Anti-20S Proteasome Antibodies in Psoriatic Arthritis

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ABSTRACT. ***Objective***. Proteasomes are targets of humoral autoimmune response in patients with connective tissue diseases and other organ-specific autoimmune diseases. The finding of circulating proteasomes in psoriasis, the multiplicity of mechanisms regulated by proteasomes that are also implicated in the pathogenesis of psoriatic arthritis (PsA), and the increasing evidence linking PsA and autoimmunity led us to evaluate whether the 20S proteasome represents an antibody target in PsA.

Methods. Serum samples from 36 patients with PsA and 30 age- and sex-matched healthy subjects were tested for the presence of anti-20S proteasome antibodies (anti-20S antibody). Additional controls included 24 patients with systemic lupus erythematosus (SLE) and 20 with rheumatoid arthritis (RA). The associations of anti-20S antibodies with clinical, laboratory, and therapeutic measures were evaluated.

Results. 27.8% of the PsA patients were positive for anti-20S antibody compared to 41.6% of the SLE group and 5% of the RA group. None of the healthy subjects were seropositive for anti-20S antibody. In PsA, anti-20S seropositivity was not associated with the presence of other autoantibodies or with a particular subgroup of articular involvement.

Conclusion. Immunoreactivity against proteasomes occurs frequently in patients with PsA. This finding supports the concept of PsA as an autoimmune disease and opens new avenues for investigating its pathogenesis. (First Release Feb 15 2008; J Rheumatol 2008;35:674–6)

Key Indexing Terms:
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The proteasome, the central enzyme complex of nonlysosome protein degradation, is an essential component of the ubiquitin-ATP-dependent proteolytic pathway[1]. Functionally, the proteasome represents the principal source of peptides presented by MHC class I molecules, influences the thymic selection of CD8+ cells and the regulation of cell-cycle control, and is involved in the processing of central transcription factors, particularly nuclear factor-κB (NF- κB)[2,3].

Circulating proteasomes and antiproteasome antibodies have been described in several autoimmune diseases[4,5]. The recent finding of elevated circulating proteasome levels in psoriasis[6], along with the known role of proteasomes in the initiation and perpetuation of the autoimmune cytotoxic T cell response[2,7], a key element of the pathogenesis of psoriatic arthritis (PsA), led us to test the hypothesis that the 20S core particle of the proteasome constitutes an antibody target in patients with PsA.

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MATERIALS AND METHODS

Patient sera. Sera samples from 36 PsA patients, 20 patients with rheumatoid arthritis (RA), and 24 patients with systemic lupus erythematosus (SLE) were examined. Thirty healthy subjects matched by age and sex with the PsA patients were also included. Sera were obtained from consecutive patients followed at the IREP, Buenos Aires, Argentina, and at LSUHSC, New Orleans, LA, USA.

Autoantibody profiles. Antinuclear antibodies (ANA) were detected by indirect immunofluorescence on HEp-2 cells (BioRad Laboratories, Mississauga, ON, Canada), anticyclic citrullinated peptide (anti-CCP) antibodies by a commercial ELISA (Quanta-lite, Inova Diagnostics, San Diego, CA, USA), and rheumatoid factor (RF) by nephelometry (Dade Behring GmbH, Marburg, Germany).

Immunoblotting. A measure of 2 μg of commercial 20S proteasome isolated and purified from human erythrocytes (Biomol International, LP) was diluted in a total volume of ~350 μl of protein loading buffer, heat denatured, loaded into a single 12 mm-wide lane (1.5 mm deep) on a 9% sodium dodecyl sulfate-polyacrylamide gel, and resolved by electrophoresis (SDS-PAGE). Subsequently the proteins were transferred onto nitrocellulose membranes (BioRad, Hercules, CA, USA), and 5 mm strips were cut. Nonspecific reactivity was blocked, and the sera samples diluted 1:400 and 1:800 were added to the strips and incubated at room temperature for 4 h. Blots were washed in Tris-buffered saline-Tween-20 (TBST) and incubated with goat anti-human IgG conjugated with horseradish peroxidase (Pierce, Rockford, IL, USA) diluted 1:10,000 in 1% non-fat milk TBST for

1 h at room temperature. Antigen-antibody complexes were detected using an enhanced chemiluminescence system (Amersham Biosciences, Buckinghamshire, UK). Positive results were confirmed in 3 independent experiments using serial serum dilutions.

