Participation of antigens related to the psoriasis associated antigen, pso p27, in immune complex formation in patients with ankylosing spondylitis

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SUMMARY Analysis of five serum samples and three synovial fluids from patients with ankylosing spondylitis (AS) and five serum samples from healthy blood donors for the presence of antibodies cross reacting with the Fc part of rabbit IgG (rheumatoid factors (RFs)) using an isotype specific, enzyme linked immunosorbent assay (ELISA) showed only insignificant amounts of free RFs, while IgG RFs were observed in alkaline dissociated circulating immune complexes (CICs). Only insignificant amounts of free antibodies reacting with the psoriasis associated antigen pso p27 could be detected in the samples, while extensive amounts of IgG antibodies and moderate amounts of IgM antibodies reacting with pso p27 were detected in alkaline dissociated CICs from the patients. Pso p27 has been reported to share a common determinant with the Fc part of human IgG. Removal of the RF activity from the CICs of patients with AS by absorption with IgG resulted in a decrease of the anti-pso p27 activity. Monoclonal anti-pso p27 antibodies in a sandwich ELISA were used to detect antigens cross reacting with pso p27. A positive reaction was observed in all serum CICs and in one of the synovial fluid CICs. The data indicate that antigens related to pso p27 participate in CIC formation in AS and may also be responsible for the elicitation of rheumatoid factors in patients with AS.

Key words: enzyme linked immunosorbent assay (ELISA), aetiology, retrovirus-like, rheumatoid factor.

Ankylosing spondylitis (AS) is a disorder of unknown aetiology characterised by proliferation of synovial cells and chronic inflammation of joint structures, particularly of the vertebral column. A chronic inflammatory reaction is also observed at the insertions of various tendons and ligaments (enthesopathy).¹

Cellular proliferation and chronic inflammation are characteristic features of several retrovirus induced disorders.² Retroviruses both of exogenous and endogenous origin may participate in the pathogenesis of chronic arthritis in animals.^{3,4}

Circulating immune complexes (CICs) are frequently observed in animals persistently infected with viruses.^{5–7} CICs have also been isolated from

humans with viral arthritis,^{8,9} and virus or virus antigens have been shown to participate in CIC formation. CIC antigens may thus reflect the pathogenetic agents causing the disease.

Psoriatic arthritis and AS are closely related disorders and may represent facets of the same syndrome.^{10,11} Genes predisposing to psoriasis may confer susceptibility both to peripheral arthritis and to AS and may also modify the clinical course of AS.^{12,13}

In a series of papers we have described the isolation of a virus-like particle from a patient with psoriasis.^{14,15} The particle has a buoyant density in sucrose and a protein composition closely resembling that of mammalian retroviruses. The major internal protein, p27, of the particle (pso p27) has been shown to participate in immune complex formation both in psoriatic scales and in CICs from patients with psoriatic arthritis.¹⁶ Psoriatic scale has

recently been shown to represent a convenient source for the isolation of pso p27 (O-J Iversen, K Åsbakk, unpublished data).

Using antiserum against pso p27 in an immunofluorescence analysis, we have previously observed cross-reacting antigens in lymphocytes and mononuclear synovial cells from patients with AS.¹⁷

Recently, we have also shown that pso p27 shares a common antigenic determinant with the Fc part of IgG and suggested a role for pso p27 in the development of rheumatoid factors (O-J Iversen *et al* unpublished data).

In this paper we have analysed CICs from patients with AS for the presence of antibodies reacting with pso p27, and antigens related to pso p27. The samples were also analysed for the presence of anti-rabbit Fc antibodies.

Patients and methods

CLINICAL SPECIMENS

Serum samples were obtained from five patients, and knee joint synovial fluid from three patients with AS. In one case (patient No 1) both serum and synovial fluid were obtained from the same patient. All patients were HLA-B27 positive and fulfilled the New York criteria for AS. In addition, serum samples were obtained from five healthy persons.

ISOLATION OF IMMUNE COMPLEXES

Immune complexes were isolated by isopycnic ultracentrifugation in 20–65% (w/w) sucrose gradients.¹⁸ After dialysis against saline the complexes were dissociated by adjusting the pH to 10.8 with 0.2 M NaOH.

