Anti-phospholipid antibodies and CD5$^+$ B cells in HIV infection

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SUMMARY

This cross-sectional study evaluates the correlation between anti-phospholipid antibodies and CD5$^+$ B cells in 110 patients infected with HIV-1. There were 89·1% of the patients who had IgG antibodies against cardiolipin and phosphatidylserine. The prevalence of IgM and IgA antibodies was $<22\%$. AIDS was associated with lower frequencies of IgM antibodies against cardiolipin ($P = 0.05$) and IgG-antibodies against cardiolipin and phosphatidylserine ($P = 0.011$). Drug users had higher IgM antibodies against phospholipids than patients from other risk groups ($P = 0.02$). A history of thromboembolic events was not accompanied by higher levels of anti-phospholipid antibodies ($P > 0.2$). No correlation between anti-phospholipid antibodies and CD5$^+$ B cells was detected. Percentage part of CD5$^+$ B lymphocytes was elevated in all patients and absolute CD4$^+$ T lymphocyte counts and HIV p24 antigen were inversely correlated. In advanced disease a significant reduction of anti-phospholipid antibodies was contrasted with persistent elevation of CD5$^+$ B lymphocytes. These observations may reflect immunological dysfunction involving apoptosis and endothelial damage rather than polyclonal B cell hyperstimulation. A possible explanation would be that in HIV infection an increased rate of spontaneous apoptosis in peripheral blood lymphocytes is accompanied by functional and structural changes of mitochondria. Therefore, structurally altered mitochondrial phospholipids could serve as antigen to induce specific humoral immune responses.

Keywords anti-phospholipid antibodies   CD5$^+$ B cells   cross-sectional study   HIV infection

INTRODUCTION

The most prominent pathogenic feature of infection with HIV is the loss of CD4$^+$ T lymphocytes and the emerging susceptibility to opportunistic infections or tumours. Recent research indicates that immune activation as well as autoimmunity may contribute to clinical features of HIV-associated disease [1]. This is particularly apparent in those HIV-infected individuals who have clinical evidence of autoimmune diseases like immune thrombocytopenia, inflammatory neuropathy and vasculitis. Polyclonal B cell activation was noted early during the AIDS epidemic [2] and was attributed, at least in part, to HIV or its peptides [3–5]. Also, spontaneous immunoglobulin synthesis *in vitro* [6] and serum autoantibodies [7,8] have frequently been detected. Antibodies against peripheral blood cells and MHC II have been frequently demonstrated, whereas antinuclear antibodies have been rarely observed [9]. This is contrasted by the observation of a disturbed generation of specific antibodies in HIV-infected individuals [10].

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Antibodies against phospholipids, particularly anti-cardiolipin antibodies (ACLA) have recently been the subject of special interest, first, because they were associated with distinct clinical symptoms, thromboembolic disease, recurrent fetal loss or thrombocytopenia [11–14], which were also frequently seen in HIV-associated disease, and second, because of their appearance during infectious disease states distinct from an association with syphilis, lyme borreliosis, helminthoses or Mycoplasma infections [15–17]. They were also highly prevalent in HIV infection [18].

B cells bearing the CD5 antigen constitute a subset which is thought to be part of the natural immune system and their role in generating natural occurring autoantibodies is still under discussion [19]. CD5$^+$ B cells are the predominant cell type found in chronic B cell lymphocytic leukaemia [20]. High numbers in the peripheral blood were also reported in rheumatoid arthritis [21], Sjögren's syndrome [22], and recent-onset insulin-dependent diabetes mellitus [23]. In the setting of HIV infection there are few reports concerning the number and the role of CD5$^+$ B lymphocytes in relation to the development of autoimmune phenomenon, lymphoid neoplasia or disease progression [24,25].

At this time, there is no study that focuses on the relationship of CD5$^+$ B lymphocytes and ACLA with each other as well as with clinical disease state, immunological progression, surrogate marker and outcome in HIV-infected individuals. The objective of this study therefore was to investigate the clinical, immunological and laboratory features associated with these laboratory autoimmune phenomena in HIV infection.

