Clinical Immunology
Vol. 90, No. 2, February, pp. 247–255, 1999
Article ID clim.1998.4620, available online at http://www.idealibrary.com on IDEAL®

C1q Autoantibodies in HIV Infection: Correlation to Elevated Levels of Autoantibodies against 60-kDa Heat-Shock Proteins

Z. Prohászka,* M. R. Daha,† C. Süsal,‡ V. Daniel,‡ J. Szlávik,§ D. Bánhegyi,§ K. Nagy,¶ V. Várkonyi,¶ A. Horváth,¶ E. Ujhelyi,‖ F. D. Tóth,** K. Uray,†† F. Hudecz,†† and G. Füst*

*Third Department of Medicine, Semmelweis Medical University, Budapest, Hungary; †Department of Nephrology, Academisch Ziekenhuis Leiden, Leiden, The Netherlands; ‡Department of Transplantation Immunology, Institute of Immunology, University of Heidelberg, Heidelberg, Germany; §Department of Immunology, Szt László Hospital, Budapest, Hungary; ¶National Institute of Dermatology and Venerology, Budapest, Hungary; ‖National Center for Blood Transfusion, Budapest, Hungary; **Institute of Microbiology, University Medical School, Debrecen, Hungary; and ††Research Group of Peptide Chemistry, Hungarian Academy of Science, L. Eötvös University, Budapest, Hungary

Antibodies to solid phase C1q (C1qAb) were determined in 295 serum samples from 132 HIV-infected subjects and in sera from 140 HIV-seronegative healthy individuals as control. An ELISA method applied for the determination of C1qAb in other diseases was used. In part of these sera, other autoantibodies (antibodies reacting with 60-kDa human heat shock protein (hsp60) or mycobacterial hsp65; IgA and IgG class antibodies against the Fab and F(ab′)2 moieties of IgG) as well as complement-mediated antibody-dependent enhancement/neutralization (C′-ADE) were also determined. Increased amount of C1qAb was found in HIV-infected subjects as compared with HIV-seronegative controls (*P* = 0.0138). In 17 of 132 (13.0%) seropositive individuals but only in 7/140 (5.0%) samples from the controls, the amount of C1qAb exceeded the upper limit (95th percentile) of the normal values (*P* = 0.031). The amount of C1qAb significantly decreased during a follow-up period of 65 months. C1qAb levels were found to strongly correlate to hsp60/65 autoantibodies but did not correlate or only weakly correlated to the amount of anti-Fab or anti-F(ab′)$_2$ autoantibodies measured in the same serum samples. Anti-C1q antibodies recognized the solid phase hsp60/65. Three predicted epitope regions of *M. paratuberculosis* hsp65 were able to bind efficiently C1q antibodies. An inverse correlation was found between C1qAb and C′-ADE, neutralization was more frequent in the sera with detectable C1qAb, whereas sera without C1qAb more likely enhanced HIV infection *in vitro.*
© 1999 Academic Press

Key Words: **anti-C1q antibodies; anti-hsp60 antibodies; cross-reaction; HIV-infection; hsp60; gp120; epitope analysis; complement-dependent enhancement/neutralization.**

INTRODUCTION

Antibodies to C1q (C1qAb), a subcomponent of the first component of complement frequently occur in different autoimmune diseases like systemic lupus erythematosus (1, 2), rheumatoid vasculitis (3), membranoproliferative glomeluronephritis (4), and hypocomplementemic urticarial vasculitis syndrome (5). C1qAb belonging to both IgG and IgA classes have been described (2, 3). Comparison of age-distribution of C1qAb in general population and in SLE patients suggests a role of these autoantibodies in the pathogenesis of the disease (6). The presence and high amount of C1qAb are associated with lupus nephritis (7), a rise in the titer of these autoantibodies predicts the development of renal involvement and relapses in SLE (7, 8).

Several types of interactions between C1q and the human immunodeficiency virus (HIV) and its recombinant proteins have been described. Physically purified HIV-1 virions and recombinant transmembrane glycoprotein gp41 bind purified C1q and activate complement (9, 10). Thielens *et al.* (11) further characterized the C1q–gp41 interactions. They demonstrated that C1q binding to gp41 is dose-dependent, saturable, and comparable with the C1q fixation to the immune complexes. In C1q, the gp41 binding site appears to be at the junction between the collagen-like stem and globular heads (11). In the gp41, the main C1q-binding site is located within the immunodominant domain (HIV-env 590–620) (9), but two additional C1q binding sites exist in the extracellular region of the HIV protein (12). The C1q binding and activation can markedly be increased by anti-gp41 antibodies (10).

Activation of the macromolecular C1 by the HIV-1 particles and binding of C1q to these particles may result in the development of C1q-coated virions that may facilitate the formation of C1qAb. This possibility was strongly supported by the observations of Stoiber *et al.* (12) on the functional and structural similarities of C1q and gp120, the external glycoprotein of HIV-1. The two proteins were found to compete for the same binding sites on gp41 (12). In addition, rabbit antibod-

1521-6616/99 $30.00
Copyright © 1999 by Academic Press
All rights of reproduction in any form reserved.

ies to human C1q recognized gp120 and rabbit antibodies to gp120 cross-reacted with C1q (12). Based on these results, Stoiber *et al.* (12) postulated the possibility of development of C1qAb in HIV infection and found nine sera from HIV-infected subjects to agglutinate erythrocytes coated with antibody and C1q (EAC1q). Metlas *et al.* (13) found a sequence similarity between V3 loop of gp120 of an HIV-1 isolate and collagen-like region of C1q, and also raised the possibility of formation of C1qAb in AIDS. They found antibodies from sera of AIDS patients purified on a V3 affinity column to bind C1q molecules as well.

In spite of these indirect evidences for the potential development of C1qAb in HIV-infection, to our best knowledge no systemic titration of C1qAb in the sera of HIV-infected subjects has been reported.

