**Lupus (2006) 15,** 865–872
www.lupus-journal.com

PAPER

# Targeting human immunodeficiency virus type 1 with antibodies derived from patients with connective tissue disease

M Scherl[1a], U Posch[1a], G Obermoser[2], C Ammann[1], N Sepp[2], H Ulmer[3], MP Dierich[1], H Stoiber[1] and B Falkensammer[1]*
[1]Department of Hygiene, Microbiology and Social Medicine, Innsbruck Medical University, Austria;
[2]Department of Dermatology and Venerology, Innsbruck Medical University, Austria; and
[3]Institute for Biostatisics, Innsbruck Medical University, Austria; [a]MS and UP contributed equally to the work.

During the budding process, human immunodeficiency virus (HIV) acquires several cellular proteins from the host. Thus, antibodies against self antigens found in sera patients with autoimmune disorders may cross react with host-derived or the HIV-specific proteins gp120 and gp41 on the viral envelope and probably neutralize HIV infection. To verify this hypothesis, 88 sera from HIV negative patients suffering from systemic lupus erythematosus (SLE) and other autoimmune disorders were analysed for cross reacting antibodies against HIV-1 by Western blot and FACS analysis indicating that antibodies cross-react with epitopes expressed on HIV infected or non-infected cells. Virus capture assays revealed that HIV-$1_{IIIB}$ was directly recognized by 60% of sera from patients with autoimmune disorders. Sera were also tested in HIV neutralization assays with stimulated T cells. Reduction of the viral load by patient sera correlated with their reactivity in Western blot analysis. Complement further enhanced the reduction of viral titres, although no complement-mediated lysis was observed. These data suggest a possible protective role of auto-antibodies against HIV infection in lupus patients. *Lupus* (2006) **15**, 865–872.

**Key words:** auto-antibodies; complement; human immunodeficiency virus; neutralization; systemic lupus eythematosus

## Introduction

In systemic lupus erythematosus (SLE) both cellular and humoral[1] abnormalities in the immune system have been identified. Current concepts on the cellular basis of autoimmunity include deficit in the active process of immunregulation, participation of oligoclonal (restricted) T-cell populations, selection or exclusion of parts of T-cell receptor repertoire that result in the expression of 'forbidden' autoreactive T-cell receptor genes, genetic factors or defects in apoptosis.[2] Increased generalized B-cell activation and the production of auto-antibodies are a hallmark in SLE patients.[3,4] Auto-antibodies can target epitopes on cell membranes and as a consequence activate the complement system inducing cell lysis. In addition, serum rheumatoid factor, auto-antibodies targeting immunoglobulines, and circulating immune complexes (CIC) can be detected.

*Correspondence: Barbara Falkensammer, Department of Hygiene, Microbiology and Social Medicine, Innsbruck Medical University, Fritz-Pregl-Str. 3, A-6020 Innsbruck, Austria. E-mail: barbara.falkensammer@i-med.ac.at
Received 27 February 2006; accepted 16 August 2006

The coexistence of SLE and infection with the human immunodeficiency virus (HIV) is very unusual. Since the first report in 1988 of five patients with AIDS and SLE,[5] 34 cases have been published.[6–28] Taken into account the prevalence of the two diseases and assuming they are independent phenomena and not mutually exclusive, it has been estimated that at least 400 patients should be prevalent with both diseases in the USA alone.[29] However only 20 cases were reported in the USA in 2000.[30]

The aim of this study was to investigate the presence of HIV-antibodies in HIV negative SLE patients and to study the functional significance of these antibodies in virus capture and neutralizing assays. Sera from patients with other connective tissue disorders, from HIV-infected individuals and healthy volunteers were used as controls.

## Material and methods

### *Cells, culture medium, HIV and antibodies*

The human T-lymphoblastoid cell line M8166, and the premonocytic line U937 were distributed by the


10.1177/0961203306071405

American Type Culture Collection (Rockville, Maryland, USA). $HIV_{IIIB}$ and $HIV_{Bal}$ were obtained through the Medical Research Council (MRC) AIDS Reagent Project (Herts, UK). FITC-conjugated and peroxidase-labelled antibodies were purchased by Dako (Glostrup, Denmark) as well as unlabeled anti-CD14, anti CD-19, anti-HLA-DR and anti-HLA-A, B, C. DV012-anti-gp120, 2G12-anti-gp120, 2F5-anti-gp41 and 1H5-anti-gp41 were kindly provided by Katinger H (IAM, Vienna).

