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Immune responses to fractionated cytomegalovirus (CMV) antigens after HIV infection. Loss of cellular and humoral reactivity to antigens recognized by HIV^-, CMV^+ individuals

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SUMMARY

In order to delineate the molecular pathogenesis of the increased susceptibility to CMV disease in HIV infection, the patterns of antigen responsiveness in HIV-infected and non-infected individuals were investigated. CMV was fractionated by SDS–PAGE and electroblotted onto nitrocellulose. Lymphoproliferative responses of healthy HIV^-, CMV^+ individuals and HIV^+, CMV^+ asymptomatic patients to a whole CMV antigen preparation and to 20 fractions of nitrocellulose-bound CMV were then compared. Three fractions of approximate molecular weight of 130–165, 65–75, and 55–65 kD appeared to contain the major T cell stimulating antigens for HIV^-, CMV^+ individuals. A statistically significant depression of responses to fractions containing antigens in the ranges of 130–165 kD and 55–65 kD but not to whole CMV was seen in HIV^+ individuals compared with controls. In healthy controls, the sum of the proliferative responses as measured by ^{3}H-thymidine uptake to these three major fractions was approximately equal to the response to a whole CMV antigen preparation, whereas it was less than half of this response in five out of six HIV^+ subjects. When antibody activities to CMV antigens were analysed by immunoblotting of sera from the two subject groups and also sera of ARC and AIDS patients, a selective loss of reactivity was revealed in 10 out of 19 HIV^+ subjects to a band of 26–28 kD whereas all 15 HIV^-, CMV^+ controls recognized this band. Serum IgG and IgM values were both significantly higher in HIV^+ individuals than in controls. These findings suggest that specific lesions in the repertoire of immune responsiveness to CMV antigens occur in HIV^+ individuals.

Keywords cytomegalovirus HIV immunoblot T cells antibodies

INTRODUCTION

CMV is a major pathogen in patients who are immunosuppressed due to drug regimens required for transplantation (Quinnan *et al.*, 1982; Meyers *et al.*, 1983) or due to infection with HIV (Drew, 1988). CMV infection may even be a factor that predisposes HIV-infected individuals to progress more rapidly to AIDS (Webster *et al.*, 1989). In recent years it has been shown that cytotoxic lymphocyte function correlates with protection against CMV disease (e.g. interstitial pneumonia) in bone marrow transplant patients (Quinnan *et al.*, 1982). Antibody may also have a role in protective immunity against CMV, since prophylaxis of transplant patients has been successfully carried out by injection of CMV hyperimmune globulin (Meyers *et al.*, 1983; Condie & O'Reilly, 1984). These latter studies as well as studies carried out in the context of congenital disease (Stagno *et al.*, 1975) and CMV mononucleosis (Drew *et al.*, 1981) indicate that both the cellular and humoral arms of the immune response have a significant role in protection against the development of disease due to CMV.

In vitro studies of immune responses to CMV have shown that whole viral antigens and also a purified 72-kD antigen stimulate the proliferation of T cells from healthy individuals and induce cytotoxic cell activity against infected fibroblast targets (Converse *et al.*, 1983; Rodgers *et al.*, 1987). Molecular weight analyses of CMV structural proteins such as the matrix and capsid polypeptides have been performed by some investigators (Gibson, 1983; Landini *et al.*, 1985). Immunoblot analyses have demonstrated that serological responses in primary CMV infection are directed against several viral antigens (Gold *et al.*, 1988). Most HIV^+ patients, in particular homosexual men, have high antibody titres to CMV (Drew *et al.*, 1981). Employing the technique (Young & Lamb, 1986; Abou-

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Zeid *et al.*, 1987) of screening for T cell epitopes that might be present on electroblotted CMV antigens, we report here the repertoire of lymphoproliferative responses in healthy individuals and the particular deficits in HIV$^+$ asymptomatic individuals. In addition, data are presented from serological CMV immunoblots showing a frequent loss of reactivity to a 28-kD antigen in sera of HIV$^+$ individuals.