In the present study we have determined the amount of C1qAb in sera of 132 HIV-infected individuals and as control, in 140 HIV-seronegative healthy subjects. As an extension of our previous studies (14, 15) we have measured autoantibodies against 60-kDa family heat shock proteins (hsp) and to Fab and $F(ab')_2$ moieties of human IgG in a subset of sera tested for C1qAb. Recently we have found that human monoclonal anti-gp41 antibodies can markedly enhance HIV infection in the presence of purified C1q (16). Therefore we have studied whether the presence of C1qAb affect complement-mediated antibody-dependent enhancement/neutralization (C′-ADE), which in our previous studies (17–19) was found to strongly correlate with immunosuppression and disease in HIV patients.

MATERIALS AND METHODS

Patients and Serum Samples

Three different series of measurements were performed: (i) Serum samples were taken from 132 HIV-seropositive patients who were enrolled in the study after informed consent. Clinical data and CD4+ cell counts were registered at each visit of the patients. Thirty-six, 33, 32, and 31 patients were in CDC stage II, III, IVa, or IVc stage of HIV disease, respectively. Levels of C1qAb were assessed in 132/132 sera, antibodies against hsp65 were determined in 120/132 sera, while anti-hsp60 autoantibodies and autoantibodies to Fab and $F(ab')_2$ fragments of human IgG were measured in 72/132 patients. One-hundred and forty serum samples from age- and gender-matched healthy subjects were applied as controls. (ii) Forty-nine of 132 patients were followed up for 13 (range 11–41) months for studying the changes in the C1qAb in parallel to the CD4+ cell counts. Two serum samples, one taken at the beginning and a second one taken approximately 1 year later were enrolled from each patient. (iii) In order to investigate the effect of the presence of C1qAb on the course of the HIV disease, 27 patients were longitudinally followed-up for 65 (range: 15–75) months. In a subset of these longitudinally tested patients viral load and complement-mediated antibody-dependent HIV-infection enhancement were also determined.

Antibodies, Heat-Shock Proteins, Purified C1q

Cross-reaction between 60/65-kDa heat-shock proteins and C1q were studied by using goat anti-human C1q antibodies (IgG) (Incstar Corp., Stillwater, MN). As control goat IgG-type antibodies against C1-inhibitor and C4b (Incstar Corp., Stillwater, MN) were used. The recombinant heat-shock protein 65 preparation (*M. bovis* BCG 65K, batch MA14) was kindly provided by Dr. M. Singh (GBF, Braunschweig, Germany, supported by the UNDP/World Bank/WHO Special programme for Research and Training in Tropical Diseases (TDR)). Human recombinant hsp60 (SPP-740) was purchased from StressGen (Victoria, Canada). C1q was purified as described by Arlaud *et al.* (20).

Measurement of C1qAb

C1qAb levels were measured as described by Siegert *et al.* (3). Briefly, 100 μl/well of C1q (2 μg) in coating buffer (0.1 M Na_2CO_3, pH 9.6) were incubated in a 96-well microtiter plates (Titertek, Zwanenburg, The Netherlands) for 2 h at 37°C. After three washes with phosphate-buffered saline (PBS) containing 0.05% Tween (PBS-Tween), serum samples diluted 1/25 in PBS-Tween with 1% newborn calf serum (NBCS) and 1 M NaCl were incubated in duplicate for 1 h at 37°C. After three more washes bound IgG was detected with mouse monoclonal anti-human IgG using the biotin-streptavidine enhancement system. After three washes *o*-phenylene-diamine (Sigma Chemical Co., St. Louis, MO) and H_2O_2 were added and 30 min later the reaction was terminated by addition of 25 μl H_2SO_4 per well. Absorbance at $\lambda = 492$ nm was measured on a spectrophotometer (Titertek, Zwanenburg, The Netherlands). IgG anti-C1qAb were expressed as units/ml (U/ml) related to a standard serum.

Measurement of HIV-1 RNA Levels in the Plasma Samples of the Patients

The HIV-1 RNA quantification was performed by using the AMPLICOR monitor test (Roche, Basel, Switzerland) according to the manufacturer's instructions. Briefly, 200 μl plasma was lysed by GuSCN solution and a quantification standard was added. RNA was precipitated by isopropanol, washed, and dissolved in 400 μl specimen diluent. Fifty microliters (equivalent to 25 μl plasma) was reverse transcribed

and amplified using the rTth thermostable polymerase with the biotinylated SK 462/431 primers (21). The amplified products from the HIV target RNA and the quantification standard were measured using an avidin/HRP ELISA type colorimetric reaction in microwell plates coated with HIV-specific probe and quantification standard specific probes in different wells. The level of HIV-1 RNA in the plasma samples of patients was estimated by linear extrapolation using the value of the quantification standard RNA and assuming that the standard curve intercepted at the origin. The AMPLICOR monitor test has a detection limit of 200 copies HIV-1 RNA per milliliter (21).

Enzyme-Linked Immunosorbent-Assay (ELISA) for the Detection of Anti-hsp60/65 Antibodies and Goat Anti-C1q Antibodies

Anti-hsp60/65 levels were measured as described before (14). Briefly, ELISA plates (Greiner, Germany) were coated with 0.1 μg/well recombinant human hsp60 or recombinant *M. bovis* hsp65. After washing and blocking (PBS, 0.5% gelatine), wells were incubated with 100 μl of serum samples diluted 1:500 in PBS 0.5% gelatine and 0.05% Tween 20. Binding of anti-hsp antibodies was determined using γ-chain specific anti-human IgG peroxidase labeled antibodies (Sigma) and *o*-phenylene-diamine (Sigma) detection system. The optical density was measured at λ = 490 nm (reference at λ = 620 nm) and means of duplicate wells were calculated. The amounts of anti-hsp60/65 antibodies were expressed as arbitrary units/ml (U/ml) related to a standard serum (StressGen SPA804, rabbit serum against hsp60). The same ELISA system was applied to assess binding of IgG-type goat anti-C1q antibodies and as control anti-C1-inhibitor and anti-C4b antibodies to recombinant hsp60 and hsp65. Plates were incubated with serially diluted goat anti-complement antibodies instead of patient's sera, and the amount of plate-bound antibodies was detected by rabbit anti-goat IgG antibodies (Incstar Corp., Stillwater, MN). The control experiment using anti-rabbit IgG polyclonal antibodies (Sigma, St. Louis, MO) and rabbit serum as source of IgG was carried out as described above.