PSO P27 ANTIGEN

Pso p27 was isolated from psoriatic scales by immunosorbent chromatography, followed by gel filtration on a Sephacryl S-300 column in 6 M guanidine hydrochloride.¹⁹

MONOCLONAL ANTI-PSO P27 ANTIBODIES

The procedure has been presented in detail elsewhere.¹⁹ Briefly, BALB/c mice were immunised with purified pso p27. Spleen cells were fused with the myeloma cell line Sp2/0-Ag-14 (Flow Laboratories). Hybrid cells were selected by cultivation in hypoxanthine, aminopterin, thymidine medium. Culture supernatants were assayed for the presence of antibodies reacting with pso p27 and psoriatic blood lymphocytes. Positive cultures were cloned and recloned by limiting dilution. Ascites fluids were obtained after intraperitoneal injection of hybridoma cells into syngeneic mice (BALB/c). None of the antibodies applied in this investigation showed any cross reaction with human IgG.

BIOTINYLATION

Immunoglobulins were purified from ascites fluid and biotinylated.¹⁹

MEASUREMENT OF HUMAN IMMUNOGLOBULINS

The amount of IgG, IgA, and IgM in serum, synovial fluid, or immune complex preparations was determined by a sandwich enzyme linked immunosorbent assay (ELISA).²⁰ Polystyrene microtitre plates (Linbro 76-381-04, Flow Laboratories) were coated with rabbit antibodies against human IgG, IgA, or IgM (Behringwerke). The assays for the respective isotypes were performed with serum samples diluted 1:10⁻⁷, 1:10⁻⁶, and 1:10⁻⁵, synovial fluids diluted 1:10⁻⁶, 1:10⁻⁵, and 1:10⁻⁴, and CIC preparations diluted 1:10⁻³, 1:10⁻², and 1:10⁻² in phosphate buffered saline pH 7.2 containing 0.05% Tween 20 (PBS T20). Bound immunoglobulins were detected by peroxidase conjugated rabbit antibodies (DAKO) against human IgG, IgA, or IgM using o-phenylenediamine (OPD) (Sigma) as substrate. The concentration of immunoglobulins was determined by referring to serial dilutions of standard serum (Behringwerke). The absorbance was recorded in a Titertek Multiscan spectrophotometer (Flow Laboratories).

DETECTION OF RHEUMATOID FACTORS

Serum samples, synovial fluids, and alkaline dissociated immune complexes were assayed for the presence of antirabbit Fc activity (RF activity) of the IgG, IgA, and IgM class.²¹ Microtitre plates were coated with human albumin and incubated with whole IgG or Fab₂ fragments of rabbit IgG against human albumin. Serum or synovial fluid samples were diluted 1:1000, and the immune complex preparations were diluted 1:20. The binding of human antibodies was detected by peroxidase conjugated rabbit antibodies against human IgG, IgA, or IgM. The RF activity was recorded as the difference in optical density (Δ OD) between the reaction with whole IgG and that with Fab₂ fragments after 30 minutes incubation with OPD. The results were expressed as RF activity per microgram immunoglobulin of the respective isotype.

HUMAN ANTIBODIES AGAINST P27

Microtitre plates were coated with purified pso p27 antigen. The amount applied in each well corresponded to the amount obtained from 10 μ g of psoriatic scale. Serum samples and synovial fluids were diluted 1:1000, and the immune complex preparations were diluted 1:20. The binding of anti-pso p27 antibodies was detected by peroxidase conjugated rabbit antihuman IgG, IgA, or IgM

antibodies (DAKO) using OPD as substrate. Uncoated wells served as negative controls.¹⁶ The colour development was stopped after four to six minutes for the IgG isotype, and after 30 minutes for the IgA and IgM isotypes. The results were expressed as anti-*pso* p27 activity per microgram immunoglobulin of the respective isotype.

DETECTION OF P27 ANTIGEN

Microtitre plates were coated with ascites fluid containing monoclonal anti-*pso* p27 antibodies 7-4G11D4 as described previously.¹⁹ The wells were incubated with alkaline dissociated immune complexes diluted 1:2. Biotinylated monoclonal anti-*pso* p27 antibodies 4-2D1H11 diluted 1:10 000, followed by peroxidase conjugated streptavidine (Bethesda Research Laboratories) diluted 1:500 were applied for the detection of bound antigens. OPD served as substrate, and the colour development was stopped after 30 minutes. Uncoated wells were used as negative controls.

IMMUNOSORBENT CHROMATOGRAPHY

Human IgG (Kabi) was coupled to CNBr activated Sepharose 6MB (Pharmacia) using the procedure recommended by the manufacturer. Immunosorbent chromatography of alkaline dissociated immune complexes was performed in PBS T20. Unbound immune complex material was assayed for the presence of rheumatoid factor activity and anti-*pso* p27 activity as described above.

