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Antibodies to Retrovirus Proteins in Scleroderma

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In 8 out of 29 patients with scleroderma we found antibodies to HIV retroviral proteins in the Western blot analysis. The sera reacted only to one or two of the following bands: p 18, p 24, p 55, p 65 in relatively weak grades. There were no evident clinical correlations with the reactivity of certain bands, nor signs of direct HIV infection in our patients. Apart from 3 cases with positive CMV reactivity (IgM), there was no cross-reactivity to HTLV I or EBV (IgM) and to topoisomerase (Scl 70) or other autoantibodies to various nuclear antigens related to scleroderma. It is not clear whether retroviruses are involved in the pathogenesis of scleroderma or whether these antibodies are due to molecular mimicry.

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In 22 of 61 (36%) patients suffering from systemic lupus erythematosus, serum antibodies to the p 24^{gag} protein of human immunodeficiency virus type I (HIV-1) have been described (1), as was also the case with 14 of 47 (30%) patients with Sjögren's syndrome (2). Sera did not react with envelope proteins of HIV-1, nor with any proteins of human T-lymphotropic virus type I (HTLV-1). The Sjögren's syndrome patients were Ro and La negative.

These findings encourage us to look for serum antibodies to HIV proteins in patients with systemic sclerosis (scleroderma) by Western blot analysis, since the involvement of virus factors has been speculated on in the pathogenetic concepts of chronic connective tissue diseases for many years (3).

MATERIAL AND METHODS

Patients

Twenty-nine patients suffering from systemic sclerosis were enrolled into this study. They fulfilled the ARA criteria and were differentiated according to the classification of the Arbeitsgemeinschaft for Dermatological Research (4). Four patients suffered from the diffuse type III (generalized form), 14 from the extremity ascending type II (intermediate form) and 11 from the acrosclerotic type I (limited form). There was no overlap to Sjögren's syndrome. As controls 120 healthy blood donors, 31 patients with psoriasis and 37 patients with atopic dermatitis were studied.

Methods

The following laboratory tests were used: human immunodeficiency virus (HIV), Western blot analysis (BIO-RAD, Munich), cytomegalovirus (CMV; Enzygnost, Behring), Ebstein-Barr-virus (EBV; Fresenius) and human T-lymphocyte virus-1 (HTLV-1; Fujirebio).

Pattern and titers of antinuclear factors (ANA) were determined by indirect immunofluorescence technique using Hp-2 cells as substrate.

Anticentromere antibodies (ACA) were detected in the same way. A clear fluorescent staining with a discernible nuclear pattern at serum dilution of 1:40 was considered to be positive.

The Scleroderma 70 (Scl-70) antibody (antitopoisomerase) was detected by immunodiffusion according to the method of Tan et al. (5).

RESULTS

Our results are listed in Table I. Eight out of 29 (28%) scleroderma sera reacted to one of two of the HIV protein bands: 2 to p 18 (inner membrane), one to p 24^{gag}, one to p 24^{gag} and p 55^{gag} simultaneously, 3 to 55^{gag} and one to p 65 (reserve transcriptase). The staining intensity of the antibodies was weak but graded as 2 or higher.

In addition, the sera with non-specific reactions in the HIV-1 Western blot were examined for the presence of antibodies against CMV, EBV and HTLV-1. In patients No. 4, 5 and 7, in whose sera the p 18-lane and p 65-lane in HIV-1 Western blot were detectable, respectively, we found a recent CMV infection. In none of the 8 investigated samples a cross-reactivity with HTLV-1 took place. There were no correlations to the type of autoantibodies such as Scl 70, ACA or ANA, nor to the clinical form of scleroderma or to the extent of the organ involvement. According to the classification of the Arbeitsgemeinschaft for Dermatological Research (4), 1 patient suffered from the diffuse type III (generalized form), 4 from the extremity ascending type II (intermediate form) and 3 from the acrosclerotic type I (limited form). There was no overlap to Sjögren's syndrome. The autoantibodies exhibited by the remaining 21 scleroderma patients with negative HIV-1 Western blot are presented in Table II. Contrary to the 28% non-specific reactions in scleroderma patients, a control group of healthy HIV-low-risk-population (n = 120) with negative HIV-1,2-EIA (Behring) exhibited only 3% non-specific reactions in the HIV-1 Western blot. This was also the case with patients suffering from psoriasis (n = 31; 2%) and atopic dermatitis (n = 37; 3%). Thus, the 28% non-specific reactions in scleroderma patients differ significantly.

