

Autoantibodies and human immunodeficiency viruses infection: A case-control study

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ABSTRACT

Objective. To determine the prevalence of organ-specific and non-specific autoantibodies in HIV-infected patients.

Design. A multicentric collaborative case-control study including 105 HIV patients and 100 sex- and age-matched HIV-negative healthy volunteers.

Methods. Antinuclear, anti-ds DNA, anti-histone, anti-Sm, rheumatoid factor (IgM), anti- β_2 glycoprotein 1, anti-neutrophil cytoplasmic, anti-LKM1, anti-LCA1, anti-gastric parietal cell, antiplatelet, anti-intermediate filament, anti-mitotic spindle apparatus, anti-Golgi, anti-ribosome and anti-thyroid autoantibodies were screened in six European laboratories.

Results. Only IgG and IgM anticardiolipin, IgG antiplatelet, anti-smooth muscle and anti-thyroglobulin antibodies were statistically more frequent in HIV patients. There was no correlation with the numbers of CD4+ cells except in the case of anti-smooth muscle antibodies. We were unable to find specific autoantibodies such as anti-ds DNA, anti-Sm, AMA, anti-LKM1, anti-LCA1 or anti- β_2 GPI antibodies in these patients.

Conclusions. Our results indicate that the autoantibody profile of HIV infections is comparable to those of other chronic viral infections. HIV does not seem to be more autoimmunogenic than other viruses.

Introduction

The presence of specific autoantibodies in HIV infected patients is a subject of controversy which can lead to misdiagnosis (1, 2). In order to define the existence and extent of autoimmunisation in HIV infection, six European laboratories, members of the GEAI (Groupe d'Etude de l'Autoimmunité), examined the frequencies in these patients of the most common autoantibodies found in organ-specific and non-specific autoimmune diseases in a case-control study.

Patients and methods

A total of 105 HIV seropositive patients were included in the study, 62 women and 43 men of mean age 38 years (range 22-56). Numbers of CD4+

T lymphocytes were determined by flow cytometry according to the recommendations of the Centre for Disease Control. The patients were divided into 3 groups as follows: less than 350 CD4+ cells/ml: 67.5% (72/105); 350-500: 21.5% (22/105); and > 500: 11% (11/105). Controls were 100 sex- and age-matched HIV negative individuals, 58 women and 42 men with a mean age of 39 years (range 21-62).

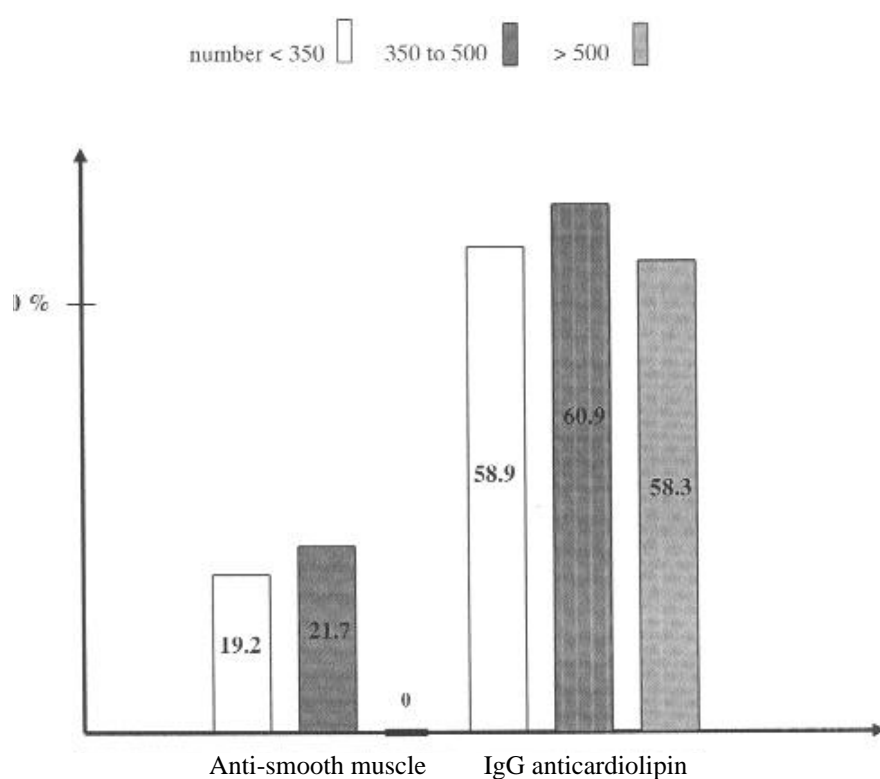
The following autoantibodies were investigated by indirect immunofluorescence on Hep2 slides (Kallestad, Biorad, Marnes la Coquette, France) using a starting serum dilution of 1/64: antinuclear (ANA), anti-ribosome, anti-mitochondrial (AMA), anti-Golgi complex, anti-intermediate filament (IF) and anti-mitotic spindle apparatus (MSA). The diluted sera (1/64) were also screened by indirect immunofluorescence on rat organ sections (liver, kidney and stomach, Biorad) to detect anti-smooth muscle, anti-mitochondrial, anti-liver kidney microsome type 1 (LKM 1), anti-liver cytosol antigen 1 (LCA1), anti-ribosome and anti-gastric parietal cell autoantibodies. Anti-double-stranded DNA, anti-Sm, anticardiolipin (IgG and IgM) and anti-histone autoantibodies were screened by ELISA (Biorad). The cut-off values fixed by the manufacturer were respectively: 30 UI/ml, 4 U/ml, 10 UGPL/ml, 10 UMPL/ml and 5 U/ml. Antithyroid autoantibodies by antithyroglobulin and antithyroperoxidase ELISA (Bio Advance kit, Emerainville, France) were detected within cut-off values of 100 UI/ml.