SUBJECTS AND METHODS

Subjects

One hundred and ten individuals with confirmed HIV infection receiving continuous medical treatment were consecutively recruited from the out-patient clinic for infectious diseases. After complete physical examination, blood was withdrawn for routine and research laboratory analysis after informed consent. Patients were seen monthly for follow-up visits. Any clinical disease during the course, including HIV-related opportunistic events, was noted. In addition, arcturial survival was calculated from start of the study to death or last patient contact.

Laboratory measurements

Detection of autoantibodies. The serum material obtained was immediately aliquoted and frozen at –70°C until analysis. Measurements of anti-cardiolipin and anti-phosphatidylserine antibodies (ACLA and APSA, respectively) were performed for all samples with the same test kits with identical lot numbers to minimize methodological errors. Antinuclear antibody (ANA) titres were determined by standard immunofluorescence assays using Hep2 cells as targets in dual logarithmic dilutions of patient's serum. ACLA and APSA were measured by ELISA. Briefly, standard samples, diluted serum samples, positive and negative control sera (each 100 μl/well) were incubated using microtitre plates precoated with cardiolipin or phosphatidylserine (IMTEC Immundiagnostika GmbH, Zepernick, Germany) for 2 h at room temperature. Plates were then washed three times with PBS. Peroxidase-conjugated rabbit antibody to human IgG, IgM, and IgA, respectively, was added in a dilution of 1:1000. After an incubation period of 1 h at room temperature and washing, 200 μl o-phenylenediamine was added as substrate. The reaction was stopped after 10 min by the addition of 100 μl 3 N H_2SO_4. Optical density (OD) at 492 nm was read and a standard curve constructed for each plate. For this method the lower detection limit was 10 U/ml, the interassay coefficient of variation was <10% (data not shown). All samples were analysed in duplicate. Given concentrations represent the mean of the two readings. Values >48 U/ml and >44 U/ml for IgG ACLA and IgM ACLA, respectively, were considered to be positive. Normal values for IgA ACLA were <45 U/ml. Positive levels of IgG APSA and IgM APSA comprised values >20 U/ml and >39 U/ml, respectively.

Flow cytometry. All measurements were performed immediately after blood was withdrawn. Complete blood counts and cell differentials were performed with an automated cell counter. Flow cytometric determination of lymphocyte surface antigens was performed by direct three-colour immunofluorescence using the FACScan flow cytometer (Becton Dickinson, Heidelberg, Germany) equipped with standard software (FACScan Research, Becton Dickinson) and commercially available MoAbs. Isotype-matched non-specific antibodies were used as negative controls. Lymphocyte phenotyping included the quantification of CD3$^+$ (pan T cell), CD4$^+$ (helper cells), CD8$^+$ (suppressor cells), CD19$^+$ (pan B cells), CD5$^+$ (T and B cell subset), κ and λ light chains on at least 5000 cells. Lymphoid cell population was detected by characteristic forward and 90° scatter light properties. Quality of the gate was determined by immunofluorescence with anti-CD45 (pan leucocyte), anti-CD14 (monocyte), and anti-CD64 (monocytes, macrophages) antibodies. Material was used for further analysis only if the number of CD45^{high}/CD14$^-$/CD64$^-$ cells was >95% in the lymphocyte gate.

Instruments settings were calibrated daily with an automated software using prepared beads (Calibrite and Autocomp Software; Becton Dickinson). Coefficient of variation (CV) for the MoAbs used in the study was <10% (for anti-κ and anti-λ <15%). Enumeration of absolute CD4$^+$ T lymphocyte counts was performed according the recommendations of the Centers for Diseases Control and Prevention (CDC) [26].

Statistical analysis

The SPSS-for-Windows Version 6.12 software package was used for data analysis. Descriptive analysis included mean, median, range and s.d. of each variable. The normal distribution of single variables was tested by Kolgomorow–Smirnov analysis. Correlations of clinical and laboratory parameters with results of the flow cytometric and autoantibody determinations were estimated by bivariate linear correlation analysis (two-sided Spearman's rank sum correlation coefficient). Differences between patient groups were compared using the Mann–Whitney U-test and the Kruskal–Wallis one-way ANOVA for independent samples. Relative risk estimations were performed for the association of clinical diseases with laboratory variables at defined cut-off points. Survival was estimated using the method described by Kaplan & Meier [27]. P <0·05 was assumed to be of significance in all tests used.