### *Patient population*

In total 88 blinded sera from 72 patients with autoimmune disorders were investigated (Table 1). These were consecutive patients of the outpatient lupus clinic of the Department of Dermatology of Innsbruck Medical University. All patients gave informed and written consent prior to study enrolment. Sera of 72 individuals had been taken from patients with diagnosed SLE, discoid lupus erythematosus (DLE), subacute cutane lupus erythematosus (SCLE), primary antiphospholipid syndrome (APS) and the mixed connective tissue diseases (MCTD). Other autoimmune disorders such as scleroderma or polymyositis accounted for 15 additional sera (Table 1). None of the patients had clinical signs of neurolupus or needed hemodialysis. Renal failure with serum creatinine values below 3 mg% was detected in one patient. In this study 81.9% of all patients were female and with a mean age of 42.7 years.

**Table 1** Baseline characteristics of 72 patients with systemic lupus erythematosus and other autoimmune disorders

| | | *Normal range* |
|---|---|---|
| Age (years) [mean (range)] | 42.7 (18–70) | |
| Female sex (%) | 81.9 | |
| Type of autoimmune disorder | | |
| • SLE (%) | 39 (54.2) | |
| • DLE (%) | 8 (11.1) | |
| • SCLE (%) | 2 (2.8) | |
| • Primary APS (%) | 6 (8.3) | |
| • MCTD (%) | 5 (6.9) | |
| • Scleroderma | 4 (5.6) | |
| • Other autoimmune disorders | 8 (11.1) | |
| Diagnosed since (years) [mean (range)] | 6.9 (1–24) | |
| Laboratory values | | |
| • C3 mg/dL [mean (range)] | 102.9 (30–206) | 84–193 mg/dL |
| • C4 mg/dL [mean (range)] | 17.8 (1.9–42.6) | 10–40 mg/dL |
| • CH-100 U/mL [mean (range)] | 82.1 (16–155) | 70–110 U/mL |
| • CIC μg/mL [mean (range)] | 60.3 (25–234) | <54 μg/mL |
| • Neopterin nmol/L [mean (range)] | 14.1 (4–48.3) | 0–10 nmol/L |
| • Soluble interleukin 2 receptor ng/mL [mean (range)] | 3.6 (0.3–16.7) | 0.0–4.8 ng/mL |
| • β2 microglobuline mg/L [mean (range)] | 2.3 (0.9–9.5) | 0.7–1.9 mg/L |

As controls, 23 sera from healthy individuals (NHS) were included. In some assays a pool of 10 sera from HIV-infected individuals was used as positive control.

Complement and immune activation parameters were determined in all patients with autoimmune disorders according to the manufacturers' instructions: CH100 and circulating immune complexes (CIC) (Imtec, Immundiagnostica Gmbh, Berlin, Germany), complement factor 3 (C3) and complement factor 4 (C4) (Dade Behring Marburg GmbH, Marburg, Germany), soluble interleukin 2 receptor (NIH, Bethesda, Maryland), serum neopterin (Henning, Berlin, Germany) and serum β2-microglobuline (β2-MG) (Pharmacia, Uppsala, Sweden) were measured by radioimmunoassays using standards provided by the manufacturer.[31] Normal values ascertained in healthy individuals are listed in Table 1.

For further characterisation of patients' sera total antinuclear antibodies (ANAs) (Bio-Rad, Herts, UK), extractable nuclear antibodies (ENAs) with anti-Smith- (Sm), anti-Sjögren-Syndrom-A- (SS-A), anti-Sjögren-Syndrom-B- (SS-B), anti-ribonuclear protein (RNP)- and anti-Scleroderma 70- (Scl70) antibodies were determined using an enzyme linked immunosorbent assay by Phadia Austria (Vienna, Austria).

### *Western blot analysis*

Lysates of $HIV_{IIIB}$-infected M8166 cells were blotted on Nitrocellulose and incubated with patient sera or sera from healthy volunteers (NHS; all sera 1:50 diluted in PBS, 0.1% skim milk) for two hours. After washing steps, bound patient IgG was visualized by peroxidase-labelled anti-human IgG (1:1000 in PBS, 0.1% skim milk) and diaminobenzidine/$H_2O_2$. As positive control, a pool of 10 HIV-infected serum samples was used.