MATERIALS AND METHODS

Subjects

Six HIV$^-$ healthy subjects and six HIV$^+$ individuals, outpatients at the Roslagstull Hospital, infected for a period of 2–8 years without symptoms of AIDS or ARC, all seropositive for CMV (reciprocal ELISA titre 10 000) with positive lymphoproliferative responses to whole CMV antigens, were tested for responses to fractionated CMV antigens. CD4:CD8 ratios (determined at the National Bacteriology Laboratory, Solna, Sweden) of the HIV$^+$ subjects ranged from 0·4 to 0·77; absolute CD3 values ranged from 1·17 to $3{\cdot}81 \times 10^9/l$ and CD4 values ranged from 0·41 to $0{\cdot}95 \times 10^9/l$. The patients included two homosexual men, three drug-abusing men, and one drug-abusing woman. Nine additional HIV$^-$, CMV$^+$ healthy controls and 13 additional HIV$^+$, CMV$^+$ individuals (six asymptomatic, five with ARC and two with AIDS) were tested by immunoblots of CMV antigens.

Antigens

Whole CMV antigen for lymphocyte stimulation assays was prepared essentially as previously described (Converse *et al.*, 1983). Briefly, human embryonic lung fibroblast cells were infected with strain AD169 and CMV and incubated until >95% cytopathic effect was observed. The supernatant was removed and cells were gently trypsinized to detach them from the surface of the flask, spun down at 450 ***g*** for 10 min and washed in phosphate-buffered saline (PBS). The cells were then fixed in 0·8% glutaraldehyde as described (Converse *et al.*, 1983). Mock-infected fibroblasts were prepared in a similar manner.

CMV antigen for SDS–PAGE and immunoblots was prepared as above, except that only the supernatants of CMV-infected cell cultures were used as a source of antigen. The supernatants of multiple flasks were pooled, spun at 750 ***g*** for 10–15 min and then ultracentrifuged at 10^5 ***g***, in a Sorvall OTB-2, for 150 min. The pelleted material was washed and resuspended in PBS. The pellet was stored with protease inhibitors (aprotinin, 0·1 TIU/ml, PMSF, 25 μg/ml) at −20°C until use, at which time it was boiled for 5 min in final sample buffer (20% glycerol, 4% SDS, 1% 2-mercaptoethanol, and 25% stacking gel buffer) and spun down to pellet insoluble material. Samples (350 μg protein determined by Pierce BCA assay) were applied to a 12-cm wide well in a 5% stacking gel and resolved on a 10% polyacrylamide 0·75 mm slab gel cross-linked with 2·6% *bis* acrylamide. The samples were electrophoresed until the tracking dye reached a distance of 8 cm. Following separation the gels were either silver-stained using the method of Morrissey (1981), or electrotransferred onto nitrocellulose (Sartorius, 0·2 μ) using high-concentration glycine buffer as described elsewhere (Fehniger *et al.*, 1990). The antigen-coated nitrocellulose sheets were air-dried and processed for lymphocyte proliferation assays or for immunoblotting.

Preparation of antigens for lymphocyte assays

Fractionated antigens for lymphocyte stimulation were prepared essentially as described by Abou-Zeid *et al.* (1987). Twenty 4-mm^2 fractions plus control nitrocellulose were cut from CMV blots, dissolved in one ml dimethyl sulphoxide, and the microparticles were precipitated by addition of carbonate/bicarbonate ELISA buffer, pH 9·6. After three washes in RPMI 1640 containing 100 U/ml penicillin and 100 μg/ml streptomycin, the particles were resuspended in a final volume of 1 ml. It was determined that 5–10 μl of this suspension gave sufficient and reproducible stimulation of $1{-}2 \times 10^5$ mononuclear cells from CMV-seropositive individuals in a final volume of 100 or 200 μl, respectively.

