are summarized in Table 1. In the CP group (two

[‡]ABI Analyst version 1.1, Applied Biosystems, Carlsbad, CA.

[§]Mascot version 2.2.0, MatrixScience, Boston, MA.

^{||}Peptide Prophet, Proteome Software, Portland, OR.

[¶]Protein Prophet, Proteome Software.

males and three females aged 51 to 60 years), $35.6\% \pm 17.1\%$ of sites had PD ≥ 4 mm, and $34.6\% \pm 10.0\%$ of sites had CAL ≥ 5 mm. In the LAgP group (three males and two females aged 12 to 19 years), $13.7\% \pm 7\%$ of the sites exhibited PD ≥ 4 mm, and $6.5\% \pm 3.1\%$ of sites had CAL ≥ 5 mm. The majority of attachment loss in subjects in the LAgP group was attributed to permanent first molars and incisors. The PDH group consisted of one male and four females, aged 21 to 28 years, with no sites exhibiting PD ≥ 4 mm or CAL ≥ 5 mm and 10% of sites exhibiting BOP.

Immunohistochemical Evaluation of Autoreactivity

Initially, to determine whether there was serum autoreactivity to components of the periodontal structures, extracted teeth and the associated tissues, such as periodontal ligament and gingival tissues, were sectioned, mounted, and probed with serum from each of the experimental groups. We detected reactivity to components of the periodontal structure in the subjects with LAgP (Figs. 1E and 1F) and CP (Figs. 1C and 1D), whereas no reactivity was detected in serum from periodontally healthy controls (Figs. 1A and 1B). The majority of reactivity seemed to be located in or adjacent to the soft tissues of the periodontal structure, including the periodontal ligament (Fig. 1, thick arrow). These data demonstrate that B-cell autoreactivity is present in CP and LAgP.

LAgP Serum Reactivity Differs in Quality and Quantity Compared to CP Serum Reactivity

To determine to what types of periodontal components these antibodies were reactive, extracts of cementum and associated tissues of extracted molars, including the periodontal ligament and gingival tissues, were probed with pools of serum ($n = 5$) from each experimental group, using a Western blot technique. Although serum from subjects with LAgP reacted to several bands of proteins, serum from subjects with CP reacted to only one of these bands of proteins (band I) (Fig. 2A). As expected, serum from periodontally healthy subjects did not demonstrate any reactivity (Fig. 2A). To determine whether the observed reactivity was representative of all members of the cohort and not due to reactivity from only one subject within the pool, the same analysis was performed on individual samples from all subjects within all experimental groups. We detected consistently higher reactivity to all bands analyzed (bands I, II, and III) from individual LAgP serum compared to individual CP and/or PDH serum (P value = 0.001; Fig. 2B). In addition, the individual serum of subjects with CP was consistently reactive with only band I of the tissue extracts, whereas as expected, individual PDH subjects demonstrated little or no reactivity. These data demonstrated that although autoreactivity is present in CP and LAgP, their quality and quantity are significantly different.

Key Components of the Periodontal Structure Are Putative Targets of Autoreactivity

To further define the protein components against which the observed autoreactivity was directed, the three reactive bands of proteins were excised and analyzed for peptide content using tandem mass spectrometry. Several peptides were identified in each band; they are summarized in Table 2. Many peptides associated with previously identified autoimmune targets, such as those associated with collagen, HSP, lipoproteins,²³ and aggregated IgG, were present (Table 2). A multitude of unexplored autoantibody targets known to be associated with the extracellular matrices and cell-to-cell interactions required for an intact periodontal structure were also detected; these include vimentin, spectrin, filamin, actin, lamin, keratin, and tubulin. Many of these proteins were identified in bands II and III (Fig. 2; Table 2), indicating that they would be involved in the autoreactive nature of LAgP but not CP, because serum from the CP group did not react with these bands. Not all peptides identified in each band are targets for autoimmunity. Further investigation is necessary to determine which peptides are autoimmune targets and may also be involved in the progression of periodontal disease and with which disease states they are associated.

Autoreactivity to Collagen Type I Is More Severe in LAgP

Because there was a significantly reduced reactivity to band I in serum from subjects with CP compared to those with LAgP (Fig. 3), and our peptide analysis of band I revealed several peptides of collagen to be present (Table 1), we wanted to better quantify the serum reactivity of these experimental groups to collagen. Therefore, we performed ELISA analysis on serum from individual subjects with CP or LAgP to determine an average collagen type I titer. Similar to what we detected with our Western blot analysis, we demonstrated that subjects with CP had a significantly lower titer to collagen type I compared to those with LAgP ($P = 0.027$), whereas PDH had no reactivity to collagen type I (Fig. 3). These data confirm that autoreactivity to collagen type I is present in CP and LAgP, although their strengths differ significantly. This suggests that although autoimmunity plays a role in periodontal diseases as a whole, its nature may differ based on the type and/or stage of these diseases.