Positive/negative controls and proof of serum antibody specificity. After SDS-PAGE, Coomassie blue staining of the gel showed the presence of bands corresponding to molecular weights between 20 and 30 kDa (expected MW of the 20S proteasome subunits). No other bands were detected due to the highly purified property of the commercial antigen. Using a monoclonal antibody to 20S proteasome subunit α7 (HC8; Biomol International, LP) as the primary antibody and a peroxidase conjugated goat anti-mouse antibody as the secondary (DakoCytomation, Glostrup, Denmark), a single band with a relative molecular weight of 30 kDa was observed. A positive control was selected from the SLE group, and a competition assay was performed to determine antibody specificity. Briefly, prior to exposure to an antigen-positive nitrocellulose strip, the serum (dilution 1:800) was incubated with purified 20S proteasome (40 µg of 20S proteasome in 1% nonfat milk TBST) at different dilutions (1:100 to 1:100,000) for 1 h at room temperature. Afterwards the strips were exposed to the sera, and immunoreactivity was determined as described above. The comparative negative control was selected from the healthy subject group.

Clinical data. At the time of serum collection, data were obtained from PsA patients on demographic features, types of articular and extraarticular involvement, psoriasis and PsA disease duration, orthopedic surgeries, and therapy.

Statistical analysis. Continuous data were assessed using a t-test with Levene's test for homogeneity of variance. Categorical data were compared by chi-square or Fisher's exact test. All statistical analyses were performed with the SPSS 11.5 software for Windows (SPSS, Chicago, IL, USA).

RESULTS

Table 1 summarizes demographic characteristics of the clinical groups in the study; Table 2 describes clinical features from the 36 patients with PsA. Anti-20S proteasome antibodies were detected in 27.8% of PsA patients (10 of 36). Using identical conditions, the seropositivity in SLE patients was 41.6% (10 of 24) and in RA patients was 5% (1 of 20). No healthy subject exhibited antiproteasome reactivity. Figure 1 illustrates that the molecular weight of the bands observed in the SLE and PsA groups corresponded to the expected molecular weight of the 20S core particle α subunits (~30 kDa). Of particular interest was the consistently stronger band intensities observed in the sera from the SLE patients as compared to PsA patients, which could be diluted as much as 1:2000 without losing signal intensity. This suggests that SLE sera may contain higher levels of anti-20S proteasome antibodies. This hypothesis remains to be tested. In addition, and similar to findings described in primary Sjögren's syndrome[8], the presence of more than one band by Western blot analysis from PsA patients suggests a polyspecific autoimmune response against the proteasome.

Table 2. Clinical and laboratory features of the PsA patients.

Feature	Mean or Percentage
Years of psoriasis, mean ± SD	17.5 ± 14.6
Years of arthritis, mean ± SD	9.8 ± 4.8
Subtype of PsA: poly/oligoarticular, %	36/27.8
Nail involvement, %	69.4
Dactylitis, %	50
Family history of PsA, %	63.9
ANA+, %	19.4
RF+, %	5.6
CCP+, %	5.6

The specificity of this response directed against the 20S proteasome subunits was confirmed by the competition assay (Figure 2). For a 3-year followup period, serial serum samples (n = 3) were tested in 5 PsA patients in which anti-20S antibody remained positive, showing the same band intensities at different timepoints (data not shown). Antiproteosomal antibodies were not associated with disease duration, type of articular involvement, or disease severity (nail involvement, dactylitis, or history of orthopedic surgeries) in PsA patients. ANA, RF, or anti-CCP antibody positivity was not associated with the presence of anti-20S antibody.

DISCUSSION

The presence of anti-20S reactivities against denatured subunits of the 20S proteasome in PsA raises questions about the mechanisms involved in their development and the pathogenic importance of antiproteasome immunoreactivity. Two mechanisms have been postulated to explain the anti-20S antibody generation[5]: (1) crossreactivity of a primary response against exogenous proteins; and (2) the proteasome driving its own autoimmune response. In psoriasis, a recent report has documented an increase in plasma proteasome levels compared with normal serum donors[6]. The same finding was previously shown in SLE, RA, myositis, primary Sjögren's syndrome, and autoimmune hepatitis. For these autoimmune conditions, circulating proteasomes were considered markers of tissue injury and cellular turnover[9]. Antiproteasome antibodies were described in these same

Table 1. Demographic characteristics and anti-20S seropositivity in different clinical groups.