Lymphocyte responses

Peripheral blood mononuclear cells (PBMC) were separated by Ficoll–Paque, washed and resuspended to a final concentration of 10^6/ml as described (Converse *et al.*, 1988). Cells (100 μl) were added to round-bottomed microtitre wells containing 5 μl nitrocellulose-bound antigens or whole CMV-infected or non-infected fibroblasts (100/well). The cells were kept in a humidified atmosphere of 5% CO_2 in air at 37°C for 6 days, at which time ^{3}H-thymidine (1 μCi/well) was added for 18 h. Cells were harvested onto filter paper and then prepared for counting in a Beckman 7000 liquid scintillation counter (Converse *et al.*, 1988).

Immunoglobulin levels

Determination of serum IgM and IgG levels (mg/ml) was carried out using a rocket electrophoresis technique (Laurell, 1972).

Immunoblot analysis

Serum antibody reactivity with nitrocellulose-bound antigen strips was determined as described elsewhere (Fehniger *et al.*, 1990). Strips were blocked with 0·05% Brij 58 detergent (Sigma, St Louis, MO) Tris-buffered saline (TBS, 50 mM, pH 7·4), incubated overnight at room temperature with human serum samples diluted 1:40 in Brij/TBS, washed in the same buffer, incubated with peroxidase-conjugated rabbit anti-human IgG, IgM, IgA (heavy and light chain specific, diluted 1:200, Dakopatts) for 2 h, washed, incubated for 1 h with a 1:100 dilution of peroxidase-conjugated swine anti-rabbit IgG (Dakopatts), finally washed with Brij/TBS, and then developed with the substrate solution (final concentration 0·01% 4-chloronaphthol (Sigma) dissolved in dimethylsulphoxide [1%] and 0·003% H_2O_2, in TBS, pH 7·4) for 10 min.

ELISA

ELISA for CMV seroreactivity was carried out using a method (Sundqvist & Wahren, 1981) detecting responses to nuclear antigens (ELISA-1). A second ELISA test (ELISA-2) was developed using the same antigen as in the immunoblot assay described above. Briefly, 100 μl containing 400 ng of SDS solubilized CMV in 0·5 M carbonate buffer, pH 9·6, were coated onto wells of plates overnight at 4°C. The wells were then blocked with 200 μl Brij/TBS for 30 min. Test serum (50 μl) diluted in Brij/TBS was added to the wells in triplicate and