RESULTS

General findings

For evaluation, the 110 patients recruited were classified into clinical categories of the disease according to the 1993

Table 1. Clinical characteristics of the patients studied

	Number of patients	(%)
Median age (range)	37	(18–63)
Male gender	91	(82·7)
Risk of HIV infection		
homosexual/bisexual	77	(70·0)
i.v. drug use	22	(20·0)
heterosexual	10	(9·1)
blood products	1	(0·9)
Clinical category*		
A (asymptomatic)	22	(20·0)
B (symptomatic, non-AIDS)	44	(40·0)
C (AIDS)	44	(40·0)
Active opportunistic infection	9	(8·2)
Opportunistic tumour	6	(5·4)
Kaposi's sarcoma	4	(3·8)
Lymphoma	2	(1·6)
History of thromboembolic events	8	(7·3)
History of syphilis	16	(14·5)
Encephalopathy	39	(35·5)

*According to 1993 CDC classification system.

classification system of the CDC [28]. Most of the patients were in an advanced stage of HIV infection. Only 14·5% of the patients had a past history of clinically apparent syphilis and 7·3% had thrombosis or thromboembolic diseases (Table 1). Malignant lymphoma was seen in only two subjects, whereas Kaposi's sarcoma was found in four. Almost all symptomatic patients received antiretroviral treatment (only two out of 22 category A individuals in contrast to 84 out of 88 in categories B and C) either with zidovudine (ZDV), didanosine (DDI), zalcitabine (DDC) or stavudine (D4T) alone or a combination therapy with either ZDV/DDC or ZDV/DDI. Regarding the baseline laboratory parameters (Table 2), severe anaemia, leukopenia or hypoproteinaemia occurred in none of the group A patients. Of the symptomatic individuals 16 (18·2%) had relevant anaemia or leukopenia, whereas none of the patients were found to have hypoproteinaemia. Thrombopenia (thrombocytes < 130/nl) was evident in one (4·5%) category A subject and in six patients each in categories B and C. Serological evidence of *Treponema pallidum* infection was present in 26 (23·6%) patients. HIV p24 antigen was detectable in all category C as well as in 90·5% and 88·6% of category A and B patients, respectively.

Autoantibodies
Of the individuals examined, 89·1% (21·8%) were found to have IgG ACLA (IgM ACLA), and 89·1% (8·2%) had positive IgG APSA (IgM APSA). Results are summarized in Table 3. Elevated IgA ACLA concentrations were found in 39·1% (41/110 patients). ACLA and APSA positivity was not associated with any of the treponemal serological tests (all *P* values > 0·2, Fisher's two-tailed exact test). In contrast to this, autoantibody screening revealed only four (3·6%) positive test results for ANA. Anti-mitochondrial and anti-smooth muscle antibodies were found in four patients (3·6%) and in one patient (0·9%), respectively. Anti-Sm antibodies were found in 6·9% of the patients. This is in agreement with our previous results that showed a prevalence of non-organ-specific autoantibodies (ANA, anti-mitochondrial and anti-smooth muscle antibodies) in HIV-infected individuals to be always < 5% (unpublished data).

A significantly lower number of patients with clinical AIDS had positive IgM antibodies against cardiolipin compared with those individuals with symptomatic HIV infection (Table 3). Furthermore, the frequency of IgG ACLA and IgG APSA was significantly lower in patients with full-blown AIDS than in those of category A or B (both *P* = 0·011, Fisher's two-tailed exact test). Similar results were obtained not only for the frequency but also for the absolute concentrations of IgG antibodies if compared according to the clinical (Fig. 1a) or immunological stage (Fig. 1b).

With regard to the risk factors for the acquisition of HIV infection, i.v. drug users had significantly higher concentrations of IgM antibodies against cardiolipin and phosphatidylserine than individuals from other risk groups (each *P* < 0·002, Kruskal–Wallis one-way ANOVA). Also, drug abuse was associated with higher concentrations of IgG APSA compared with homo- or bisexual patients (*P* < 0·05, Mann–Whitney *U*-test).