DISCUSSION

The importance of these findings is uncertain, the interpretation difficult. The false-positive detection rate of HIV-1 antibodies among healthy population with a low HIV-1 prevalence also depends on the Western blot test system used. Midthun et al. (6) examined healthy adult volunteers with Biotech/DuPont Western blot and found more than 30% false-positive results with poor reproducibility. Therefore this test system could not be recommended. In our studies, in agreement with other authors (7), the rate of non-specific reactions was less than 3%. The test system we used proved to be more specific.

Clearly there are no signs of direct HIV infection in scleroderma patients in general, and no clinical features resembling it in our patients in particular. The CD_4/CD_8 ratio was within

Table I. *Clinical and laboratory findings in scleroderma patients with antibodies to retroviral proteins*

Pat No.	Age	Sex	Type of sclero-derma	Organ involve-ment	Autoanti-bodies	Antibodies to retroviral proteins (Western blot)	CMV/IgM		EBV/IgM	HTLV I
							EIA	IF	IF	
1	60 Y	♂	II	Pulmo	ANA, speckled 1:1280 RNP ACA	p 55	neg.	neg.	neg.	neg.
2	54 Y	♀	I	Esophagus Pulmo	ANA 1:10240 Scl 70	p 55	neg.	neg.	neg.	neg.
3	53 Y	♀	I	Joints	ANA 1:5120 Scl 70	p 24, 55	neg.	neg.	neg.	neg.
4	52 Y	♀	II	Esophagus	ANA 1:5120 Scl 70	p 18	pos.	≥1:10 pos.	neg.	neg.
5	43 Y	♂	II	Joints	ANA 1:2560 ACA	p 18	+/−pos.	neg.	neg.	neg.
6	27 Y	♂	III	–	ANA 1:640	p 55	neg.	neg.	neg.	neg.
7	64 Y	♂	I	Pulmo, Esophagus	ANA 1:1280 ACA	p 65	pos.	neg.	neg.	neg.
8	41 Y	♂	II	Pulmo, Esophagus	Scl 70 1:800	p 24	neg.	neg.	neg.	neg.

normal ranges and the IL 2 receptor levels were increased in the sera of our patients (8, 9). In addition, we found signs of activated T-lymphocytes, as CD 25 (IL-2 receptor), CD 71 (transferrin-receptor) and HLA-DR Ia 1 were increased in peripheral blood lymphocytes (10).

In general, a polyclonal stimulation of B-lymphocytes responsible for the synthesis of a wide range of autoantibodies has been discussed in scleroderma. Such an antigen-independent polyclonal B-cell activation may also account for the antibodies to retroviral proteins. For instance, HIV-1 seronegative individuals are able to produce antibodies to HIV-1 in vitro after pokeweed mitogen stimulation of the peripheral blood mononuclear cells (11). However, the antibodies produced in vitro only reacted with the envelope glycoproteins gp 160 and gp 120, apart from p 66 of HIV-1.