### *Reactivity of patient sera against HIV-infected and non-infected cells*

Binding of IgG to infected or non-infected M8166 and U937 cells was determined by FACS analysis. For this, $5 \times 10^5$ cells were washed in PBS, supplemented with 3% bovine serum albumin (BSA) and 0.1% $NaN_3$ (FACS-buffer) and exposed to heat-inactivated patient sera (1:50 in FACS-buffer) for 30 minutes at 4°C. Antibodies 2F5, 2G12 and a pool of HIV positive sera were used as positive controls as well as NHS as negative control. Cell bound IgG was assessed by FITC-conjugated rabbit-anti-human IgG (1:100 in FACS buffer) on formalin fixed cells. Specific median fluorescence intensities (MFIs) of a representative experiment are given. These experiments were done in duplicates.

*Virus capture assay*

The virus capture assay (VCA) was performed as described previously.[32] In brief, microtiter plates (Greiner, Kremsmünster, Austria) were coated with monoclonal anti-human IgG (Dako, Glostrup, Denmark) at a concentration of 2 μg/mL in 0.1 M $NaHCO_3$, pH 9.5. The plates were washed, blocked with 1% skim milk (Difco, Kansas City, USA) in PBS and incubated with the patient samples and sera from healthy donors (1:50 in 0.1% skim milk) for two hours at room temperature. The plates were then washed three times and incubated at 4°C with virus (600 pg/mL p24 antigen) purified by ultracentrifugation on a sucrose gradient. The plates were washed five times with PBS and bound virus was lysed with 120 μL PBS supplemented with 1% Nonidet P-40 (NP40) and 2% BSA. Obtained supernatant were transferred to a p24 ELISA to determine whether HIV bound to SLE specific IgG.

*Monitoring viral replication*

$HIV_{IIIB}$ replication in M8166 cells was determined by ELISA. For this, a double monoclonal antibody sandwich ELISA (developed at the Institute of Applied Microbiology, Vienna, Austria) was used to determine the amount of HIV-1 p24 core protein (p24 ELISA) in supernants from the HIV neutralization assays.[33]

*HIV neutralization assay*

To determine neutralizing activity of serum antibodies for potential inhibition of $HIV_{IIIB}$ infection, heat inactivated sera from patients were diluted serially in cell culture medium, starting from 1:125 in a 96-well plate (Greiner, Kremsmünster, Austria) to neutralize a fixed concentration (1: 5000 final dilution) of a $HIV_{IIIB}$ virus-stock containing 0.7 μg/mL of p24 antigen. The viral stock used for infection has been prepared as a cell-free supernatant of infected M8166 cells. The virus-antiserum mixture was added to $2.5 \times 10^4$ M8166 cells in a total volume of 200 μL per well. Experiments were performed in triplicates, and the average values of three independent experiments are given. Indicated samples were incubated with NHS (1:10 in RPMI-1640) as a source of complement. As a negative control a pool of heat inactivated sera from healthy donors were included (iNHS), as a positive control the HIV-specific neutralizing monoclonal antibody 2F5 was used. To monitor virus production, cell culture supernatants were harvested on day 3 post-infection. The relative amount of $HIV_{IIIB}$ replication in the presence of different sera was determined by a HIV p24 antigen assay.

*Detection of complement-mediated virolysis*

Complement-mediated lysis was determined as described previously.[34] In brief, virus was loaded with antibodies (1:25 in RPMI 1690) and ultra-centrifuged (Beckman L-60 Ultracentrifuge; $50.000 \times \boldsymbol{g}$ SW 41 Ti rotor, one hour) to remove unbound antibodies. Virus antibody complexes were resuspended and aliquots were incubated with 10% (v/v) pooled NHS for one hour at 37°C in RPMI. Lysis of the samples was quantified by determining the amount of the intraviral protein p24 ELISA, which is released in the supernatant upon complement-mediated destruction of the viral envelope integrity. To avoid complement activation in control samples, 10 mM EDTA was added in parallel probes. Total lysis was achieved by the addition of 1% (v/v) Triton X-100 in RPMI, while background (free p24 by 'spontaneous lysis') was determined in the presence of RPMI alone. Virolysis was calculated by the following formula:

$$\text{Virolysis } (\%) = \frac{(p24_{\text{sample}} - p24_{\text{background}})}{(p24_{\text{TX100}} - p24_{\text{background}})} /$$