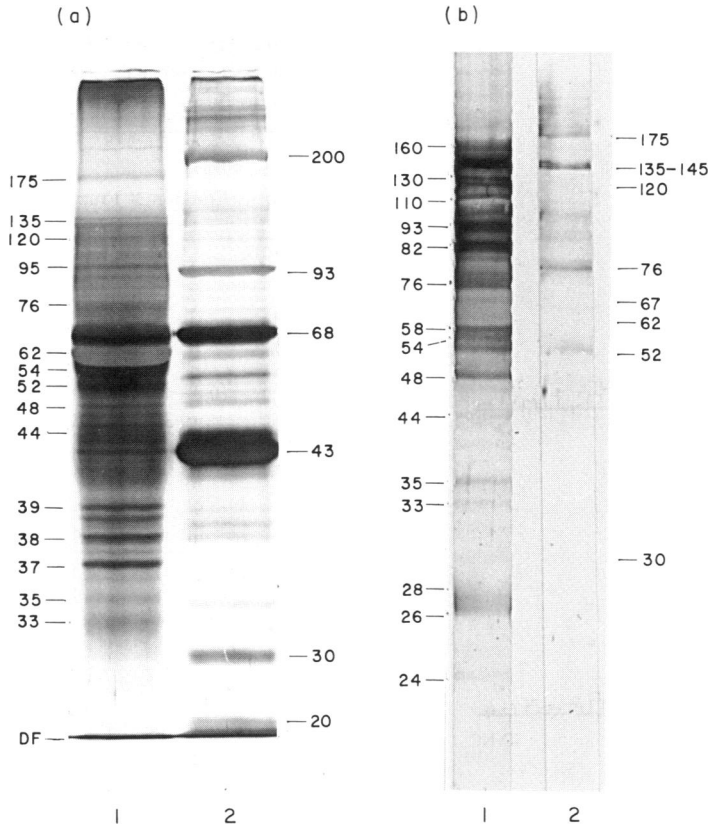

Fig. 1. Demonstration of fractionated cytomegalovirus (CMV) antigens by silver stain and immunoblotting. (a) Silver-stained 10% SDS–PAGE gel showing 1, CMV strain AD-169 proteins (DF, dye front) and 2, molecular weight protein markers (Pharmacia low range and myosin); (b) representative immunoblots of healthy subjects; lane 1, CMV ELISA seropositive individual, lane 2, CMV ELISA seronegative individual.

incubated at 37°C for 60 min. After washing, the wells were incubated with alkaline-phosphatase-conjugated goat anti-human IgG (1:1000, Calbiochem) for 60 min at 37°C, again washed and then developed in a substrate solution of 1 mg/ml *p*-nitrophenyl phosphate in 10% diethanolamine, pH 9·8, for 30 min. The reaction was stopped by adding 50 μl of 3 M NaOH to each well. The plates were read at an absorbance of 405 nm in a Labsystems Multiskan Plus Reader.

Statistical analysis

Data are presented as the arithmetic mean ± s.e.m. of triplicate wells. The Student's *t*-test, Mann–Whitney *U*-test and Wilcoxon rank sum test were used to test for significant differences between stimulating fractions and subject groups as appropriate. Significant correlations were determined by linear regression analysis.

RESULTS

Cytomegalovirus proteins

Silver staining of the SDS–PAGE gels used for fractionating CMV revealed multiple protein bands (Fig. 1a). There were major bands at 55–75 kD presumably corresponding to the CMV matrix proteins (Gibson, 1983) and numerous bands, in the range of 33–135 kD. Representative immunoblots of HIV$^-$ individuals are shown in Fig. 1b. Serum from CMV ELISA seronegative subject (lane 2) showed weak to moderate reactivity with (probably cross-reactive, non-specific for CMV) bands at about 175, 135, 78 and 52 kD. The CMV ELISA seropositive subject (lane 1) reacted strongly to over 20 bands including those of 175, 135–145, 76, and 48 kD as well as to a broad band of 26–28 kD that was just visible in the original silver-stained gel. In contrast, there was no significant antibody reactivity to four CMV proteins between 37 and 39 kD which were distinctly stained on the silver-stained gel.

Lymphocyte responses to whole and fractionated CMV

A remarkably consistent pattern with little intra- or inter-individual variation was observed in PBMC responses to the 20 fractionated CMV antigens in HIV$^-$, CMV-seropositive individuals. The principal fractions to which responses were observed were numbers 3, 7, and 8, corresponding to M_r of 130–165, 65–75, and 55–65. Figure 2a shows the response to these fractions of a repesentative individual and Table 1 shows the results for all six CMV-seropositive control subjects. Responses to the matrix proteins (M_r 55–75) presumably present in fractions 7 and 8 appeared to account for the major part of the response to whole CMV-infected fibroblasts. Mean Δ ct/min were 8·6, 19·6, and 43×10^3 in the control subjects. Together the sum of the responses to the three fractions was $\geq 80\%$ of the response to the whole CMV antigen at the time of cell harvesting. Responses to non-infected fibroblasts were comparable to responses to control nitrocellulose or medium alone in all cases. Lymphocytes of CMV seronegative subjects did not respond to CMV-infected fibroblasts, indicating that