One probable explanation for the occurrence of antibodies to viral proteins is "molecular mimicry". In diffuse scleroderma, antibodies that bind to a synthetic peptide corresponding to a common sequence found in DNA topoisomerase I and several mammalian p 30^{gag} proteins (12) have been described. This speaks in favour of the antigenic cross-reactivity of these peptide-binding antibodies. In the present study we could not

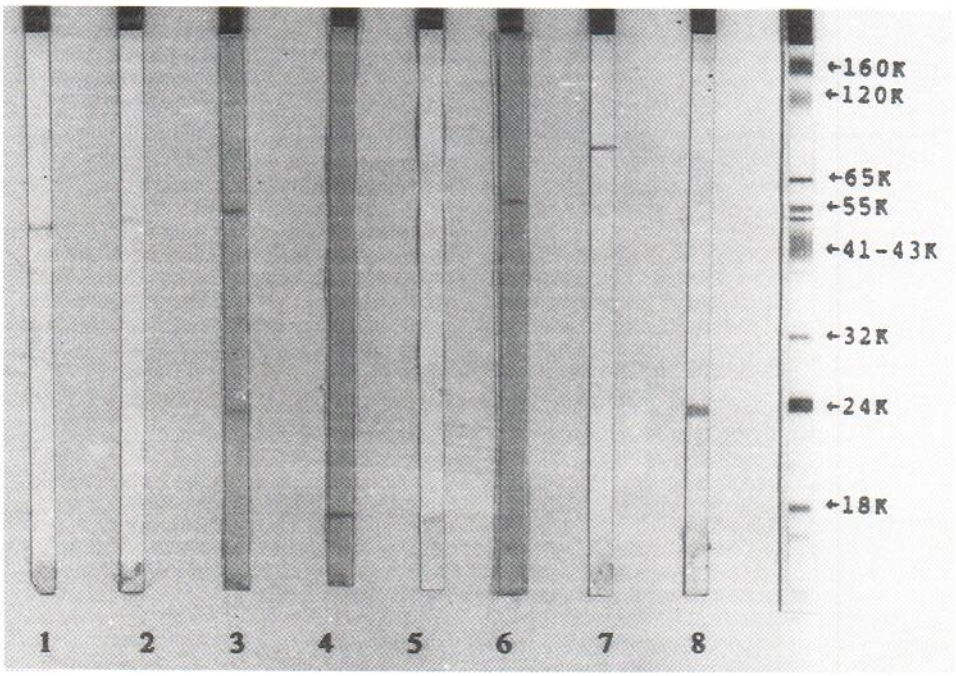

Fig. 1. HIV Western blot analysis in 8 scleroderma patients.

Table II. *Autoantibodies in scleroderma patients with negative HIV Western blot*

	n	ANA	Scl. 70	ACA
Diffuse type III	3	3	3	–
Extremity ascending type II	11	11	6	4
Acrosclerotic type I	7	5	2	3
Total	21	19	11	7

see a close correlation between antibodies to retroviral proteins and antibodies to nuclear antigens and topoisomerase. It is still unknown whether antibodies to HIV-1 proteins cross-react with DNA topoisomerase or other autoantibodies related to scleroderma. Studies with synthetic peptides have shown that the antigag antibody in autoimmune diseases is directed toward a peptide epitope (12, 13). Epitope mapping of the HIV-1 proteins is necessary to clarify the question of molecular mimicry.

The antibody directed against the reverse transcriptase is of particular interest and may also suggest a prolonged latent retrovirus infection in these patients. On the other hand, the relation to prooncogens must be clarified in the future.

Last but not least, in a subset of autoimmune patients seropositive for anti-RNP antibodies the reported cross-reactivity with M1 protein of influenza B virus should be mentioned (14). The human anti-p 68 autoantibodies recognized a common epitope of U_1 RNA containing small nuclear ribonucleoprotein and influenza B viruses (14).

Although the sporadic occurrence and the absence of small endemies speak against an infectious etiology, a slow virus infection or a latent retrovirus infection should still be considered in scleroderma as well as in other autoimmune diseases. The evaluation of the role of retroviral proteins in the modulation of the immune system, in the stimulation of autoantibody production and in the pathogenetic events of autoimmune diseases and scleroderma needs further research.

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