Differences regarding the levels or the positivity of autoantibodies were not found in relation to: gender or age, acute or active opportunistic infections, and clinical signs of HIV-related encephalopathy or radiological features suggesting cerebral vasculopathy. Patients with a history of thrombotic or thromboembolic events or thrombopenia had no higher levels of serum autoantibodies (all *P* > 0·2, Mann–Whitney *U*-test) except for IgM APSA, where a

trend towards higher concentrations was found in thrombopenic subjects (*P* = 0·06).

Considering the association of survival in individuals with severe immunodeficiency (CD4$^+$ T lymphocytes < 0·2/nl), those with positive IgG APSA tended to have a longer survival compared with IgG APSA$^-$ subjects. The difference between these groups, however, was found to be not statistically significant (*P* = 0·106, log-rank test; Fig. 2). Survival time was not affected by the presence of any other autoantibody determined in this study (all *P* > 0·20, log-rank test).

Cellular immunology
As expected, progression in clinical symptoms was accompanied by a decrease in lymphocyte and T lymphocyte numbers (Table 4). Also, B lymphocyte counts were reduced in advanced HIV infection. CD5 antigen-positive B lymphocytes showed a proportional increase relative to all B lymphocytes in all three patient groups compared with levels from healthy subjects. A decrease in absolute numbers similar to the total B lymphocyte population with advanced clinical stage of HIV infection was also detected. No significant difference was seen regarding gender, age, risk for the acquisition of HIV infection or full-blown AIDS. Immunoglobulin light chain expression on B cells showed no differences between either group, male or female gender, different risk groups and clinical AIDS *versus* non-AIDS.

CD5$^+$ B lymphocytes were inversely correlated with absolute CD4$^+$ T lymphocyte counts ($r^2 = -0·18$, *P* = 0·037; Fig. 3) and the immune complex-dissociated HIV p24 antigen in serum ($r^2 = -0·19$, *P* = 0·029), whereas no correlation was found with other
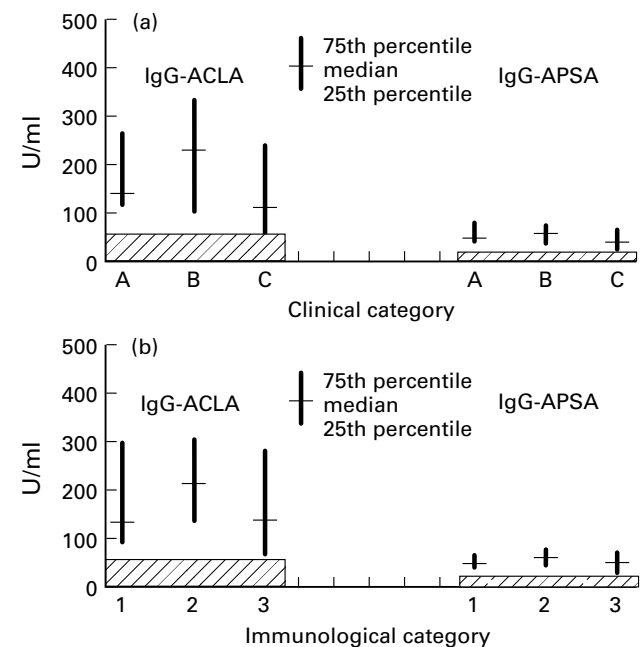

Fig. 1. IgG anti-cardiolipin antibodies (ACLA) and IgG anti-phosphatidylserine antibodies (APSA) levels according to the clinical (a) and immunological (b) stage. Measurements of ACLA and APSA were performed as described in Subjects and Methods. The frequency of IgG ACLA and IgG APSA was significantly lower in patients with full-blown AIDS than in those of category A or B (both *P* = 0·011, Fisher's two-tailed exact test). Similar results were obtained comparing the different immunological groups. The hatched area indicates the negative range.