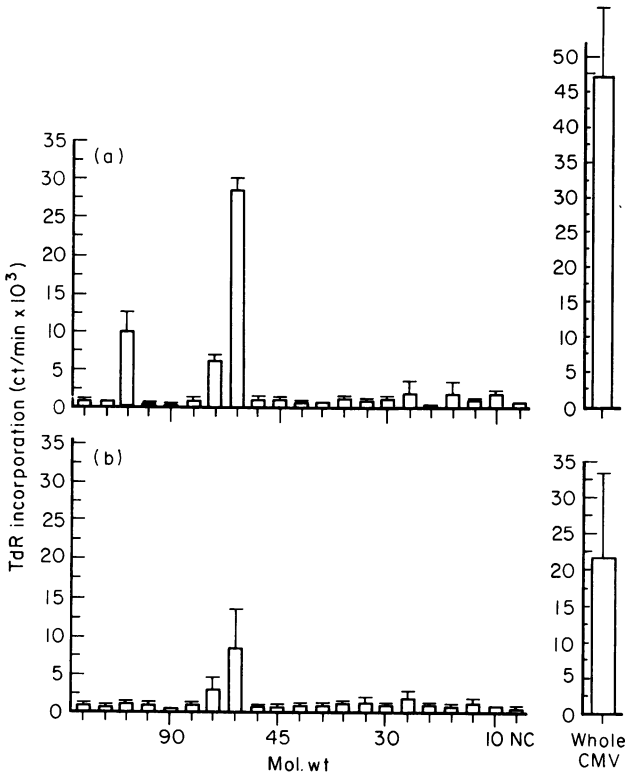

Fig. 2. Pattern of proliferative responses of mononuclear cells to SDS-PAGE fractionated antigens of cytomegalovirus (CMV) electroblotted onto nitrocellulose (NC). Response to NC control is shown adjacent to fraction 20. Fractions 3, 7, and 8 contained proteins of 130–165, 65–75, and 55–65 kD, respectively. (a) Response pattern of a representative HIV$^-$, CMV$^+$ subject; (b) response pattern of a HIV$^+$, CMV$^+$, asymptomatic individual.

Table 1. Lymphoproliferative responses of six healthy HIV$^-$ controls and six HIV$^+$ asymptomatic patients to the principal stimulating fractions of cytomegalovirus (CMV), whole CMV, and nitrocellulose (NC) alone

Group	Fraction 3	Fraction 7	Fraction 8	NC alone	Whole CMV
Controls					
1	7·6±1·3 (7·2)	8·5±1·3 (8·1)	24·8±0·2 (24·4)	0·4±0·1	34·0±0·9 (33·6)
2	24·5±4·9 (24·1)	59·5±19·5 (59·1)	92·4±9·1 (92)	0·4±0·1	93·1±2·2 (92·7)
3	10·0±1·9 (9·3)	6·1±0·6 (5·4)	28·5±1·2 (27·8)	0·7±0·6	49·4±11·3 (48·7)
4	1·6±0·3 (0·6)	2·5±0·6 (2·0)	8·2±4·7 (7·7)	0·5±0·1	13·2±2·9 (12·7)
5	4·9±1·9 (4·4)	23·8±6·1 (23·3)	63·6±5·7 (63·1)	0·5±0·1	68·3±5·6 (67·8)
6	6·4±1·8 (5·7)	20·5±14·9 (19·8)	44·4±14·7 (43·7)	0·7±0·3	71·2±14·4 (70·5)
HIV$^+$					
1	1·1±0·2 (0·6)	3·1±1·1 (2·6)	8·5±3·8 (8·0)	0·5±0·1	21·7±8·8 (21·2)
2	2·9±0·5 (0)	2·2±0·3 (−0·7)	8·8±0·1 (5·9)	2·9±1·1	25·9±4·8 (23·0)
3	0·3±0·1 (−0·3)	2·2±0·9 (1·6)	1·2±0·5 (1·6)	0·6±0·2	17·8±3·9 (17·2)
4	2·2±0·6 (2·0)	12·1±2·3 (11·9)	15·9±2·2 (15·7)	0·2±0·1	102·8±12·6 (102·6)
5	2·3±0·8 (1·6)	4·5±3·9 (3·8)	3·6±1·3 (2·9)	0·7±0·2	40·1±4·3 (39·4)
6	15·7±4·7 (9·7)	59·0±22·0 (53·0)	43·1±32·9 (37·1)	6·0±1·7	48·3±12·9 (42·3)