Measurement of IgG and IgA Autoantibodies to Fab and F(ab')$_2$

Anti-Fab and anti-F(ab')$_2$ autoantibodies of the IgG and IgA isotypes were determined in specific ELISA assay as described previously (15).

Prediction of Secondary Structure

Chou-Fasman analysis (22) was applied on the hsp65 sequence of *Mycobacterium paratuberculosis* to predict the potentially antigenic β-turn sites. We have used the procedure and values of Prevelige and Fasman (23). Also tetrapeptide sequences were studied for their ability to form β-turn structure based on the probability of each amino acid to accept the first, second, third, or fourth position of β-turns (24). Tetrapeptide sequences with calculated product of the probability numbers for the amino acids higher than $7.5.10^{-5}$ were considered as predicted β-turn sites (24).

Synthesis of Peptides on the Tips of Polyethylene Pins

To encompass the predicted epitopes of hsp65, 46 decamer peptides with an overlap of 5 amino acids were synthesized using Fmocβ-alanine-glycine ester derivatized pins obtained from Chiron Technologies (Australia). The Fmoc protecting groups were removed with 20% piperidine in DMF (v/v) for 20 min. The side chains were protected. As side chain protecting groups of the trifunctional amino acids *tert*-butyl for Ser, Thr and Tyr, trityl for His and Gln, *tert*-butyloxi for Asp and Glu, acetamidomethyl for Cys, and 2,2,5,7,8-pentamethylchromane-6-sulphonyl for Arg were applied. Coupling was performed with 1-hydroxy-benzotriazole/diisopropyl-carbodiimide method in DMF. After the final coupling cycle the Fmoc protecting group was removed and the N-terminus of the peptides was acylated using Ac_2O-DIEA-DMF 5:1.50 (v/v/v) for 90 min. The side chain protection groups were cleaved from the peptides with TFA containing 2.5% ethanedithiol and 2.5% anisole. The peptides were prepared in duplicates. As nonrelated, negative control GLAQGGGGGG peptide was also synthesized.

Enzyme-Linked Immunosorbent-Assay for Epitope Scanning of C1q Antibodies

Antibody binding to the hsp65 peptides immobilized on polyethylene pins was detected by using a modified ELISA. After blocking (PBS, 0.5% gelatine) pins were incubated with 150 μl of 1:500 diluted antibodies in PBS 0.5% gelatine and 0.05% Tween 20 for 1 h in room temperature. Binding of anti-C1q antibodies was determined using anti-goat IgG peroxidase-labeled antibodies (Incstar Corp., Stillwater, MN) and *o*-phenylene-diamine (Sigma) detection system. The optical density was measured at λ = 490 nm (reference at λ = 620 nm) and means of duplicate wells were calculated. To assess specificity of binding of anti-C1q antibodies to the hsp65 peptides, the antibody was preincubated with 30 μg/ml purified C1q for 1 h at 37°C and thereafter used as described above. Pins were used repeatedly after thorough cleaning by sonification in disruption buffer (PBS, 1% SDS, 0.1% 2-mercapto-ethanol).

Measurement of HIV Enhancement/Neutralization in the Presence of Complement

The neutralizing/enhancing effect of the serum samples in the presence of active human complement was assessed as described previously (17, 19). Briefly, MT-4 target cells were infected wit HIV-1_{IIIB} in the presence of 1:64 dilution of heat-treated sera to be tested and 1:4 final dilution of pooled fresh normal human serum (NHS) as complement source. RT-activity was measured in the culture supernatants. The results were expressed by an index value (CO E/N I) calculated by dividing the peak c.p.m. valued measured in the presence of the tested serum + NHS by the c.p.m. value measured in the control sample (HIV only). Index value of <0.5 was considered as neutralization, >2 as enhancement.

Statistical Analysis

Differences between the means of different groups were calculated by the one-sample *t* test, by the two-sample *t*-test with Welch's correction, by the nonparametric Mann–Whitney test, and by the nonparametric Wilcoxon signed rank test. Correlations were calculated by the nonparametric Spearman method, differencies among the frequency of parameters in the groups were assessed by the Fisher's exact test using the GraphPad Prism 2.0 software (San Diego, CA; www.graphpad.com; Serial No. GPA 21889-988).

RESULTS

Amount of Antibodies to C1q in the Sera of HIV-Seropositive and -Seronegative Subjects

Serum samples from 132 HIV-infected persons and from 140 HIV-seronegative healthy subjects were tested for C1qAb. The results of the titration performed with an ELISA were expressed in arbitrary units (U/ml) related to a standard serum (Fig. 1). Marked differences were observed between the two groups. Sera from the HIV-infected subjects contained C1qAb at significantly ($P = 0.0138$, two-sample *t* test with Welch correction) higher levels (0; 0–713 U/ml, median; range) than those from the HIV-seronegative control subjects (0; 0–233 U/ml). Since C1qAb could not be detected in the majority (124/140) of HIV-seronegative individuals, the 95th percentile of data (80.5 U/ml) was taken as the upper limit of normal values. The amounts of C1qAb exceeded this limit in 17/132 (13%) of the HIV-seropositive subjects but only in 7/140 (5.0%) of the HIV-seronegative individuals ($P = 0.031$; odd's ratio, 2.81 (1.13–7.01, Fisher's exact test)).

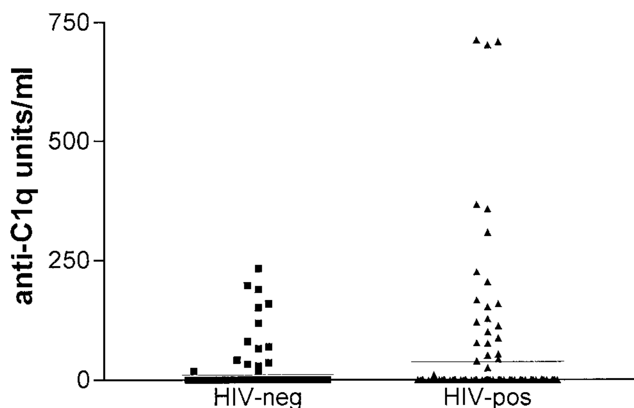

FIG. 1. C1qAb measured in the sera of HIV-seronegative persons ($n = 140$) and of HIV-infected individuals ($n = 132$). The difference between the two groups is statistically significant ($P = 0.0138$) as calculated by the two-sample *t*-test with Welch correction. Each point represents mean U/ml value of parallel measurements.