Table 2. Baseline laboratory data according to the clinical stage

	Clinical category		
	A ($n = 22$)	B ($n = 44$)	C ($n = 44$)
Haemoglobin	140.4 ± 13.1	132.0 ± 16.2	112.8 ± 15.2
(g/l)	137 (118–163)*	133 (87–166)	112.5 (76–147)***
Leucocytes	6.65 ± 2.06	4.35 ± 1.60	2.67 ± 1.43
($\times 10^6/l$)	6.4 (3.9–11.1)	4.2 (1.5–9.6)**	2.45 (0.7–7.1)***
Thrombocytes	213.3 ± 66.3	174.2 ± 68.3	177.4 ± 89.2
($\times 10^6/l$)	199 (73–344)	174.5 (20–336)**	165 (31–442)**
Surrogate markers			
IgA	319.7 ± 279.4	491.7 ± 410.2	536.8 ± 379.0
(mg/dl)	259 (59–1440)	357.5 (107–1860)**	393 (113–1600)**
Neopterin	15.1 ± 6.7	21.2 ± 10.3	36.1 ± 22.6
(mmol/l)	13.8 (6.6–32.4)	18.5 (8.5–52.2)**	28.4 (10.4–116.0)***
β_2-microglobulin	2.86 ± 0.87	3.68 ± 1.31	4.74 ± 1.67
(mg/l)	2.7 (1.7–4.9)	3.3 (1.7–7.0)**	4.6 (2.3–11.1)***
p24 antigen	44.7 ± 38.6	95.8 ± 93.5	128.8 ± 89.4
(pg/ml)	36 (0–182)	57 (0–250)**	97 (19–250)***
Syphilis serology			
TPHA$^+$	3/22 (13.6%)	14/44 (31.8%)	9/44 (20.5%)
VDRL$^+$	0	9/44 (20.5%)	4/44 (9.1%)
FTA Ab$^+$	2/22 (9.1%)	14/44 (31.8%)	8/44 (18.2%)

*Values indicate mean $\pm$ s.d. and median with range except for treponemal tests.

**$P \leq 0.05$ (Mann–Whitney U-test) compared with category A.

***$P \leq 0.05$ (Mann–Whitney U-test) compared with categories A and B.

surrogate markers like serum neopterin, β_2-microglobulin ($P > 0.2$), and IgA (all P values > 0.2).

Individuals with a high proportion of CD5$^+$ B lymphocytes and only moderate CD4$^+$ T lymphocyte depletion tended to have a longer survival than those with CD5$^+$ B lymphocytes $< 50\%$ of all B lymphocytes (Fig. 4; log-rank test; the difference was not statistically significant: $P = 0.087$).
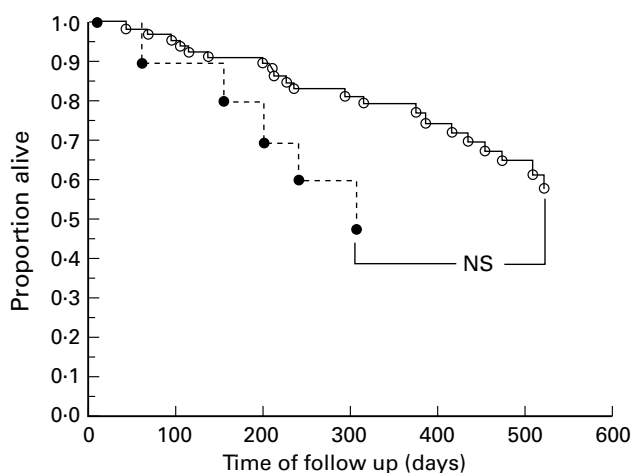

Fig. 2. Survival estimations comparing IgG anti-phosphatidylserine antibody (APSA)$^+$ (○) and IgG APSA$^-$ (●) individuals. Individuals with severe immunodeficiency (CD4$^+$ T lymphocytes < 0.2/nl) and positive APSA IgG tended to have a longer survival compared with subjects with severe immunodeficiency, but negative APSA IgG ($P = 0.106$, log-rank test).

Comparisons of cellular and humoral markers of autoimmunity

No correlation could be detected between the absolute or relative number of B lymphocytes or CD19$^+$CD5$^+$ lymphocytes (B1 cells) and ACLA and APSA concentrations. Symptomatic individuals (clinical categories B and C) with positive results for ACLA and APSA IgG testing had lower levels of CD5$^+$ B lymphocytes ($P = 0.028$, Mann–Whitney U-test). Similar data were obtained in individuals with severe immunodeficiency (CD4$^+$ T lymphocytes < 0.2/nl).

