Antigens related to the major internal protein, p27, of a psoriasis associated retrovirus-like particle are expressed in patients with chronic arthritis

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SUMMARY A rabbit antiserum against the major internal protein, p27, of a psoriasis associated retrovirus-like particle has been applied in an immunofluorescence assay for the detection of antigens cross reacting with p27 in patients with psoriatic arthritis, seronegative rheumatoid arthritis, or ankylosing spondylitis. Antigens reacting with anti-p27 antibodies were present in lymphocytes from blood or synovial fluid from all patients examined. However, the expression was restricted to 0.01–0.1% of the cells. Among the positive p27 cells were cells reacting with markers for T, B, or NK cells. The anti-p27 antibodies also reacted with mononuclear cells in the synovial membrane and with the internal wall of some small or medium sized vessels in sections of synovial biopsy specimens from the patients with chronic arthritis. The reaction with mononuclear synovial membrane cells was restricted to approximately 0.1% of the cells. Blood lymphocytes or synovial sections from healthy persons did not react with the anti-p27 antibodies. The implication of these observations in the pathogenesis of chronic arthritis in man is discussed.

Key words: psoriatic arthritis, rheumatoid arthritis, ankylosing spondylitis, lymphocytes, synovial membrane, immunofluorescence assay.

Chronic inflammatory rheumatic disorders of man are a heterogenous group of disorders where the aetiology has remained obscure. In animals comparable conditions are sometimes observed as a consequence of retrovirus infections.^{1,2}

Recently, we described the isolation of a retrovirus-like particle from a patient with psoriasis.³ The major internal protein, p27, of the particle is expressed by a subpopulation of epidermal cells both in the psoriatic lesion and in clinically uninvolved skin.⁴ p27 is also expressed by approximately 0.1% of peripheral blood lymphocytes in patients with psoriasis.⁵

Psoriasis is complicated by arthritis in approximately 5% of all cases,⁶ the majority presenting with symptoms from peripheral joints. The prevalence of psoriasis among patients with seronegative rheumatoid arthritis (RA) is 9% compared with 1–2% in

population controls.⁷ Spinal involvement is observed in 20% of cases with psoriatic arthritis (PsoA).⁸ Among patients with ankylosing spondylitis (AS) the prevalence of psoriasis is 16%.⁹ Psoriasis, PsoA, uncomplicated seronegative polyarthritis, and AS show an inter-related familial aggregation.⁸

This paper describes the presence of antigens cross reacting with p27 in sections of synovial biopsy specimens and in lymphocytes isolated from peripheral blood or synovial fluid from patients with PsoA, RA, or AS.

Material and methods

p27 ANTIGEN AND ANTI-p27 ANTIBODIES *Purification of p27*

Retrovirus-like particles were isolated from the urine of a patient with extensive psoriasis by sucrose gradient ultracentrifugation. The major internal protein, p27, of the particles was purified by fractionation on a Con A Sepharose column (Pharmacia), immunosorbent chromatography, and gel

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filtration on a Sephacryl S-300 column (Pharmacia) in 6 M guanidine hydrochloride (Sigma) as described previously.^{3, 10} The antigen was purified to homogeneity as judged by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of labelled antigen.⁵

Rabbit antiserum against p27

A hyperimmune antiserum with specificity for p27 was obtained by immunising a rabbit with purified p27 isolated from four litres of urine.⁵

CLINICAL SPECIMENS

Synovial biopsy specimens

Biopsy specimens were obtained from synovectomy specimens from two patients with RA, two patients with PsoA, and one patient with AS. Synovial biopsy specimens were also obtained from two otherwise healthy persons subjected to arthroscopy due to a mechanical disarrangement.

Blood or synovial fluid

Heparinised blood was obtained from five patients with RA, five with PsoA, and five with AS. Heparinised synovial fluid was obtained from two patients with RA, two with PsoA, and two with AS.

Heparinised blood was also collected from five healthy persons without known cases of chronic inflammatory disorders among their first degree relatives.

FLUORESCENCE MICROSCOPY

Preparation of blood or synovial fluid lymphocytes

Lymphocytes were isolated by the one step sodium metrizoate/Ficoll procedure (Lymphoprep, Nye-gaard & Co.), and the cells were washed in phosphate-buffered saline pH 7.2 (PBS). The lymphocytes were adsorbed to coverslips that had been

pretreated with 0.1% poly-L-lysine (Sigma) and fixed in methanol.