Changes in the C1qAb Titers at Longitudinal Investigation of HIV-Infected Subjects

C1qAbs measured in the first and last samples obtained in a longitudinal investigation lasting for 65 months (median value) in 27 HIV-infected subjects were compared. The amount of C1qAb was significantly ($P = 0.0342$ Wilcoxon test) lower in the last serum samples (0; 0–614) as compared to those measured in the first samples (0; 0–2502) obtained during the follow-up period. Seven patients exhibited an elevated level of C1qAbs; C1qAbs were detectable in 15/27 patients at the beginning of the observation period. CD4+ cell counts of the 27 patients declined from 409 ± 235 cells/μl to 242 ± 179 during the observation period, 10 patients remained untreated, 6 patients were treated with zidovudine monotherapy, 3 patients with ddI or ddC therapy, 8 patients received combination therapy (4 zdv/3TC, 4 zdv/ddC).

Lack of Correlation between C1qAbs and Clinical Course of HIV Disease

No significant difference in the occurrence of C1qAbs was found between the patients with CDC stage II (11/36, 30.5%), stage III (4/33, 12.1%), stage IVa (6/32, 18.8%), or stage IV c (5/31, 16.1%) HIV disease. Although there was a tendency ($P = 0.083$, Fisher's exact test) of the C1qAb to occur more frequently in the asymptomatic (CDC II) stage as compared to the symptomatic (CDC III, IVa, and IVc) stages. In 49 patients, two samples were taken at 13 (11–41) month intervals and tested for the presence of C1qAb. Sera of 12 patients contained C1qAb (Group A), while those from 37 patients did not contain C1qAb (Group B). Progression

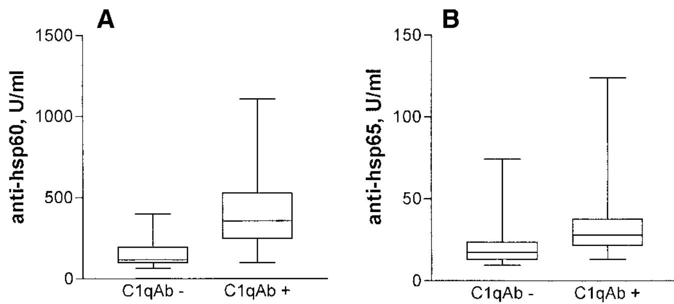

FIG. 2. Amount of anti-hsp60 (A) and anti-hsp65 (B) antibodies in sera with or without C1q antibodies. The difference between the two columns is significant in both cases: (A) $P = 0.013$, (B) $P = 0.036$, two-sample *t* test with Welch's correction. Box and whiskers diagram showing min., max., 25th–75th percentile and median values.

from stage II to stage III or from stage III to stage IV was observed in 5/12 (41.6%) and 11/37 (29.7%) patients of Group A and B, respectively (Fisher's exact test: $P = 0.48$). In 38 patients, CD4+ cell counts were determined at the time of both blood samples, which allowed study of the predictive value of the C1qAb measurement. During the 13-month follow-up period CD4+ cell counts decreased by 122.8 ± 73.5 and 104.3 ± 38.0 cells/μl in patients of Group A and B, respectively ($P = 0.945$).

Similarly, the presence of C1qAb did not predict the extent of the CD4+ cell decline in the 27 patients longitudinally tested for 65 months. In the 15/27 patients with detectable C1qAb at the beginning of the follow-up a CD4+ cell decline of 133.5 ± 47.1 cells/μl, while 12/27 patients without C1qAb a CD4+ cell decline of 185.2 ± 98.9 cells/μl were registered. The difference between the two groups was not significant. In 12 of 27 patients, the concentration of HIV-1 RNA was also determined in plasma samples obtained at the beginning and at the end of the observation period. No significant correlation between C1qAb and the viral load was found. In samples with ($n = 12$) and without ($n = 12$) C1qAb plasma HIV-1 RNA concentration of 14040 ± 8064 and 16320 ± 7642 copies/ml were measured: the difference was not significant.

Relationship of the C1qAb Titers to the Amounts of Other Autoantibodies

In 120/132 serum samples, titers of antibodies against mycobacterial hsp65 and, in 72/132 samples, titers of autoantibodies against human hsp60 as well as IgA and IgG class anti-Fab and anti-F(ab′)$_2$ antibodies were determined. The amounts of the hsp60/65 specific and other autoantibodies in the sera with and without C1Ab were compared to each other (Fig. 2; Table 1). In the case of hsp60-specific antibodies the difference was significant ($P = 0.013$): 11 serum samples with C1qAb contained 357 (100–1109, median, range) units/ml while the 61 serum samples without C1qAb contained 119 (66–398) U/ml anti-hsp60 antibodies (Fig. 2A). The amount of anti-hasp65 antibodies was also significantly ($P = 0.036$) higher in the 18 sera with C1qAb (28; 13–124 units/ml) than in the 102 sera with no C1qAb (17; 10–74 units/ml) (Fig. 2B). A strong positive correlation (Spearman correlation coefficient $r = 0.460$, $P < 0.0001$) was observed between the C1qAb- and the hsp60-specific antibodies, and to a lesser extent ($r = 0.272$), $P = 0.0023$) between C1qAb- and hsp65-specific antibodies.

By contrast, no significant difference was found between sera with or without C1qAb in the amounts of autoantibodies against Fab or F(ab′)2 fragments of IgG (Table 1).