DISCUSSION

Antibodies against cardiolipin and phosphatidylserine are known to be associated with certain clinical conditions including autoimmune diseases like systemic lupus erythematosus [29],

Table 3. Positivity for anti-cardiolipin antibodies (ACLA) and anti-phosphatidylserine antibodies (APSA) and clinical stage

	Clinical category		
	A ($n = 22$)	B ($n = 44$)	C ($n = 44$)
ACLA number positive (%)			
IgG class	22 (100)*	41 (93.2)*	35 (79.5)
IgM class	3 (13.6)	15 (34.1)*	6 (13.6)
APSA number positive (%)			
IgG class	21 (95.5)*	42 (95.5)*	35 (79.5)
IgM class	1 (4.5)	6 (13.6)	2 (4.5)

*$P <$ or $= 0.05$ *versus* group C (Fisher's two-tailed exact test).

Table 4. Flow cytometric results of immunophenotyping

	Clinical category		
	A ($n = 22$)	B ($n = 44$)	C ($n = 44$)
T cell markers			
CD3$^+$ cells ($\times 10^6/l$)	1·642 (0·647–3·870)*	1·152 (0·255–3·430)**	0·309 (0·004–2·386)***
CD4$^+$ T cells ($\times 10^6/l$)	0·501 (0·207–1·414)	0·142 (0·004–0·811)**	0·009 (0–0·307)***
CD8$^+$ T cells ($\times 10^6/l$)	1·093 (0·502–3·197)	0·908 (0·186–2·340)	0·240 (0·004–2·044)***
B cell markers			
CD19$^+$ cells ($\times 10^6/l$)	0·190 (0·059–0·622)	0·135 (0·016–0·432)	0·050 (0·002–0·402)***
CD19$^+$CD5$^+$ cells ($\times 10^6/l$)	0·095 (0·038–0·307)	0·086 (0·006–0·351)	0·024 (0·001–0·248)
CD5$^+$ cells relative (%CD19$^+$)	54·1 (10·3–90·0)	55·0 (14·3–96·3)	62·4 (7·9–97·9)
κ^+CD19$^+$ cells ($\times 10^6/l$)	0·016 (0–0·098)	0·009 (0–0·206)	0·003 (0–0·045)**
λ^+CD19$^+$ cells ($\times 10^6/l$)	0·049 (0·007–0·129)	0·032 (0–0·206)	0·006 (0–0·084)**
κ^+/λ^+ ratio	0·38 (0–5·82)	0·43 (0–6·00)	0·46 (0–1·75)

*Values indicate median with range.