Results

RHEUMATOID FACTOR ACTIVITY

Serum samples from five patients with AS and five healthy persons, as well as synovial fluid from three patients with AS were assayed for the presence of free antirabbit Fc antibodies (RF activity). All samples were assayed in a dilution of 1:1000, and the isotype RF activity was recorded as ΔOD of antibodies reacting with IgG and Fab₂ fragments per microgram immunoglobulin of the respective isotype. Only insignificant amounts of free RF activity of the IgG, IgA, or IgM class were detected (ΔOD per μg immunoglobulin isotype <0.05).

Immune complexes were isolated from the AS sera and the synovial fluids by isopycnic ultracentrifugation in sucrose gradients. The complexes were dissociated at high pH, neutralised, and assayed for RF activity in a dilution of 1:20. Antibodies of the IgA or IgM class reacting with the Fc region of rabbit IgG could not be detected in any of the immune complex preparations. Considerable RF activity of the IgG class was observed in two of the patients (Nos 1 and 7). From patient No 1 we

analysed both serum and synovial fluid immune complexes, and IgG rheumatoid factor activity was observed in both (Table 1). The RF activity of CICs from patients 2, 4, and 5 was also significantly positive when the mean RF activity in serum of healthy blood donors plus three standard deviations ($\Delta OD/\mu g$ IgG=0.06) was used as cut off point.

ANTIBODIES AGAINST *PSO* P27

The same serum samples and synovial fluids were analysed for the presence of free antibodies reacting with *pso* p27. The samples were assayed in a dilution of 1:1000. Only insignificant amounts ($OD <0.2/\mu g$ immunoglobulin isotype after 30 minutes incubation with OPD) of IgG, IgA, or IgM antibodies reacting with *pso* p27 were observed.

Both undissociated and alkaline dissociated immune complex preparations were then assayed for the presence of antibodies reacting with *pso* p27. Significant anti-*pso* p27 activity was not observed using undissociated immune complexes. Table 2 shows, however, that extensive amounts of IgG antibodies reacting with *pso* p27 were revealed after alkaline dissociation of the immune complexes in all samples studied. Moderate amounts of anti-*pso* p27 IgM antibodies were also detected, but no anti-*pso* p27 IgA antibodies were observed. Fig. 1 shows IgG anti-*pso* p27 activity of serially diluted alkaline dissociated CICs from patient No 1. The anti-*pso* p27 activity recorded after 30 minutes' incubation with OPD of dissociated serum immune complexes from patient No 2 corresponded to 50.63/ μg IgG, while that of serum was 0.05/ μg IgG, indicating that the anti-*pso* p27 IgG activity of dissociated immune complexes exceeded that of free serum IgG by a factor of 1000.

CICs from patient No 1 contained antibodies reacting with both the Fc region of rabbit IgG and *pso* p27. We have shown that antigenic determinants

Table 1 *IgG rheumatoid factor activity of alkaline dissociated immune complexes from sera and synovial fluids from patients with AS*

	Patient No						
	1	2	3	4	5	6	7
Serum ICs ($\Delta OD_{492}/\mu g$ IgG)	2.26	0.10	0.05	0.19	0.15	ND*	ND
Synovial fluid ICs ($\Delta OD_{492}/\mu g$ IgG)	1.47	ND	ND	ND	ND	0.06	0.79

The change in optical density (ΔOD) represents the difference between IgG antibodies reacting with IgG and Fab₂ fragments of IgG. The RF activity is expressed as ΔOD per μg IgG of the CIC sample.

*ND=not done.

Table 2 Anti-pso p27 activity of alkaline dissociated immune complexes from patients with AS

Patients	Anti-pso p27/ μ g IgG	Anti-pso p27/ μ g IgM
Serum ICs		
1	4.63	1.80
2	6.75	1.22
3	5.53	1.22
4	2.92	0.87
5	2.42	0.80
Synovial fluid IC		
6	1.81	0.68
1	1.88	1.36
7	7.16	1.75

The figures obtained for IgG antibodies were recorded after four minutes' incubation with o-phenylenediamine (OPD), whereas the figures for IgM antibodies were recorded after 30 minutes' incubation with OPD.

Fig. 1 Serial dilution showing IgG anti-pso p27 activity of serum immune complexes from patient No 1. The figures were recorded after six minutes' incubation with o-phenylenediamine.

Table 3 Immunosorbent chromatography of alkaline dissociated serum CICs from patient No 1 for the absorption of RFs to human IgG coupled to CNBr activated Sepharose 6MB

	RF	Anti-pso p27
Unabsorbed	2.26	4.63
Absorbed with IgG	0.15	1.62

RF and anti-pso p27 activity/ μ g IgG was recorded both for unabsorbed CICs and for CICs absorbed with human IgG.