Binding of anti-C1q Antibodies to 60-kDa Heat Shock Proteins

Since a highly significant positive correlation between C1qAb and anti-hsp60/65 antibodies was observed in the HIV-infected patients we found it of interest to test whether C1qAb cross-react with hsp60 or hsp65. ELISA plates were coated with recombinant *M. bovis* hsp65 or human recombinant hsp60 and different dilutions of goat antisera (IgG fractions) against C1q or against C1-inhibitor and C4b (as control) were added (Fig. 3). Anti-human C1q antibodies were found to bind to the plates coated with human hsp60 (Fig. 3A) and *M. bovis* hsp65 in a dose-dependent manner (Fig. 3B). Neither anti-C1-inhibitor nor anti-C4b antibodies

TABLE 1

Autoantibodies to Fab and F(ab′)$_2$ Fragments of Human IgG in the Sera of 72 HIV-Infected Patients with or without Autoantibodies to C1q (C1qAb) (Mean ± SEM OD × 1000)

	IgG a-Fab	IgG a-F(ab′)$_2$	IgA a-Fab	IgA a-F(ab′)$_2$
C1qAb absent ($n = 61$)	307 ± 31	813 ± 50	278 ± 38	538 ± 72
C1qAb present ($n = 11$)	471 ± 109	878 ± 158	262 ± 44	586 ± 204
P values (Mann–Whitney test)	0.064	n.s.	n.s.	n.s.

Note. n.s., not significant.

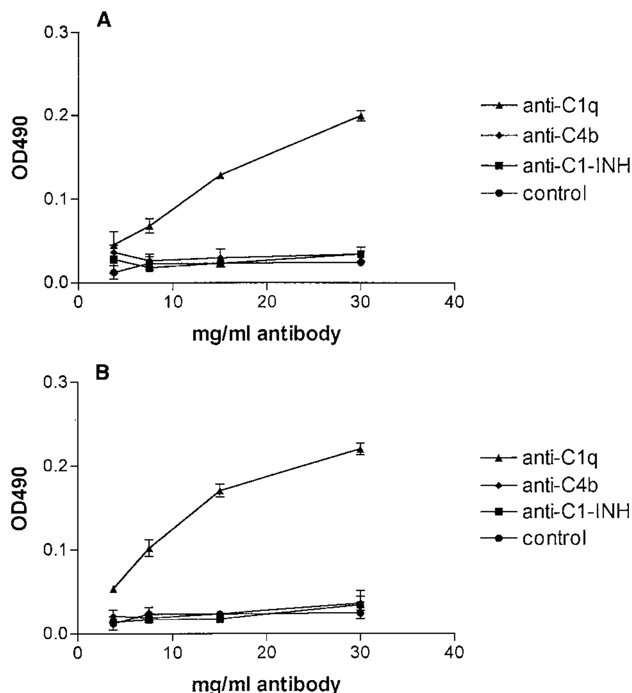

FIG. 3. Binding of goat anti-complement factor antibodies to solid phase human hsp60 (A) and *M. bovis* hsp65 (B). Control means uncoated plate. Each point represents mean ± SD value of two parallel measurements. The experiment was repeated three times with similar results.

were found to bind to the solid phase heat shock proteins.

Epitope Scanning of the Binding Site(s) for C1qAb on hsp65

Next, we have studied the binding of goat anti-C1q antibody to overlapping synthetic peptides immobilized on polyethylene pins, corresponding to nine different predicted epitope regions of *M. paratuberculosis* hsp65. O.D. values exceeding cut-off (2 × O.D. of the nonrelated negative control peptide) were considered as significant binding. The anti-C1q antibody bound to three of nine predicted epitope regions of the hsp65 (AA271–300, AA331–360, and AA451–475) (Fig. 4). Preincubation of the antiserum against C1q with purified human C1q fully abrogated the binding of C1q antibodies to all of the three epitopes of hsp65 (Fig. 4). Since C1q did not eliminated reactivity of a different antibody (anti-rabbit IgG) with an unrelated antigen (rabbit IgG, Fig. 5.) the inhibition seems to be specific. Based on these binding data three epitopes were located with AA 276–290, 341–355, and 456–475.

Correlation of the Autoantibodies Measured in the HIV Patients to the Complement-Dependent HIV-1 Enhancing Antibodies

C1qAb and other autoantibodies were measured in aliquots of sera which were previously tested for HIV-1 infection enhancement/neturalization in the presence of complement (C′-ADE). The extent of HIV-infection enhancement/neutralization (expressed by the CO E/NI index value, for details see Materials and Methods) was found to negatively correlate to the amounts of both C1qAb (Spearman correlation, $r = -0.274$, $P = 0.021$) and anti-hsp60 antibodies (Spearman correlation, $r = -0.309$, $P = 0.0076$). By contrast, the same index value positively correlated to the anti-Fab autoantibodies (e.g., $r = 0.449$, $P < 0.0001$, $r = 0.393$, $P < 0.0001$ for the IgA and IgG class anti-F(ab′)2 antibodies, respectively). Since this significant negative correlations do not necessarily give any sense of biological relevance we tested if the sera with or without C1qAb or above or below median level of hsp60/65-specific antibodies are more likely neutralizing or enhancing,

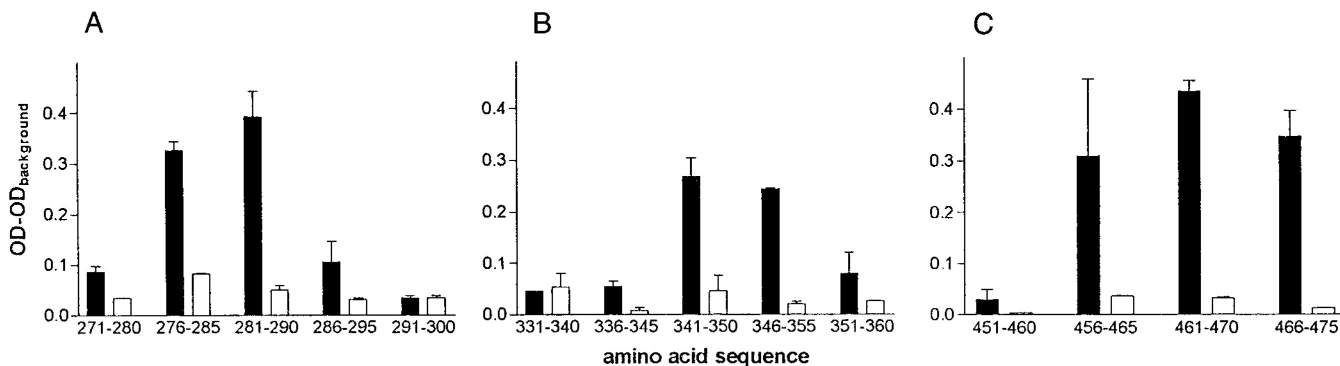

FIG. 4. Scanning of hsp65 protein from *M. paratuberculosis* for the binding sites to antibodies against human C1q. Binding sites for anti-C1q antibody were located in 271–300 (A), 331–360 (B), and in 451–475 (C) regions. Bars represent mean ± SD OD values of overlapping peptides, corrected with control OD values. ■ indicates no preincubation, □ indicates competition of antibody binding by 10 μg/ml C1q. The experiment was repeated three times with similar results.