*Determination of $TCID_{50}$ and $IC_{50}$*

The $TCID_{50}$ (reciprocal of viral dilutions that causes infection of 50% of samples to be infected) and the $IC_{50}$ (antibody concentration which causes 50% inhibition of viral replication) were calculated using IC-50 software, freely available from the National Center for Biotechnology Information (National Library of Medicine, NIH, author JL Spouge).

*Statistical analysis*

Mann–Whitney *U*-test and the Fischer's exact test were used as appropriate. *P*-values smaller than 0.05 were considered to indicate statistical significance. All analyses were performed with SPSS 11.0 (SPSS Inc., Chicago, 2003).

## Results

*Complement, immune activation parameters and auto-antibodies*

In 72 patients with autoimmune disorders complement- (C3, C4, CH100), CIC- (IgG, A, M) and immune activation (Neopterin, soluble interleukin 2 receptor, $\beta_2$-MG) parameters were investigated (Table 1). In 35 of 88 sera reduced complement values were detectable. In the study group eight patients had clearly elevated immune activation parameters like elevated serum neopterin and confirming the clinical signs of an active stage of disease during blood

sampling. In 15 sera one, in 16 sera two and in four sera all three complement parameter were reduced (data not shown). Mean CIC, Neopterin and $\beta_2$-MG results were elevated compared to values achieved by healthy donors.

In 79.5% of patients with connective tissue disease ANAs were detected. ENAs were observed in 52.4%. Anti-Sm antibodies were measured in 27.3%, anti-SS-A in 63.6%, anti-SS-B in 31.8%, anti-RNP in 31.8% and anti-Scl70 in 9.1%.

*Western blot analysis*

In total, 88 patient samples and 23 NHS obtained from healthy volunteers were tested by Western blot analysis for their reactivity to lysates from $HIV_{IIIB}$-infected M8166 cells. According to the pattern obtained in this assay, sera were categorized into four groups (Table 2). SLE sera, which recognized lysates from $HIV_{IIIB}$-infected M8166 cells are referred to as group 1 (0 up to three bands), while sera which recognized four to six proteins in the Western blot were combined in group 2. Group 3 consisted of sera, which gave seven to nine bands, group 4 had more than nine. The patterns of sera from five patients were not clearly defined (high background) and therefore excluded from further analysis. The 83 remaining patient samples were compared to the reaction of sera from healthy controls. From the 23 healthy donors tested, 17 were negative, while five sera recognized one band and one of the sera reacted with two proteins from the cell lysates. Thus, patient sera in group 1 were defined as non specific (negative), while 63 (76%) patient sera (groups 2 to 4) were scored as positive in the Western blot analysis. The number of SLE patients compared to DLE was higher in groups 2 to 4 than in group 1. SLE patients with more than six Western blot bands had a higher number of accompanied ENAs compared to patients with DLE. There was no correlation between special ENA subtypes (eg, SS-A. SS-B) and the detected number of Western blot bands.

As positive control, the HIV-blots were incubated with sera from HIV-infected individuals.

**Table 2** Reactivity of patient sera in Western blot analysis

| | *Group 1* ≤*3* | *Group 2* *4 to 6* *bands* | *Group 3* *7 to 9* *bands* | *Group 4* ≥*10* *bands* |
|---|---|---|---|---|
| SLE ($n = 44$) | 9 | 13 | 14 | 8 |
| DLE ($n = 11$) | 5 | 3 | 3 | 0 |
| SCLE ($n = 2$) | 0 | 0 | 2 | 0 |
| APS ($n = 7$) | 2 | 3 | 1 | 1 |
| Others ($n = 19$) | 4 | 9 | 2 | 4 |
| Total ($n = 83$) | 20 | 28 | 22 | 13 |
| Controls ($n = 23$) | 23 | 0 | 0 | 0 |

*FACS analysis of infected/non-infected cells*

To further evaluate the presence of cross-reacting antibodies from patient sera with autoimmune disorders against conformational epitopes FACS assays were performed with $HIV_{IIIB}$ and $HIV_{BaL}$-infected and non-infected M8166 and U937 cells. Binding was scored as positive, when the signal obtained with patient sera exceeded more than 200% of the background given by NHS. In both assays, more patient sera bound to infected compared to non infected cells. Out of 88 patient sera, 15 (10 SLE patient samples) reacted with uninfected M8166 cells (Figure 1A), significantly more (24 sera; 18 SLE samples) bound to $HIV_{IIIB}$-infected cells (Mann–Whitney *U*-test: $P = 0.046$) (Figure 1B).