Preparation of sections from synovial biopsy specimens

The biopsy specimens were fixed in 96% ethanol at 4°C for 18 h, embedded in paraffin and routinely cut into 2–3 µm sections prepared. The slides were incubated at 56°C for 30 min, deparaffinised in xylene (2×5 min), washed with absolute ethanol, and rehydrated with decreasing concentrations of ethanol in saline.

Immunofluorescence assay

Lymphocytes adsorbed to coverslips or sections of synovial biopsy specimens were incubated with anti-p27 antiserum or preimmune serum diluted 1:60 in PBS at 37°C for 45 min. The binding of rabbit antibodies was detected by fluorescein-conjugated swine antibodies to rabbit immunoglobulin (DAKO) (20 min, 37°C).

Identification of lymphocyte subpopulations

T cells, T_h cells, T_s cells, and NK cells were identified by anti-leu 4, anti-leu 3, anti-leu 2, and anti-leu 11, respectively (Becton Dickinson monoclonal antibodies). B cells were identified by B220 0120 (Behringwerke AG, monoclonal antibodies). Lymphocytes were isolated as described above, incubated with monoclonal antibodies in concentrations recommended by the manufacturers at 20°C for 45 min, and washed with PBS. Texas Red conjugated antimouse immunoglobulins (Amersham) served as second antibodies. The cells were then adsorbed to coverslips, fixed in methanol, and labelled with anti-p27 antibodies and fluorescein conjugated antirabbit immunoglobulins as described above.

Fig. 1 Blood lymphocytes from a patient with AS isolated by the one step sodium metrizoate/Ficoll procedure. The cells were doubly labelled with anti-leu 2 antibodies (left) and anti-p27 antibodies (right) as described above. The arrows indicate a p27 positive cell (×400).

Results

BLOOD AND SYNOVIAL FLUID LYMPHOCYTES
After the incubation of blood lymphocytes from patients with RA, PsoA, or AS with anti-p27 antiserum followed by fluorescein-conjugated anti-rabbit immunoglobulins as second antibodies a bright cytoplasmatic fluorescence was observed in a minority of the cells (Fig. 1). The frequency of cells expressing antigens cross reacting with anti-p27 antibodies was 0.01–0.1% in all cases examined. Anti-p27 antibodies also reacted with synovial fluid lymphocytes (Fig. 2). No reaction was observed with preimmune serum (Fig. 3). Blood lymphocytes from healthy persons did not react with anti-p27 antibodies (frequency <0.01%).

For the identification of the lymphocytes reacting with anti-p27 antibodies a double labelling with monoclonal antibodies against various cell surface

markers was performed. The lymphocytes were incubated with antibodies against markers for T cells, B cells, or NK cells followed by Texas Red conjugated sheep antimouse immunoglobulins before fixation in methanol and subsequent incubation with anti-p27 antiserum and fluorescein-conjugated swine antirabbit immunoglobulins. Markers for either T, B, or NK cells were present on lymphocytes reacting with anti-p27 antibodies (Figs 1 and 2). The frequency of anti-p27 reactive cells was approximately the same for the three cell populations. Among the anti-p27 reactive T cells were cells with markers for T helper as well as T suppressor cells (Fig. 1).

SECTIONS OF SYNOVIAL BIOPSY SPECIMENS
When sections of the synovial biopsy specimens from patients with RA, PsoA, or AS were incubated with anti-p27 antiserum followed by fluorescein-

Fig. 2 Synovial fluid lymphocytes from a patient with AS isolated by the one step sodium metrizoate/Ficoll procedure. The cells were doubly labelled with anti-leu 4 antibodies (left) and anti-p27 antibodies (right) as described above. The arrows indicate a p27 positive cell. ($\times 400$).

Fig. 3 Blood lymphocytes from a patient with AS isolated by the one step sodium metrizoate/Ficoll procedure. The cells were incubated with rabbit preimmune serum. ($\times 400$).