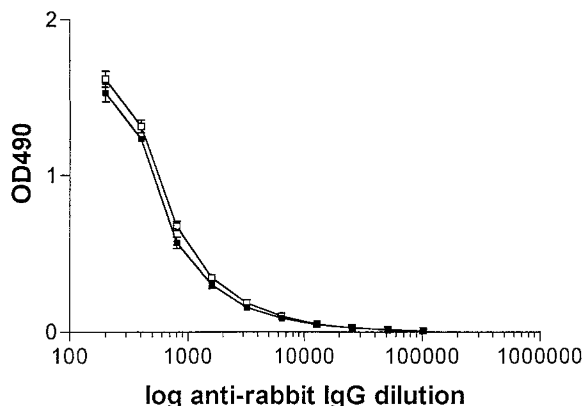

FIG. 5. Lack of inhibition of anti-rabbit IgG antibody binding to rabbit IgG after preincubation with 10 μg/ml C1q. Rabbit IgG was incubated with anti-rabbit IgG antibodies after preincubation with C1q (■) or with control PBS (□). Values represent mean and SEM of four parallel measurements.

respectively. As shown in Table 2, complement-dependent infection enhancement was observed less frequently while neutralization measured in the presence of complement occurred more frequently in the sera with C1qAb as compared to those without C1qAb. Similar findings were obtained in the case of anti-hsp60/65 autoantibodies (Table 2).

DISCUSSION

The amount of C1qAb was found to be significantly higher in the sera of the 132 HIV-infected individuals as compared to the 140 HIV-seronegative control subjects. Since 88% of the sera in the later group did not contain measurable C1qAb and the data did not exhibit a Gaussian distribution the 95th percentile of the data (80.5 U/ml) was considered as the upper limit of normal values. This value is very close to the mean + 2SD (85.73) U/ml) of the C1qAb measured in the 140 healthy control subjects in the present study or to the upper limit (mean + 2SD) of normal values (90 U/ml) of C1qAb calculated on the basis of a previous study (8). Sera of 13% of the HIV patients but only 5% of those from the HIV seronegative controls contained C1qAb in titers exceeding the upper limit of the normal values.

Presence of antibodies reacting with the solid phase C1q can be the consequence of a cross-reaction between antibodies to an HIV protein with C1q or the development of autoantibodies to the complement protein.

Of the specific antibodies against different HIV-1 proteins most probably anti-gp120 antibodies can react with solid phase bound C1q. Stoiber *et al.* (12) also demonstrated that antibodies isolated on a C1q-affinity column from HIV patient's sera reacted not only with C1q but also with HIV-1 gp120. Similar results were reported by Metlas *et al.* (13). Therefore it can be assumed that due to the homologies between the two proteins (12), anti-gp120 reacted with solid phase C1q in our system. Some observations of the present study, however, are difficult to reconcile with this assumption. Antibodies against gp120 can be detected in the sera of most HIV-infected persons (25) and titers are rather constant and decline only at the very advanced stage of HIV disease (25–28). By contrast, we did not find detectable C1qAb in almost 80% of all patients tested or in 70% of the 36 patients in the early (CDC stage II) stage of HIV disease, although anti-gp120 antibodies are expected to be present in high amounts in this stage. It should be noted that we cannot exclude that cross-reactive anti-gp120 antibodies are not detected by the C1qAb assay. However, it seems more probable, that autoantibodies to C1q in HIV infection are similar to those that develop in other diseases (1–4). HIV virion can activate C1 (9), interaction of the virion with anti-gp120 and atni-gp41 antibodies can lead to release of gp120. Consequently more C1q binding sites are available that might enhance C1 activation. In addition, C1q may bind to gp41 expressed on HIV-1-infected cells (29). Exposure of free C1q to the immune system may facilitate C1qAb formation. Clearly further experiments are needed to clarify the mechanism of C1qAb development in HIV infection.

TABLE 2

Frequency of Neutralization (CO E/N Index <0.5) or Enhancement (CO E/N Index >2) in Sera with or without C1q Antibodies and with High or Low Levels of Anti-hsp60/65 Antibodies

	Group	Neutralization CO E/NI <0.5	Enhancement CO E/NI >2	Fisher's exact test *P*, odd's ratio (CI)
C1qAb	0	8	13	0.0406, 3.79 (1.03–13.92)
	>0	14	6	
anti-hsp60	<Median	15	18	0.032, 3.6 (1.139–11.376)
	>Median	18	6	
anti-hsp65	<Median	15	18	0.032, 3.6 (1.139–11.376)
	>Median	18	6	

Nothing is known about the significance of C1qAb and their possible influence on the progression of HIV disease. One of us (C.S.) with others described two classes of autoantibodies (and other molecules) in HIV infection (30). One class of these antibodies such as antibodies against the Fab and $F(ab')_2$ moieties of human IgG molecules seems to be strongly associated with the progression of HIV disease (15, 31). Our previous (31) and present studies suggest that these antibodies correlate positively with the complement-dependent HIV-1 enhancing antibodies which, in turn, were found to be associated with a rapid progression of HIV disease (18, 19). C1qAb, however, did not correlate to the Fab and $F(ab')_2$ antibodies and the presence of C1qAb did not predict the rate of decline of CD4+ cell counts or the progression of HIV disease to a subsequent stage. Consequently it is likely that C1qAb does not belong to the autoantibodies associated with the progression of HIV disease.