Uninfected U937 cells were recognized only by three SLE positive sera (Figure 1C). With the R5 tropic $HIV_{BaL}$-infected U937 cells, 13 sera (nine SLE samples) gave a signal, higher than 200% (Figure 1D).

*Virus capture assay*

Virus capture assays were performed to demonstrate the recognition of free viral particles by sera from patients with autoimmune diseases. Heat-inactivated sera of these patients as an antibodies source, NHS as negative control and sera from HIV-infected individuals as positive control were bound to ELISA plates and incubated with $HIV_{IIIB}$ derived from M8166 cells. Compared to background signal obtained with NHS, 53 (60%) of 88 patient sera interacted with HIV particles in the assay. Ten sera (11%) bound more than 150% virus compared to NHS and three sera exceeded 200% (Table 3). With sera from HIV-infected individuals and average virus binding of 150% was obtained, when compared to the NHS background signals (not shown).

*Neutralization assays*

In order to examine the ability of patient sera to inhibit HIV-1 infection, heat-inactivated patient samples from groups 1 to 4 or healthy control sera were incubated with $HIV_{IIIB}$ and M8166 cells. If indicated a pool of active human serum was added as complement source. The results of neutralization assays monitored by HIV p24 ELISA at three days post-infection are shown in Table 4. In the absence of complement, none of the patient sera of groups 1 and 2 was able to reduce the infection more than 50%, while two patients in group 3 and one patient in group 4 were able to reduce viral replication. In all patient groups tested, complement enhanced the neutralization of HIV significantly. The effect correlated with the results obtained in Western blot analysis and was significantly higher in group 4