**$P \leq 0.05$ (Mann–Whitney U-test) compared with category A.

***$P \leq 0.05$ (Mann–Whitney U-test) compared with categories A and B.

thromboembolic events [30], recurrent fetal loss [13], and infectious disease states. Their occurrence in the setting of HIV infection was noted early in the course of the epidemic [18,31,32]. In our work we have detected in almost all HIV-infected individuals at least one variety of anti-phospholipid antibody. The presence of these autoantibodies was not accompanied by evidence of polyclonal B cell stimulation or production of non-specific antibodies such as antinuclear antibodies. Therefore, it appears that anti-phospholipid antibodies are of significance in HIV infection. Anti-phospholipid antibodies may well play a role in HIV-related immunopathogenesis: in the setting of HIV infection an increased rate of spontaneous apoptosis in peripheral blood lymphocytes is reported [33]. Initial events that characterize lymphocyte apoptosis are alterations of the mitochondrial function such as changes of mitochondrial transmembrane potential and increased mitochondrial generation of superoxide anion [34]. This is found in a significant proportion of peripheral lymphocytes in HIV-infected subjects. Furthermore, the same group reported a structural defect of the cardiolipin-containing inner mitochondrial membrane in lymphocytes of HIV-infected individuals. Structurally altered mitochondrial phospholipids are transported by a phospholipid flippase/translocase mechanism [35,36] to the cell surface and could then act as new antigens which induce humoral immune response in the HIV-infected host who already presents with polyclonal B cell activation. This is interesting because the transporter protein belongs to the mdr gene products which encode P-glycoproteins. These proteins are up-regulated in cells which are infected with HIV-1 *in vitro* [37] and in the presence of opportunistic
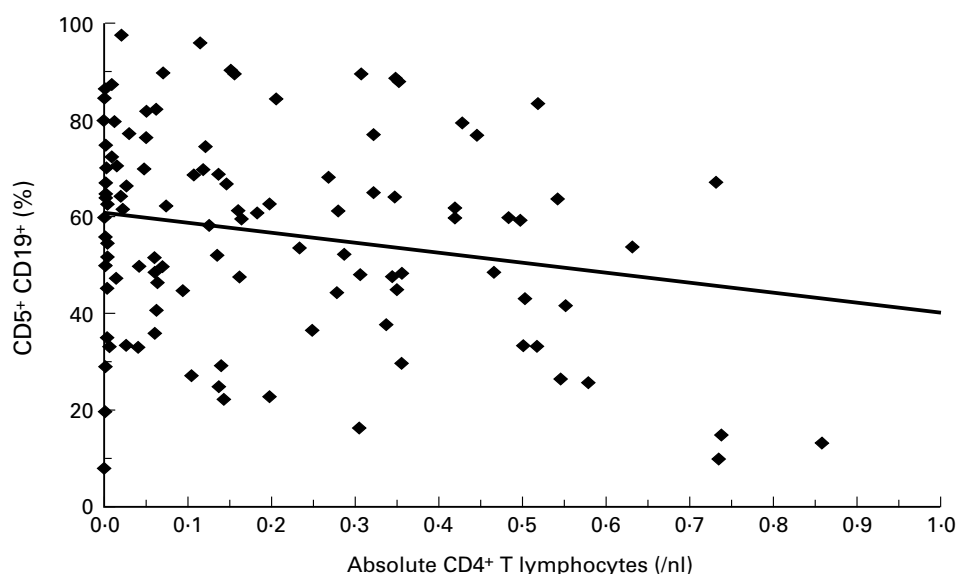

Fig. 3. Correlation of CD4$^+$ T lymphocytes with the proportion of B1 lymphocytes in the peripheral blood. CD5$^+$ B lymphocytes were inversely correlated with absolute CD4$^+$ T lymphocyte counts ($r^2 = -0.18$, $P = 0.037$) and immune-complex-dissociated HIV p24 antigen in serum ($r^2 = -0.19$, $P = 0.029$).

pathogens in chronically infected cell lines [38]. This hypothesis is strongly supported by results recently published by Silvestris *et al.* [39], who demonstrated a clear association between the occurrence of APSA and apoptosis in HIV-infected individuals. Additionally, this group also reported an augmented antibody-dependent cell-mediated cytotoxicity (ADCC) of autologous macrophages using IgG APSA compared with non specific IgG, which could increase the clearance of apoptotic T cells.

With regard to the prognostic significance of our results, we found in contrast to Coll Daroca *et al.* [25] significantly less positive ACLA and APSA in patients with full-blown AIDS. This is also accompanied by a statistical trend for better survival comparing IgG APSA$^+$ with IgG APSA$^-$ individuals. Therefore, we hypothesize that the disappearance of ACLA and APSA may reflect the increasing immunodeficiency with loss of T helper cell type 2 functions resulting in a progressive B cell immunodeficiency in the late course of HIV disease. Furthermore, the presence of anti-phospholipid autoantibodies was not associated with thromboembolic diseases or thrombocytopenia, nor was there an increased incidence of other non-specific autoantibodies. In our study, patients with positive ACLA or APSA did not have a higher risk for the development of thromboembolic events or thrombocytopenia. This finding is in accordance with the data published by Weiss *et al.* [32]. A previously published paper suggests an association between cerebrovascular perfusion deficits as detected by ^{99}mTc-HMPAO SPECT and anti-phospholipid antibodies [40]. In our study 99 patients had cerebral imaging studies (computed tomographic or magnetic resonance imaging scans) within 3 months from the determination of ACLA and APSA. However, these imaging techniques did not reveal an association of micro-angiopathy with antibodies. Neither was there a correlation to the presence of clinical symptoms of encephalopathy and the occurrence of autoantibodies.