Responses to fractions 3 and 8 expressed relative to the response to whole CMV are significantly depressed in HIV$^+$ individuals compared with controls ($P<0·05$ and $P<0·025$, respectively, Wilcoxon rank sum test).

Data are presented as mean ct/min × 10^3 ± s.e.m. In parentheses are the mean Δ ct/min × 10^3 compared with NC alone.

neither infected nor non-infected fibroblasts express sufficient MHC class II molecules to induce an alloreactive response (Converse *et al.*, 1983).

The proliferative responses of six HIV$^+$ asymptomatic subjects were tested using the same antigen preparations as those above. Paired comparisons showed that responses to whole CMV antigen were not significantly different from responses of healthy controls (Table 1).

Responses to the putative matrix proteins present in fractions 7 and 8 (55–75 kD range) were observed, at least weakly, to at least one of the two fractions in all of the patients tested (Fig. 2b shows the pattern seen in one of these patients). The response to fraction 8, however, was significantly lower in HIV$^+$ than in HIV$^-$ individuals (Wilcoxon rank sum test, $P<0·025$) and there was a similar tendency observed in the response to fraction 7. Similarly, there was a low or absent response to fraction 3 in five of the six HIV$^+$ subjects and this response was also significantly lower than in the HIV$^-$ controls ($P<0·05$). In the five patients the response to fraction 3 ranged from 1% to 5% of the response to whole CMV with one exception whose response to nitrocellulose alone was as high as to that fraction. In contrast, the response to fraction 3 in the healthy controls ranged from 7 to 27% of the response to whole CMV. Cells from the sixth HIV$^+$ individual displayed a high background activity (Table 1). The sum of the responses to fractions 3, 7, and 8 in the HIV$^+$ patients ranged from 19% to 53% in five of the six patients; in the sixth patient the sum of the responses to the fractions was greater than the response to whole CMV antigen, as also observed in three of the six control subjects. Statistically significant ($P<0·01$) correlations were found between absolute CD3 and CD8 but not CD4 numbers and proliferative responses to whole CMV antigen.

Immunoglobulin evaluations

HIV$^+$ patients as a group or asymptomatic patients alone had significantly higher levels of both serum IgG ($P<0·0003$) and IgM ($P<0·04$) than did healthy HIV$^-$ controls ($n=8$). In the healthy control group IgG values (mg/ml) were 11·5±1·0 and IgM values 1·6±0·2 while in the 12 asymptomatic patients the values were 20·7±1·7 and 3·0±0·3, and in the seven ARC/AIDS patients these were 18·9±2·1 and 1·7±0·4, respectively.