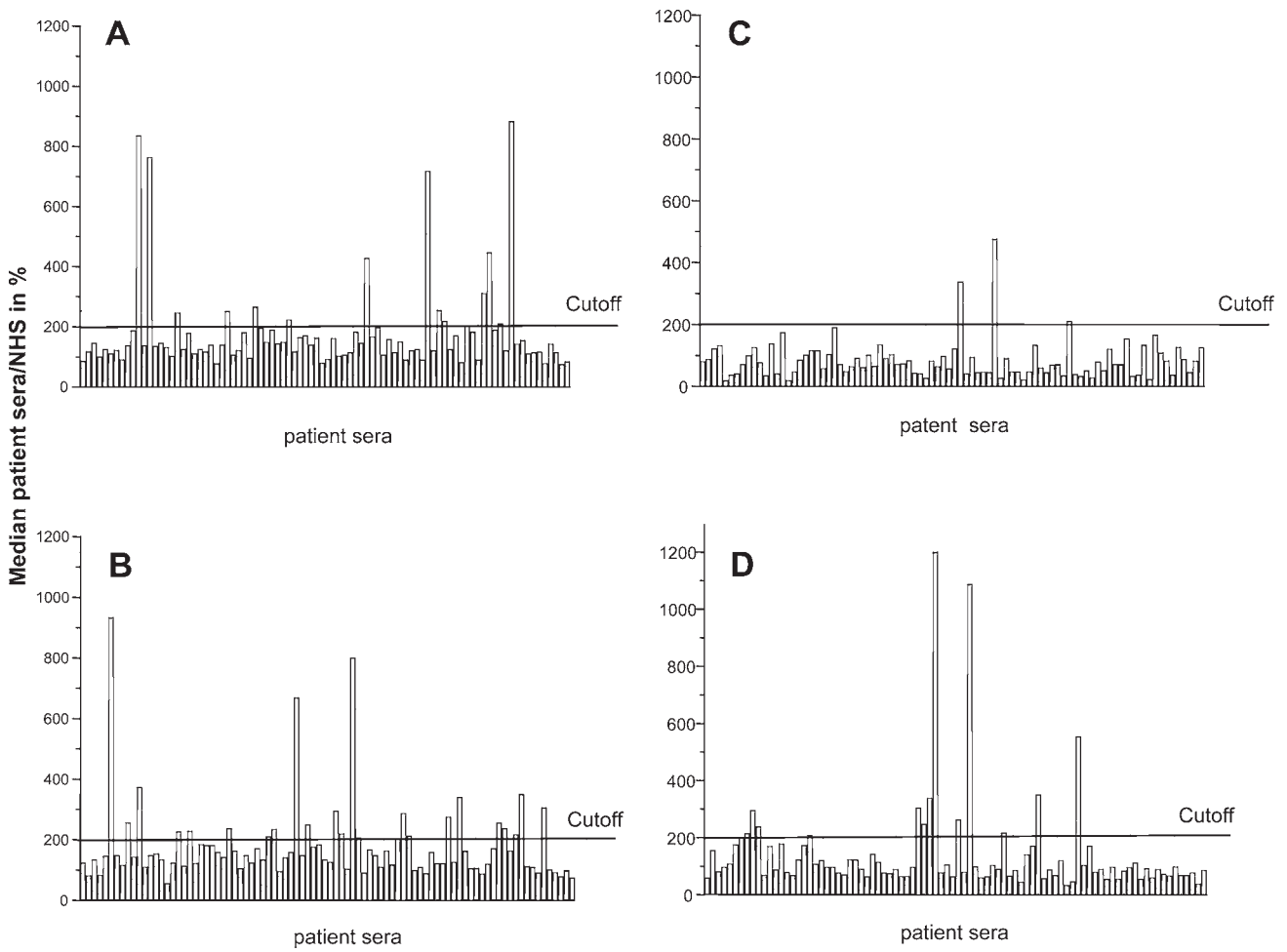


**Figure 1** Determination of the reactivity of patients' sera with HIV-infected and uninfected cells by FACS analysis. (A) Non-infected or (B) $HIV_{IIIB}$ infected M8166 cells were incubated with sera derived from patients with SLE or other autoimmune disorders and the amount of bound IgG was analysed by FACS. Similarly, (C) not infected and (D) $HIV_{Bal}$ infected U937 cells were compared. For analyses, the median reactivity of a pool of 20 sera from health individuals was set at 100%. The medians of the patient samples are given relative to the values obtained with to pool of NHS. The cut off was defined by the two-fold binding obtained with NHS and therefore set at 200%. Patient samples were scored as positive (reactive) when the median binding exceeded the cut off. Each bar represents the median of one patient serum from two independent experiments; the order of the samples in all assays is unchanged, due to limitations in space, the code of the patients is to shown.

(Fisher's exact test: $P = 0.012$), which represented the patients with more than nine bands in Western blot (Table 2). In the latter group five out of six individuals were able to decrease the viral burden. Patients with more than six positive Western blot bands showed significantly more neutraliziation of $HIV_{IIIB}$ in the presence of NHS than patients of groups 1 and 2 (Fisher's exact test: $P = 0.017$). All 10 control sera from healthy individuals had no neutralizing capacity at all dilutions tested independently on the presence or absence of complement (Table 4).

*Lysis*

The significant enhancement of viral neutralization in the presence of active human serum may be due to complement-mediated lysis. To clarify a possible mechanism of the observed complement-mediated inactivation of $HIV_{IIIB}$ in concert with patient sera, the complement-mediated virolysis on HIV was examined. For this assay patient sera with neutralizing capacity in the presence of complement were selected (see Table 4) and compared with unreactive patient samples or healthy controls. After loading with cross-reacting antibodies present in patient sera, between 2% and 15% of viral particles were lysed by complement (Figure 2), independent on their reactivity in the neutralization assay. Lysis was dependent on active complement since incubation with heat-inactivated or EDTA-containing serum totally abrogated virolysis. Incubation with DV012, an HIV-specific sheep anti-gp120 antibody, as positive control induced 15% lysis of $HIV_{IIIB}$.