DISCUSSION

The results of the present pilot study demonstrated that subjects with CP or LAgP exhibited serum autoreactivity to components of periodontal structures. However, serum autoreactivity was greater in patients with LAgP compared to those with CP, and healthy individuals did not demonstrate any reactivity at all. Many peptides associated with collagen, extracellular matrices, and cell-to-cell interactions were identified as putative targets for autoreactivity (Table 2). Finally, it was determined that the autoreactive repertoire in LAgP is more diverse than that observed in CP.

Periodontal lesions contain large amounts of leukocytes, among which B cells seem to be a predominant cell type. These cells and their products, which are associated with tissue destruction, are controlled by immunoregulatory mechanisms. The equilibrium or imbalance established between the biofilm and the inflammatory process determines the severity of the periodontal lesion. Although the timing and progression of CP and LAgP differ greatly, they exhibit similar features with respect to the cellular composition of the lesions.⁵ B1 cells, otherwise known as unconventional B-lineage cells, and more precisely, B1a cells, are elevated compared to PDH individuals.^{10,15,24} B1 cells have the capacity to produce autoantibodies with various degrees of affinity. These cells are abundant in subjects with autoimmune diseases, such as Sjögren's syndrome and rheumatoid arthritis (RA).^{25–27} Periodontal diseases can be epidemiologically linked to and have similar pathways of pathogenesis to autoimmune diseases, such as RA, diabetes, and systemic lupus erythematosus.^{28,29}

It has been determined that autoimmune reactions are involved in periodontal diseases, where one of the main evaluated autoantigens is collagen type I.⁵ A recent review³⁰ highlighted the conflicting results that have plagued this field of study. Although our study demonstrated that serum from subjects with CP or LAgP exhibited autoreactivity to collagen, serum autoreactivity was greater in subjects with LAgP (Figs. 2 and 3). Therefore, we suggest that conflicting reports on collagen autoimmune reactivity are due to the differences in periodontal disease status of the subjects in these studies. This pilot study demonstrated a distinct difference in the quality and quantity of autoreactivity between CP and LAgP (Figs. 2 and 3; Table 2).

In addition to antibodies to collagen type I, several other autoreactive antibodies have associations with periodontal disease, such as antidesmosomal, antineutrophil cytoplasmic, antiphospholipid, and anti-HSP60 antibodies.⁵ We demonstrated reactivity to known autoantigens, such as collagen, HSP, and aggregated IgG (Table 2) by LAgP and CP sera and described several molecules associated with extracellular matrices and cell–cell

interactions that were previously unknown putative targets for autoreactivity (Table 2). Although the validity of their autoantigen status is still to be determined, many of these molecules only demonstrated reactivity with serum from subjects with LAgP (Table 2). These data suggest that the autoreactive repertoire between LAgP and CP differs and may be responsible for the differences in clinical presentation and disease progression.

Some interesting putative targets identified include alpha-enolase and calreticulin. Both of these molecules have been implicated in other autoimmune processes.³¹ Alpha-enolase has been identified as an autoantigen in Hashimoto's encephalopathy,³² severe asthma,³³ and Behcet's disease.³⁴ Calreticulin was demonstrated to bind antibodies in sera of some patients with systemic lupus or Sjögren's syndrome.^{31,35,36} In addition to these autoimmune-associated proteins, epithelial cell (keratin) and osteoblast (periostin)-associated proteins were detected during our analysis and, therefore, are molecules of interest for autoimmune targets in periodontal diseases (Table 2). Periostin was originally identified as a protein secreted only by osteoblasts (OSF-2),³⁷ where its expression was localized in the periosteum and periodontal ligament.³⁸ It has since been classified as a matrixcellular protein because of its expression in other tissues and pathologies.³⁹ The potential autoreactivity to all of these molecules was demonstrated only in LAgP serum; CP serum was not reactive to the band in which these molecules were identified (Fig. 3; Table 2).

Although this study demonstrated the presence of autoreactive antibodies to extracts from the periodontal structures, the putative autoimmune targets need to be confirmed using purified proteins and ELISA technology. It is an ongoing area of research in our laboratory to identify novel targets within the periodontium for autoimmune reactivity. This study was the first step in identifying candidates to investigate further (Table 2).

CONCLUSIONS

This pilot study confirmed that autoimmunity is involved in the disease progression of periodontal diseases, yet it demonstrated that the quality and quantity of autoreactivity are related to disease classification and/or disease state. Most importantly, although their validity needs to be confirmed, novel targets associated with the periodontium for autoimmune reactivity were described.

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Figure 1.

Immunohistochemical analysis of autoreactivity. Five-micron sections of PDH teeth and associated soft tissues were probed with 1:500 dilution pooled serum ($n = 5$) from PDH subjects (**A** and **B**) and those with CP (**C** and **D**) or LAgP (**E** and **F**). 3,3'-diaminobenzidine was used to detect autoreactivity, indicated by the brown coloring. Data are representative sections of experiments performed on tissues from four donors. * = dentin; thin arrow = cementum; thick arrow = associated soft tissues (periodontal ligament and gingiva). (Original magnification: A, C, and E, $\times 10$; B, D, and F, $\times 20$.)

Figure 2.