Antibodies against autologous human IgG rheumatoid factor and β_2 glycoprotein 1 (β_2 GPI) were detected by ELISA (the cut-off value was OD < 0 and < 12, respectively) and antineutrophil cytoplasmic autoantibodies (ANCA) by indirect immunofluorescence on human neutrophils, using procedures developed in the participating laboratories. Serum immunoglobulin binding to platelets was investigated by flow cytometry using fluorescein isothiocyanate (FITC) conjugated Fab'2 antibodies against human IgG or IgM (Dako, France). According to the 95th percentile test, the cut-off value was

Table I. Autoantibodies in seropositive subjects and controls.

Auto Ab	HIV	Control (Chi 2)	Auto Ab	HIV	Control (Chi 2)
ANA	6.9%	3% (3.57)	Thyro.	11.4%	3% (5.35)
dsDNA	0.5%	0% (1.45)	TPO	0%	1% (1.54)
histones	6.3%	4% (1.02)	₂ Gpl	1.9%	2% (0)
Sm	0%	0%	Mito.	0%	0%
Cardio IgG	64.1%	5% (95.02)	LKM1	0%	0%
Cardio IgM	17.5%	5% (9)	GPC	1.9%	1% (0.28)
SM	18.5%	8% (3.99)	Plat. IgG	21.4%	0% (12.69)
ANCA	2.09%	0% (2.92)	IF	5.8%	3% (1.1)
RF IgM	0.9%	1% (0)	MS	3.8%	1% (2.5)
RF IgA	2.8%	0% (2.8)	Golgi	6.3%	1% (3.6)

ANAb: antinuclear antibodies; dsDNA: double stranded DNA; Cardio: cardiolipin; SM: smooth muscle; ANCA: antineutrophil cytoplasm antibodies; RF: rheumatoid factor; Thyro: thyroglobulin; TPO: thyroperoxydase; Mito: mitochondria; LKM: liver-kidney microsome; GPC: gastric parietal cell; Plat.: platelet; IF: intermediate filament; MS: mitotic spindle; Golgi: Golgi complex.

**Fig. 1.** Proportion of anti-smooth muscle and IgG anticardiolipin in the different CD4+ groups.

fixed at 1850 arbitrary units (antibody binding capacity).

The autoantibody frequencies in HIV-positive patients were compared to those in controls by the Pearson 2 x 2² test and a p value of < 0.05 was considered to be statistically significantly significant (Table I).

Results

Only IgG and IgM anticardiolipin, anti-smooth muscle, antithyroglobulin and

IgG antiplatelet autoantibodies appeared to be statistically more frequent in HIV seropositive patients than in controls. The value obtained were also elevated and not borderline. There was no correlation with the number of CD4+ cells, except in the case of anti-smooth muscle antibodies which were not detectable in the group with >500 CD4+ cells/ml (Fig. 1). ANCA, IgA anti-rheumatoid factor, anti-Golgi complex and anti-gastric parietal cell auto-anti-

bodies seemed to be a little more common in HIV patients, but this difference was not statistically significant. Anti-thyroglobulin antibodies and serum immunoglobulin binding to platelets were more often found in women.

Discussion

The present results are in agreement with previous studies concerning anti-smooth muscle, anticardiolipin IgG and antiplatelets autoantibodies in HIV infection (3-7). Several different authors have reported a higher incidence of anticardiolipin IgG antibodies in HIV patients (4, 8, 9), whereas anti-2 glycoprotein 1 antibodies were no more frequent than in control groups. Thus, the anticardiolipin antibodies found in HIV infection could be related to those observed in syphilis, but not to those described in systemic lupus erythematosus or primary phospholipid syndrome (9). There is no correlation between anticardiolipin antibodies and thrombosis. The abnormal presence of IgG binding to platelets in 21.4% of HIV positive serum samples also agrees with previous reports (5), as does the lack of correlation between this finding and thrombocytopenia. Finally, the anti-mitochondrial and anti-LKM1 antibodies usually found in hepatopathy were not present in the serum of HIV positive patients in any study including ours (3). None of these autoantibodies found in seropositive patient could be related to clinical complication such as thrombosis hypothyroid or thrombocytopenia.

On the other hand, our results reveal some discrepancies with previous work. Muller *et al.* (1), using ELISA methods, observed a high frequency of antibodies directed against nuclear antigens, particularly double-stranded (ds) DNA. No anti-ds DNA antibodies could be found in our study, or by Lafeuillade *et al.* (2) or Viard *et al.* (10) using various detection methods including ELISA. The results of Muller *et al.* could have been related to antibodies against single-stranded (SS) DNA, if the DNA they used contained large amounts of ss DNA. Antibodies against soluble nuclear antigens (Sm, RNP and SS-A) were likewise more often present only in the study of Muller *et al.*, but their use of small peptides instead of whole antigens might explain this discrepancy. Since the spatial configuration of a small peptide bound to a polystyrene microtiter plate is completely modified with respect to its configuration in the parent molecule, the antibodies detected could in fact be directed against epitopes absent from the native antigen. Despite the absence of anti-ds DNA antibodies, Viard *et al.* (10) observed anti-nucleosome and anti-histone antibodies in HIV infected patients. In our

patients, we found no anti-histone antibodies and did not perform anti-nucleosome analyses.

In conclusion, the autoantibodies we found to occur more frequently in HIV positive serum (anti-smooth muscle and anticardiolipin antibodies) were those usually observed in chronic viral infection (11-13). Moreover, HIV does not seem to be more autoimmunogenic than other viruses with regard to the development of common autoantibodies.

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