Fig. 4 Section of a synovial biopsy specimen from a patient with seronegative rheumatoid arthritis incubated with anti-p27 antiserum. The arrow indicates vascular fluorescence. Some mononuclear p27 positive cells appear scattered in the synovial tissue. ($\times 400$).

conjugated antirabbit immunoglobulins a bright cytoplasmatic fluorescence was observed in approximately 0.1% of the cells. The reaction was quite similar in all of the biopsy specimens examined. The cells reacting with anti-p27 antibodies were mononuclear and appeared single or in small clusters scattered throughout the synovia (Fig. 4). A bright fluorescence was also observed on the internal surface of vessel walls (Fig. 4). The reaction was restricted to a minority of the vessels, but it was observed both in small and medium sized vessels. No reaction was observed with cells or vessel walls when preimmune serum was applied instead of anti-p27 antiserum. Synovial biopsy specimens from apparently healthy persons did not react with anti-p27 antiserum.

Discussion

In animals, retroviruses constitute a disease potential for the induction of several disorders either of chronic inflammatory or neoplastic character.¹¹ Chronic inflammation is the hallmark of infections with the subfamily lentivirinae,¹¹ which comprise the visna, maedi, and caprine arthritis encephalitis virus (CAEV).¹²

The pathology of the lesions observed after infection with CAEV is very similar to that observed in chronic polyarthritis in humans.¹³ This includes the presence of synovitis with a mononuclear cell infiltrate and hyperplasia of synovial lining cells. There are also skin changes (emaciation and exfoliation) and an increased susceptibility for the deposition of amyloid in various organs.

The isolation and characterisation of a retrovirus-like particle from patients with psoriasis has been

described previously.^{3 10} The major internal protein, p27, of the particle is expressed in epidermal cells in psoriatic lesions and in blood lymphocytes from patients with psoriasis.^{4 5} We have also shown the presence of p27 antigen and anti-p27 antibodies in immune complexes from patients with PsoA.¹

In this study we have used anti-p27 antibodies as a probe to examine specimens from patients with chronic arthritis for the presence of antigens cross reacting with p27.

The anti-p27 antibodies reacted with lymphocytes and synovial mononuclear cells from patients with PsoA, RA, and AS. The reaction was restricted to a minority of cells, an observation also encountered in psoriasis.^{4 5} In infections with retroviruses a characteristic feature is the restricted expression of virus antigens. In visna expression of p30 is observed only in 0.03% of the cells.¹⁵ A frequency of the same order of magnitude was observed for cells reacting with anti-p27 antibodies in our patients with chronic arthritis. This low but persistent expression of antigen could provide the stimulus for chronic inflammation. The expression of viruses in lymphocytes is frequently observed in infections with the lentivirinae.¹⁶ The lymphotrophism of pathologic retroviruses in humans, the HTLV I, II, and III, is well reported.¹⁷

The reaction of anti-p27 antiserum was restricted to cells, but in sections of synovial tissue a bright fluorescence was observed on the internal wall of some vessels. Similar deposits have also been observed in the dermis of psoriatic lesions.⁴ The vascular reaction in psoriatic skin is probably due to the deposition of immune complexes but could also be explained by a local expression of antigen.^{4 14} In RA others have shown the presence of immunoglobulins,¹⁸ complement factors,¹⁸ and virus-like particles¹⁹ in synovial vessel walls. These observations indicate that the reaction of anti-p27 antibodies with synovial vessel walls could be explained correspondingly to the reaction with psoriatic dermal vessels.

The properties of the psoriasis associated retrovirus-like particle have been described in detail elsewhere.^{3 10} Despite several attempts, we have not been able to show the presence of RNA dependent DNA polymerase or high molecular weight polyadenylated RNA in preparations containing purified particles from patients with psoriasis. Thus there is no evidence for an infectious particle.

Both complete and incomplete genomes of retroviruses are present as endogenous genetic elements in nearly all species, including humans.^{21 22} A pathogenetic role of endogenous retrovirus antigens has been reported both in birds and in inbred strains of mice.^{23 24} Whether the psoriasis associated par-

ticle is of endogenous or exogenous origin remains to be clarified. However, this topic and the possible association of the antigens cross reacting with anti-p27 antiserum in patients with RA or AS with a particle structure is currently being investigated in our laboratory.

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