**Table 3** Virus capture assay: 10 sera bound more than 150% virus compared to NHS and three sera exceeded 200%

| *Serum number* | *Diagnosis* | *Amount of p24 compared to NHS (%)* | *Amount of p24 compared to HIV$^+$-sera (%)* |
|---|---|---|---|
| 1662/99 | APS | 248 | 169 |
| 2202/99 | MCTD | 211 | 144 |
| 1488/99 | SLE | 204 | 138 |
| 1597/99 | SLE | 196 | 133 |
| 2371/99 | SCLE | 183 | 124 |
| 1723/99 | MCTD | 179 | 122 |
| 942/99 | SLE | 165 | 131 |
| 2226/99 | SLE | 160 | 109 |
| 309/99 | SLE | 154 | 122 |
| 2205/99 | SLE | 152 | 103 |

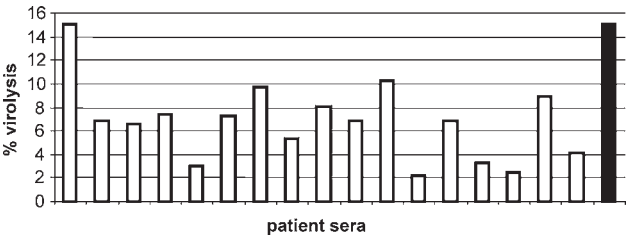


**Figure 2** Determination of complement-mediated lysis. Patients' sera which were positive in the neutralization assays were analysed in lysis assays. Lysis was determined by measuring the viral core protein p24 in the supernatant, which can be detected only when the viral envelope is destroyed. The amount of lysis was calculated as indicated it the material and method section. The mean of three independent experiments performed in duplicate is given. Open bars represent patients' sera while the black bar is the positive control with the DV012 antibody.

## Discussion

Autoimmune disorders like SLE and HIV infection share several similarities. Elevated parameters of immunactivation (elevated serum neopterin, soluble interleukin 2 receptor, $\beta$2-MG) and acquired immunosuppression (eg, low CD4+T-cell counts, susceptibility to 'opportunistic' infections) are present in both disorders. Although SLE patients in general have a higher risk of acquiring infections, HIV can be rarely found in these individuals. Since the first report in 1988, 34 cases have been published.[6–28] Apart from underreporting bias, several hypotheses have been suggested to explain this discrepancy: SLE affects predominantly young women versus young males, who have been the major risk group for HIV in the last decade. Further epidemiological factors like closer medical monitoring of SLE patients compared to other patients or reduced sexual intercourse due to fear of pregnancies may reduce the risk of acquiring HIV infection in SLE patients. Also blood transfusions are usually avoided as there is a high risk of haemolytic anaemia and anti-platelet auto-antibodies. It has been observed that acquired immunosuppression by HIV counteracts the emergence of SLE.[15,35] Lupus flares and/or primary manifestations of SLE have been observed after initiation of highly active antiretroviral therapy with consequent, immunoreconstitution and recovery of CD4+T-cell counts.[36–38] Conversely, the polyclonal B-cell activation and auto-antibody production may encompass HIV protective antibodies.[3]

HIV infection can manifest itself with multiple autoimmune signs and symptoms which may significantly overlap with SLE and other autoimmune diseases making it difficult to distinguish them.[29,35,39] Lupus sera may account for false positive HIV-ELISA and indeterminate Western blot results.[40,41] Thus SLE patients can induce antibodies to various retroviral proteins including gag, env and nef of HIV and HTLV[42] in the absence of retroviral infection. In addition HIV patients can produce ANAs, antibodies against double stranded DNA, small RNP complexes, platelets, lymphocytes and granulocytes, Coomb's positivity, CIC, rheumatoid factor and lupus anticoagulant.[42–45]

In this study SLE- and HIV-patient antibodies were studied by Western blot, FACS analysis, VCA and neutralization assays. As the Western blot was performed with M8166-cell-lysates, positive bands might not have been exclusively caused by binding antibodies towards HIV but also by SLE antibodies which recognize cellular antigens. The fact that the number of positive Western blot bands do not correlate with FACS analyses of uninfected M8166 may indicate this as well. Further, antibodies in Western blots bind linear epitopes only. These linear epitopes in the Western blot may not necessarily be available in the native state.