In this study we have found significantly higher antibody levels in i.v. drug users compared with homosexual patients. This could be due to a stronger activation of endothelial tissues by drug injection, the high incidence of bloodstream infections as well as the higher amount of antigen presented to peripheral blood cells in

this risk group. All factors may lead to B cell activation with augmented production of ACLA and APSA. Biological false-positive syphilis serology was only rarely seen in our population and reached an incidence of 0·9%. Similar data were recently published in a large retrospective analysis which showed no association with ACLA [41].

The pathogenicity of ACLA and APSA is probably mediated by the interaction of the antibodies with membrane phospholipids or phospholipid-bound cofactors (e.g. apolipoprotein H) from endothelial cells or platelets resulting in increased release of platelet-activating factor, interference with phospholipid-dependent anticoagulant factors (protein S, protein C) and imbalance in the amount of arachidonic acid metabolites. Thus, cofactor binding may be the crucial point in the pathogenesis of the anti-phospholipid antibody syndrome-related clinical manifestations [42,43]. In infectious diseases, including HIV infection, detectable anti-phospholipid antibodies have been shown to be apolipoprotein H-independent [44–46]. This fact may explain the lack of association of clinical symptoms with the detection of ACLA and APSA in HIV-infected individuals. On the other hand, the determination of both subtypes (ACLA and APSA) enabled us to show positivity for both cofactor-dependent or -enhanced (ACLA) and cofactor-independent (APSA) antibodies.

Elevated proportions of B1 lymphocytes expressing the CD5 antigen have been documented in the peripheral blood of HIV-infected individuals [47,48]. These cells are thought to be involved in autoantibody production [49,50] and may represent a part of the natural immune system [51]. However, the prognostic or clinical significance in the setting of HIV infection has not been evaluated so far. In this study, no statistically significant correlation or association of CD5$^+$ B lymphocytes with ACLA and APSA, clinical disease or progression of HIV infection was detected. The observed (i) weak inverse correlation to the levels of CD4$^+$ T lymphocytes and the HIV core antigen serum, and (ii) tendency of a better survival of patients with mild to moderate immunosuppression if CD5$^+$ B lymphocytes comprise the lesser part of the B cell are both of limited value in the clinical assessment of HIV infection.

We conclude that anti-phospholipid antibodies are frequently observed in HIV-infected individuals. These antibodies may be involved in immunopathogenic events during HIV-induced lymphocyte apoptosis and may reflect loss of B cell functionality in the course of the disease. There is no correlation between CD5$^+$ cells and ACLA/APSA or clinical disease.

Fig. 4. Survival in individuals with immunological stages 2 and 3 compared according to the proportions of CD5$^+$ B lymphocytes. Individuals with a high proportion of CD5$^+$ B lymphocytes and only moderate CD4$^+$ T lymphocyte depletion (O) tended to have a longer survival than those with CD5$^+$ B lymphocytes <50% of all B lymphocytes (●) ($P = 0·087$, log-rank test).

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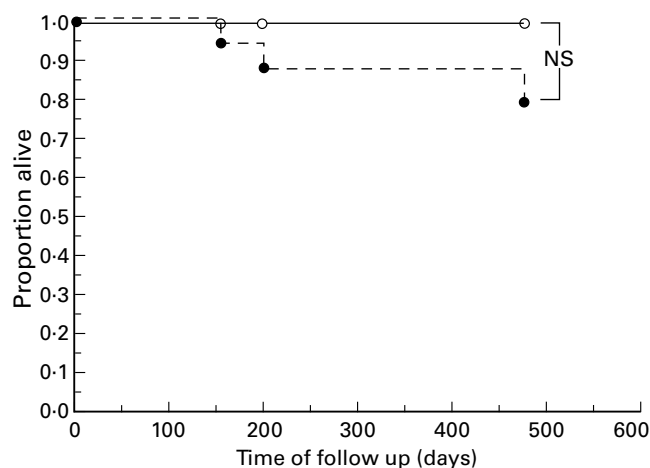
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