LAgP serum demonstrated differential reactivity compared to CP serum. **A)** Extracts from cementum scrapings and the associated gingival tissue from eight premolar teeth were probed with 1:500 dilution pooled serum ($n = 5$) or 1:500 dilution of individual serum of subjects with LAgP, CP, or PDH subjects using a standard Western blot protocol. Representative blot of experiments performed three times. **B)** A densitometer was used to capture the developed image of blots from experiments performed with individual serum ($n = 5$) from each experimental group, and the band densities were semiquantified. * $P = 0.001$: LAgP versus CP and LAgP versus PDH band I; † $P = 0.001$: CP versus PDH band.

Figure 3.

Autoreactivity to collagen type I is more severe in LAgP. Twenty micrograms/well of collagen type I was probed with serial dilutions of individual sera ($n = 5$) of subjects with LAgP (squares), CP (triangles), or PDH (circles) subjects using a standard ELISA protocol. $^{\dagger}P = 0.027$: LAgP versus PDH and LAgP versus CP; $^{\S}P = 0.0251$: CP versus PDH. Data are a compilation of three separate experiments performed in duplicate.

Table 1Clinical Characteristics (mean $\pm$ SD) of Study Subjects

Group	Age (years)	PD (mm)	CAL (mm)	BOP (% sites)	PD 4 mm (% sites)	CAL 5 mm (% sites)
CP (n = 5)	55 $\pm$ 4.2	3.5 $\pm$ 1.6	4.0 $\pm$ 2.0	49.4 $\pm$ 7.2	35.6 $\pm$ 17.1	34.6 $\pm$ 10.0
LAgP (n = 5)	16.2 $\pm$ 2.9	2.9 $\pm$ 1.4	1.5 $\pm$ 1.4	54.3 $\pm$ 9.5	13.7 $\pm$ 7.0	6.5 $\pm$ 3.1
PDH (n = 5)	23.4 $\pm$ 3.0	1.7 $\pm$ 0.6	0.6 $\pm$ 0.5	8.1 $\pm$ 2.7	0	0

Table 2**Putative Targets of Autoreactivity**

Previously Identified Classes of Targets in Periodontal Disease	Band I	Band II	Band III
Lipoproteins			
Apolipoprotein A-I precursor	×		×
Immunoglobulins			
IGHA1			×
IGHG2	×		×
IGHG1	×	×	
IGHG1	×	×	×
IGKV1	×		
Heat shock proteins			
HSP90	×		
HSP70	×		
HSP70		×	
Collagens			
Collagen alpha-1(I) chain precursor	×		
Collagen alpha-1(XII) chain precursor (isoform 4)	×		
Collagen alpha-1(XIV) chain precursor (isoform 1)	×		
Collagen alpha-2(I) chain precursor	×		
Glycoproteins			
Alpha-1-antitrypsin precursor	×		×
Alpha-2-HS-glycoprotein precursor			×
Beta-2-glycoprotein 1 precursor			×
Alpha-1B-glycoprotein precursor		×	
Fibrinogen precursor (gamma chain)	×	×	×
Inter-alpha-trypsin inhibitor precursor (heavy chain H2)	×		
Blood proteins			
Alpha-2-macroglobulin precursor	×		
C3 (187 kDa protein)	×		
Hemoglobin subunit alpha	×	×	×
Hemoglobin subunit beta	×	×	×
Hemopexin precursor		×	
Serum albumin precursor (isoform 1)	×	×	×
Nuclear proteins			
Elongation factor 1 (alpha 2)	×		
Putative Autoimmune Targets of Interest in Periodontal Disease	Band I	Band II	Band III
Cell-cell interactions			
Actin (cytoplasmic 1)	×	×	×
Fibrinogen precursor (beta chain)			×
Filamin (alpha)	×		
Lamin (isoform A)	×	×	×

Putative Autoimmune Targets of Interest in Periodontal Disease	Band I	Band II	Band III
Spectrin (isoform b)	×		
Tubulin (beta chain)	×		
Vimentin	×	×	×
Proteoglycans			
Lumican precursor		×	×
Versican precursor (isoform V0)			×
Epithelial cell-associated proteins			
Keratin type I (cytoskeletal 10)	×		
Keratin type II (cytoskeletal 1)	×	×	×
Putative Autoimmune Targets of Interest in Periodontal Disease	Band I	Band II	Band III
Osteoblast-associated proteins			
Periostin precursor (isoform 1)	×	×	
Autoimmune-associated proteins			
Alpha-enolase			×
Calreticulin precursor			×
Enzymes			
Aldehyde dehydrogenase (member A1)			×
Glyceraldehyde-3-phosphate dehydrogenase	×		
Peroxiredoxin-2	×		×
Protein disulfide-isomerase precursor			×
Binding proteins			
Anion transport protein	×		
Selenium-binding protein 1			×
Serotransferrin precursor		×	×
Vitamin D-binding protein precursor			×
Proteins of unknown function			
Uncharacterized protein SPTA1	×		

HS = homo sapiens; SPTA1 = spectra alpha chain.