On the contrary antibodies in FACS- and VC-assays bind conformational epitopes. This might be a possible explanation why not all Western blot positive sera had positive results in FACS- and VC-assays. Additionally, no serum with the maximum number of Western blot bands reacted with HIV-infected M8166. Beside differences in the access of linear and conformational eptiopes, target of SLE antibodies are differentially

**Table 4** Neutralization assay: patients with more than six positive Western blot bands showed significantly more neutralization of $HIV_{IIIB}$ in the presence of NHS than patients of groups 1 and 2 (Fischer's exact test: $P = 0.017$)

| *Group* | *Amount of sera* | *Day 3 (−NHS)* | *Day 3 (+NHS)* | *In %* |
|---|---|---|---|---|
| 1 | 8 | 0 | 1 | 13 |
| 2 | 6 | 0 | 1 | 17 |
| 3 | 5 | 2 | 2 | 40 |
| 4 | 6 | 1 | 5 | 83 |
| Control | 10 | 0 | 0 | 0 |

accessible in the various assays used. Anti-Sm-antibodies, for example, may bind to p24 in Western blots, but not in VCA as in this assay the intraviral core protein is not accessible.[32]

More detailed information concerning auto-antibodies towards specific HIV surface proteins could be acquired by FACS analyses using M8166 cells. The binding of IgG from SLE sera to infected M8166 was significantly increased (Mann–Whitney *U*-test: $P = 0.046$). In contrast no major differences between the various patients groups were observed when using uninfected M8166. This might indicate that SLE patients have more HIV specific antibodies than patients with other connective tissue disorders.

Some interesting correlations between HIV and SLE antibodies could be found. On the one hand there are antigen similarities between HIV-gp120 and nuclear antigens, which have been reported by Pinto *et al.*[46] Similar results were obtained by Talal *et al.*,[47] describing cross-reactions between the HIV-p24 and anti-Sm-antibodies. Additionally, between HIV- and HLA-antigens some corresponding regions like gp120 and HLA-antigens[46] or C1q[48] have been reported. Gp41 shares homology to the complement protein C3[49] or the HLA-class II $\beta$1-chain.[50,51] Still, the frequency of specific antibodies to HIV-surface proteins gp41 and gp120 in SLE sera needs to be determined. Gül *et al.*[52] described reactivity towards HIV antigens in 52% of Turkish SLE patients. In our study 88 sera were analysed by Western blots. Positive reactions with HIV specific proteins were found in 63 sera (72%).

In contrast to Western blots, where single viral proteins and peptides can be tested, VCA target the intact HIV surface structure. Specific anti-HIV-antibodies in the serum seem to be responsible for virus binding in the VCA. Therefore those sera, that bound better to infected than to uninfected M8166 cells, were expected to give superior results in the VCA. In these experiments a significant correlation between VCA- and FACS-results with uninfected M8166 cells could be found (Mann–Whitney *U*-test: $P = 0.032$).

These results may indicate that viruses are not only captured by HIV-targeting antibodies, but also by antibodies that bind cell surface structures. Due to the budding process from double lipid layers of host cells, HIV particles acquire several different receptors such as HLA I- and II-molecules,[34,53] ICAM-1 or CD44.[32] Further receptors like CD55 and CD59 can protect viral particles against complement mediated lysis.[54] Some host cell surface receptors are up-regulated upon HIV-infection, such as complement receptor 3 (CR3), CD55 and HLA-I antigens.[32] CR3 can be detected on HIV after budding from host cells (U937), however, it is absent on this cell line if uninfected.

Neutralization assays were performed in order to assess specifically the inhibitory effect of SLE antibodies and $HIV_{IIIB}$. SLE patients sera were heat inactivated in order to avoid immune modulating effects of cytokines, interferons and complement factors. Results indicate that patient sera with more than six positive Western blot bands had some $HIV_{IIIB}$-neutralizing capacity providing a certain degree of protection with their neutralizing antibodies against an HIV infection similar to polytransfused individuals.[34]

Our study confirms previously published results that antibodies derived from SLE patients interact with HIV[15,29,40] for example, in Western blot analyses. In addition, we could also show that the same antibodies have neutralizing capacity towards $HIV_{IIIB}$, which is significantly higher in patients with more than six positive Western blot bands. The results of this study suggest a possible protective role of these auto-antibodies, thereby contributing to a low incidence of HIV infection in SLE patients.

## Acknowledgement

The experiments were supported by the EU (TIP-VAC 012116, DEC-VAC 018685), the FWF (17914 to HS), the Ludwig Boltzmann Gesellschaft and the federal state of Tyrol.

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