Table 4 Absorption recorded after the analysis of alkaline dissociated immune complexes diluted 1:2 in the assay for the detection of antigens cross reacting with pso p27

	Patient No						
	1	2	3	4	5	6	7
Serum ICs (OD ₄₉₂)	0.31	0.35	0.27	0.40	ND*	ND	ND
Synovial fluid ICs (OD ₄₉₂)	0.10	ND	ND	ND	ND	0.29	0.08

*ND=not done.

of pso p27 may cross react with Fc (O-J Iversen *et al*, unpublished data). It was therefore of interest to remove the anti-IgG antibodies and study the anti-pso p27 activity of the absorbed sample. Immunosorbent chromatography with human IgG coupled to CNBr activated Sepharose 6MB efficiently removed the rheumatoid factor activity. In the case of patient No 1 this was accompanied by a 65% reduction of the anti-pso p27 activity (Table 3).

DETECTION OF ANTIGENS CROSS REACTING WITH PSO P27

A sandwich ELISA with two sets of monoclonal anti-pso p27 antibodies, one solid phase for capture of antigen and one biotinylated for the detection of bound antigen, was applied as an antigen detection test for pso p27. The immune complex preparations were tested in a dilution of 1:2 after alkaline dissociation. Table 4 shows the activity recorded in the assay for each of the immune complex preparations. The presence of antigens cross reacting with pso p27 was evident in all of the serum immune complex preparations tested and in one of the synovial fluid immune complexes.

Discussion

This paper presents further details of the analysis of CICs from patients with AS. Using immunoblot

analysis, we identified, in a former study, antigens cross reacting with the envelope glycoprotein (gp70) of a psoriasis associated retrovirus-like particle in AS immune complexes.²² Previously, we analysed immune complexes from patients with psoriatic arthritis using ELISA techniques and showed that pso p27 participates in immune complex formation both in psoriatic scales and in CICs.¹⁶

The presence of RF activity, mainly of the IgG class, in sera from patients with AS has been reported, though it appears that such antibodies are either absent or present in relatively small amounts in most patients.²³⁻²⁴ These observations were made using relatively crude techniques. In this report we applied a sensitive isotype-specific ELISA for the identification of RF.²¹ Still, only insignificant amounts of free RF activity were observed in the serum samples or synovial fluids analysed. Some IgG RF activity was detected in CICs, indicating that although the presence of RF is not a prominent feature, RF may participate in immune complex formation in patients with AS.

Free antibodies reacting with pso p27 were also undetectable in any of the serum samples or synovial fluids examined, but extensive amounts were present in all immune complex preparations. The observations correspond to those seen in patients with psoriasis or psoriatic arthritis.¹⁶ The anti-pso p27 activity of the immune complex preparation of patient No 1 was reduced when the immune complexes were absorbed with IgG. In a previous paper we reported that pso p27 shares a common antigenic determinant with the Fc region of IgG. Thus the RF activity of the CICs from patient No 1 could be due to antibodies originally synthesised against an antigen related to pso p27.

In addition to the large amounts of anti-pso p27 antibodies, antigens reacting with monoclonal antibodies against pso p27 were observed in immune complex preparations from all of the serum samples studied, and from one of the synovial fluids. The antigens cross reacting with pso p27 are not IgG as the monoclonal antibodies applied do not react with IgG. Antigens related to pso p27 could not be detected in two of the synovial fluid immune complex preparations. Alkaline dissociated CICs, however, are applied for the detection of antigens cross reacting with pso p27. Thus the binding of antigen to the solid phase monoclonal anti-pso p27 antibodies occurs in the presence of CIC anti-pso p27 antibodies. Depending on the avidity as well as affinity of the CIC antibodies, they may compete with the solid phase monoclonal anti-pso p27 antibodies in the immobilisation of the antigen, and may also block the binding site for the biotinylated 'second' antibodies. This could explain the low

activity observed in two of the synovial fluid immune complex preparations.

The identification of antibodies reacting with pso p27, as well as antigens cross reacting with pso p27 in CICs, shows that antigens related to pso p27 participate in immune complex formation in AS. In psoriasis pso p27 has been observed to participate in immune complex formation both locally in psoriatic scales and in CICs.¹⁶ In an immunofluorescence analysis using antiserum against pso p27¹⁷ we observed deposition of cross-reacting antigens in synovial vessel walls closely resembling immune deposits. This observation further supports the suggestion that antigens related to pso p27 could be important for the elicitation of inflammatory reactions in patients with AS.

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