Characteristic	Controls	Patients with PsA	Patients with SLE	Patients with RA
No.	30	36	24	20
Female/male	20/10	23/13	20/4	16/4
Age, mean ± SD yrs	49.5 ± 11	51 ± 12	41.6 ± 10	52.4 ± 10
Anti-20S antibody, %	0	27.8	41.6	5

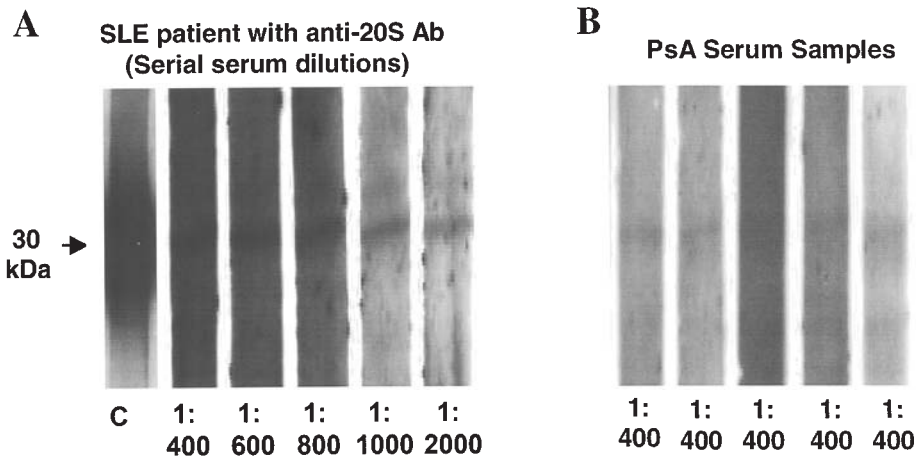

Figure 1. Anti-20S antibody in SLE and PsA. 2 μg of purified human 20S proteasome was resolved by SDS-PAGE and immunoblotted as described in Materials and Methods. An anti-20S proteasome subunit, α7 (anti-HC8 monoclonal antibody), was included as a positive control (C). Healthy serum sample was included as a negative control (data not shown) A. Serial dilutions from anti-20S-positive SLE serum. B. Seropositive PsA samples.

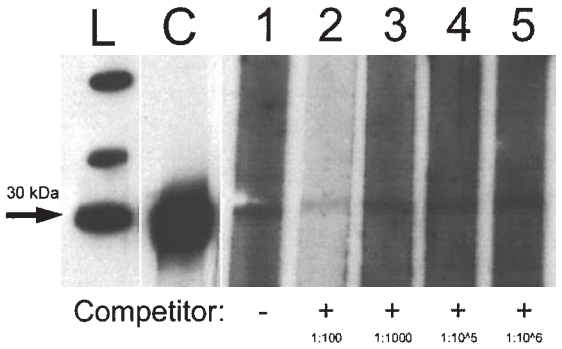

Figure 2. Competition assay shows specificity of the anti-20S antibody. A monoclonal antibody to 20S proteasome subunit α7 (HC8; Biomol International) was used as a positive control. L: ladder; C: anti-HC8 monoclonal antibody-positive control; (+): presence of competitor; (—): absence of competitor.

diseases, suggesting an antigen-driven mechanism as a trigger of proteasome autoimmunity[4,8,10,11].

The pathogenic significance of anti-20S antibodies in PsA, as well as in other autoimmune conditions, remains uncertain. However, antiproteasome antibodies have been shown *in vitro* to alter proteasome activation by competing with the specific proteasome activator PA28[12]. This could lead to an altered cleavage pattern of the proteasome, with the generation of potentially immunogenic new self-antigens.

Our findings demonstrate an autoimmune response to the 20S proteasome in 27.8% of patients with PsA that was not associated with the production of other antibodies, a particular type of articular involvement, or the use of anti-tumor necrosis factor agents. Proteasomes are not only the targets and modulators of immune responses, they also play a central role in the critical mechanisms implicated in the pathogenesis of PsA. Further investigations are justified to determine the influence of proteasome function in this disease.

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