The second class of autoantibodies (30) that develop in HIV infection like anti-anti-gp120 antibodies are dominant in the early phase of HIV disease and their titers decrease with the progression. In the present study, the amount of C1qAb significantly decreased during a 65-month follow-up period of the patients. In addition C1qAb tended to occur more frequently in the patients in early (CDC stage II) as compared to those in the late (CDC stage III or IV) stage of HIV disease. Previously (14), we have found similar changes with antibody to hsp60 and its amount was also found to negatively correlate to the titers of complement-dependent HIV-1 enhancing antibodies. In addition, the amount of C1qAb and anti-hsp60 antibodies correlated strongly. Therefore it seems probable that both the C1qAb and anti-hsp60 antibodies belong to the class II of the autoantibodies and other molecules described by Süsal *et al.* (30). These compounds might have a protective effect or at least capability to inhibit the progression-facilitating effect of class I autoantibodies. Recently (16) we have described a new type of HIV infection enhancement route. This was found to be dependent exclusively on the presence of antibodies and C1q. It is tempting to speculate that anti-C1q antibodies can abrogate this type of enhancement and inhibit in this way the disease progression-facilitating effect of C-ADE (18, 19).

Maybe the most surprising finding of the present study is the real cross-reaction of the goat anti-human C1q antibodies with the solid phase bound 60-kDa heat shock protein. We used overlapping peptides to determine binding sites in the hsp65 protein. As shown on Fig. 4, three of nine predicted epitope domains were recognized as specific binding epitopes for goat anti-human C1q antibodies. The binding was specific since it could be inhibited by purified C1q. We have analyzed the binding of anti-hsp autoantibodies present in IVIG (intravenous IgG) preparations prepared from plasma of several thousand blood donors and in HIVIG (IgG preparation prepared from sera of several hundred of asymptomatic HIV infected persons) to the same predicted epitope regions of hsp65. Interestingly enough, IVIG and HIVIG recognized the same three (AA271–300, AA331–360, AA451–475) epitope regions, but in addition a strong binding to five other epitope regions was also observed (Prohászka *et al.,* manuscript in preparation). This finding indicates that anti-hsp60 antibodies can be distinct of C1qAb. Experiments aiming the further study of the relationship between C1q and anti-hsp60 autoantibodies are in progress in our laboratories.

ACKNOWLEDGMENTS

We thank all subjects who participated in the study. This work was supported by the PECO'94 grant to the European Union Concerted Action "Development and Evaluation of Immunological and Virological Progression Markers to be Used for Monitoring of Therapy of HIV Infection"; by the 1/95, C4/96, and 204/1997 grants of the Hungarian National AIDS Committee and by the FKFP 0101/1997 (Ministry of Education) grant. We are indebted to Mrs. N. Verhagen, Margit Kovács, and Erika Farkas for their excellent technical assistance. The recombinant hsp65 *M. bovis* BCG 65K (batch MA14), was kindly provided by Dr. M. Singh, GBF, Braunschweig, Germany, financially supported by the UNDP/World Bank/WHO Special programme for Research and Training in Tropical Diseases (TDR). We are highly indebted for Drs. G. Arlaud and N. Thielens (Inst. Biol. Struct., Grenoble, France) for the kind gift of purified C1q.

REFERENCES

1. Uwatoko, S., Aotsuka, S., Owaka, M., Eugsa, Y., Yokohari, R., Aizawa, C., and Suzuki, K., Characterization of C1q binding IgG complexes in systemic lups erythematosus. *Clin. Immunol. Immunopathol.* **30,** 104–116, 1984.
2. Antes, U., Heinz, H.-P., and Loos, M., Evidence for the presence of autoantibodies to the collagen-like region of C1q in systemic lupus erythematosus. *Arthritis Rheum.* **31,** 457–464, 1984.
3. Sieger, C. E. H., Daha, M. R., van der Voort, E. A. M., and Breedvelt, F. C., IgG and IgA antibodies to the collagen-like region of C1q in rheumatoid arthritis. *Arthritis Rheum.* **33,** 1646–1654, 1990.
4. Strife, C. H., Leahy, A. E., and West, C. D., Antibody to a cryptic, solid phase C1q antigen in membranoproliferative nephritis. *Kidney Int.* **35,** 836–842, 1989.
5. Wisniewski, J. J., Baer, A. N., Christensen, J., Cupps, T. R., Flagg, D. N., Jones, J. V., Katzenstein, P. L., McPhadden, E. R., McMillen, J. J., and Pick, M. A., Hypocomplementemic urticarial vasculitis syndrome. Clinical and serological findings in 18 patients. *Medicine (Baltimore)* **74,** 24–41, 1995.
6. Siegert, C. E. H., Daha, M. R., Swaak, A. J. G., van der Voort, E. A. M., and Breedvelt, F. C., The relationship between serum titers of autoantibodies to C1q and age in the general population and in patients with systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **67,** 204–209, 1993.
7. Siegert, C. D. H., Daha, M. R., Tseng, C. M. E. S., Coremenans, I. E. M., van Es, L. A., and Breedveld, F. C., Predictive value of IgG autoantibodies against C1q for nephritis in systemic lupus erythematosus. *Ann. Rheum. Dis.* **52,** 851–856, 1993.

8. Coremans, I. E., Spronk, P. E., Bootsma, H., Daha, M. R., van der Voort, E. A., Kater, L., Breedveld, F. C., and Kallengerg, C., Changes in antibodies to C1q predict renal relapses in systemic lupus erythematosus. *Am. J. Kidney Dis.* **24,** 595–601, 1995.
9. Ebenbichler, C. F., Thielens, N. M., Vornhagen, R., Marschang, P., Arlaud, G. J., and Dierich, M. P., Human immunodeficiency virus type 1 activates the classical pathway of complement by direct C1 binding through specific sites in the transmembrane glycoprotein gp41. *J. Exp. Med.* **174,** 1417–1424, 1991.
10. Hidvégi, T., Prohászka, Z., Ujhelyi, E., Thielens, N. M., Dierich, M. P., Hampl, H., Arlaud, G., Nagy, K., and Füst, G., Studies on the mechanism of complement-mediated inhibition of antibody binding to HIV gp41. *Clin. Exp. Immunol.* **94,** 490–493, 1993.
11. Thielens, N. M., Bally, I. M., Ebenbichler, C. F., Dierich, M. P., and Arlaud, G. F., Further characterization of the interaction between the C1q subcomponent of human C1 and the transmembrane envelope glycoprotein gp41 of HIV-1. *J. Immunol.* **151,** 6583–6592, 1993.
12. Stoiber, H., Thielens, N. M., Ebenbichler, C., Arlaud, G. J., and Dierich, M. P., The envelope glycoprotein of HIV-1 gp120 and human complement protein c1q bind to the same peptides derived from three different regions of gp41, the transmembrane glycoprotein of HIV-1, and share antigenic homology. *Eur. J. Immunol.* **24,** 294–300, 1994.
13. Metlas, R., Skerk, V., Velikovic, V., Colombatti, A., and Pongor, S., Immunoglobulin-like domain of HIV-1 envelope glycoprotein gp120 encodes putative internal image of some common human proteins. *Viral. Immunol.* **7,** 215–219, 1994.
14. Prohászka, Z., Bánhegyi, D., Ujhelyi, E., Karádi, I., and Füst, G., Antibodies against 60kD heat-shock proteins in human immunodeficiency virus infection. *In* "Stress of Life from Molecules to Man" (Peter Csermely Ed.), Vol. 851, pp. 94–98, Ann. N.Y. Acad. Sci., 1998.
15. Süsal, C., Daniel, V., Doerr, C., Zimmerman, R., Huth-Kühne, H., and Opelz, G., IgA-anti-Fab antibodies and disease progression in AIDS. *Immunol. Letts.* **36,** 27–30, 1993.
16. Prohászka, Z., Nemes, J. Hidvégi, T., Tóth, F. D., Kerekes, K., Erdei, A., Szabó, J., Ujhelyi, E., Thielens, N., Dierich, M. P., Späth, P., Ghebrehiwet, B., Hampl, H., Kiss, J., Arlaud, G., and Füst, G., Two parallel routes of the complement-mediated antibody-dependent enhancement of HIV-1 infection. *AIDS* **11,** 949–958, 1997.
17. Tóth, F. D., Szabó, B., Ujhelyi, E., Pálóczi, K., Horváth, A., Füst, G., Kiss, J., Bánhegyi, D., and Hollán, S. R., Neutralizing and complement-dependent enhancing antibodies in different stages of HIV infection. *AIDS* **5,** 263–268, 1991.
18. Füst, G., Tóth, F. D., Kiss, J., Ujhelyi, E., Nagy, I., and Bánhegyi, D., Neutralizing and enhancing antibodies measured in complement-restored serum samples from HIV-1 infected individuals correlate with immunosuppression and disease. *AIDS* **8,** 603–609, 1994.
19. Prohászka, Z., Tóth, F. D., Bánhegyi, D., Ujhelyi, E., Kiss, J., Nemes, J., and Füst, G., Complement-Mediated Antibody-Dependent Enhancement of HIV Infection Correlates to Viral Load and Predicts Progression of HIV Disease. 12th World AIDS Conference, Geneva, Switzerland June 28–July 3, 1998, Abstract book, p. 265.
20. Arlaud, G. J., Sim, R. B., Duplaa, A. M., and Colomb, M. G., Differential elution of C1q, C1r and C1s from human C1 bound to immune aggregates. Use in rapid purification of C1 subcomponents. *Mol. Immunol.* **16,** 445–448, 1979.
21. Mulder, J., McKinney, M., Christopherson, C., Sninsky, J., Greenfield, L., and Kwok, S., Rapid and simple PCR assay for quantitation of human immunodeficiency virus type 1 RNA in plasma: Application to acute retroviral infection. *J. Clin. Microbiol.* **32,** 292–300, 1994.
22. Chou, P. Y., and Fasman, G. D., Prediction of protein conformation. *Biochemistry* **13,** 222–245, 1974.
23. Prevelige, P., and Fasman, G. D., Chou-Fasman prediction of the secondary structure of proteins. *In* "Prediction of Protein Structure and the Principles of Protein Conformation" (G. D. Fasman, Ed.), pp. 391–416, Plenum Press, New York/London, 1990.
24. Uray, K., Kajtár, J., Vass, E., Price, M. R., Hollósi, M., and Hudecz, F., The effect of solution conformation on antibody recognition of a protein core epitope from gastrointestinal mucin (MUC2). *Biochem. J.,* submitted.
25. Turbica, I., Posner, M., Druck, C., and Barin, F., Simple enzyme immunoassay for titration of antibodies to the CD4-binding site of human immunodeficiency virus type 1 gp120. *Clin. Microbiol.* **33,** 3319–3323, 1995.
26. Ebenbichler, C., McNearney, T., Stoiber, H., Most, J., Zangerle, R., Vogetseder, W., Patsch, J. R., Ratner, L., and Dierich, M. P., Sera from HIV-1 infected individuals in all stages of disease preferentially recognize the V3 loop of the prototypic macrophage-tropic glycoprotein gp120 ADA. *Mol. Immunol.* **32,** 1039–1045, 1995.
27. Lawoko, A., Johansson, B., Dash, R., Falch, L., Dietrich, U., Pipkorn, R., Nilehn, B., and Blomberg, J., Continuity and discontinuity in the anti-V3 IgG response of human immunodeficiency virus type 1-infected persons in a cross-sectional and longitudinal study using synthetic peptides. *J. Infect. Dis.* **172,** 682–690, 1995.
28. Langedijk, J. P., Zwart, G., Goudsmit, J., and Meloen, R. H., Fine specificity of antibody recognition may predict amino acid substitution in the third variable region of gp120 during HIV type 1 infection. *AIDS Res. Hum. Retroviruses* **11,** 1153–1162, 1995.
29. Marschang, P., Gütler, R., Tötsch, M., Thielens, N. M., Arlaud, G. J., and Dietrich, M. P., HIV-1 and HIV-2 isolates differ in their ability to activate the complement system on the surface of infected cells. *AIDS* **7,** 903–910, 1993.
30. Süsal, C., Daniel, V., and Opelz, G., Does AIDS emerge from a disequilibrium between two complementary groups of molecules that mimic the MHC? *Immunol. Today* **17,** 114–119, 1996.
31. Tóth, F. D., Süsal, C., Ujhelyi, E., Bánhegyi, D., Kiss, J., Daniel, V., Nagy, I., Opelz, G., and Füst, G., Comparative studies of antibodies that are associated with disease progression in HIV disease. *Immunol. Letts.* **41,** 33–36, 1994.

Received February 10, 1997; accepted September 18, 1998