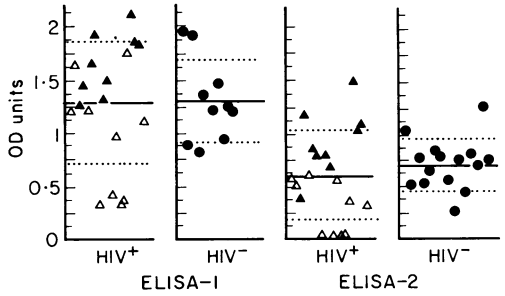

Fig. 3. Reactivity to cytomegalovirus (CMV) nuclear antigens (ELISA-1), CMV virions (ELISA-2), and CMV p28 in HIV$^+$ subjects and CMV$^+$, HIV$^-$ healthy controls. All 19 HIV$^+$ individuals tested were CMV seropositive at a reciprocal serum dilution of at least 1000 by ELISA-1. The figure shows optical density (OD) values obtained with sera diluted 1:1000 (ELISA-1) or 1:250 (ELISA-2). Of the four HIV$^+$ individuals who were negative by ELISA-2 at the 1:250 dilution, two were asymptomatic, one had ARC and one had AIDS. Solid symbols indicate sera that were positive and open symbols those that were negative for reactivity to CMV p28 in immunoblot analyses. Mean ± 3 s.d.

CMV ELISAs

All 19 HIV$^+$ individuals evaluated in the ELISA-1 detecting CMV nuclear antigens were positive at reciprocal serum dilutions of 1000–100 000. All healthy CMV$^+$ controls had titres from 1000 to 10 000. Healthy controls ($n=7$) were considered negative in this system at dilutions of 100 or less. All ELISA-1 CMV$^+$ healthy controls were clearly positive at reciprocal serum dilutions of 250 in ELISA-2 using the CMV virion antigen as were all HIV$^+$ patients who were positive in ELISA-1 at dilutions of $\geq 10\,000$. There was a highly significant ($P=0{\cdot}0001$) correlation between the two ELISAs. There was a much wider range of OD values for HIV$^+$ individuals but there was no statistically significant difference observed between the mean points for HIV$^+$ and HIV$^-$ individuals (Fig. 3). However, within the HIV$^+$ individuals significantly lower ($P<0{\cdot}025$) mean OD values were found in the group that lacked antibody in immunoblots to p28 (see below).

CMV immunoblot analyses

Sera of 15 HIV$^-$, CMV$^+$ healthy individuals and the 19 HIV$^+$ patients in various stages of infection were analysed by immunoblotting to determine the pattern of recognition of CMV antigens. The analysis showed an apparent loss or reduction of reactivity against three of the 22 serologically detectable antigenic bands. Most notably 10/19 HIV$^+$ subjects (six out of 12 asymptomatic and four out of seven ARC/AIDS) had no seroreactivity to a broad band of 26–28 kD whereas all 15 HIV$^-$, CMV$^+$ individuals clearly recognized this band (Table 2 and Fig. 3). On a scale of 0–3, the reactivity of 19 HIV infected individuals was significantly less intense when compared with 15 healthy CMV seropositive subjects to the bands of 120, 54, and 26–28 kD ($P<0{\cdot}001$, 0·02, and 0·02, respectively). A positive correlation with ELISA titre and band recognition was found within the HIV$^+$ group but, remarkably, some patients with ELISA titres of $\geq 10\,000$ still completely lacked reactivity with the 26–28 kD band (Figs 3 and 4). A band of 33 kD was clearly recognized by all three ARC/AIDS patients who had symptoms compatible with ongoing CMV infection but only by one asymptomatic patient in this group; all four had reciprocal ELISA titres of $\geq 50\,000$. Weak reactivity against the 33-kD antigen was seen in nine out of the 15 HIV$^-$, CMV$^+$ individuals and in four asymptomatic patients, only one of whom had a high ELISA-1 titre. One asymptomatic patient with a titre of 100 000 by ELISA-1 did not react with the 33-kD band. However, none of the observations regarding this band were statistically significant. No correlations with serum IgG or IgM levels and band recognition were observed. No correlations of band recognition with T cell numbers or CD4:CD8 ratios or other disease parameters were detected in the HIV$^+$ group.

Table 2. Percentage of individuals showing clear reactivity to cytomegalovirus (CMV) antigens detected by immunoblotting

Molecular weight (kD)	Asymptomatic ($n=12$)	ARC/AIDS ($n=7$)	Healthy CMV$^+$ ($n=15$)
175	17	43	33
160	42	57	67
135–145	100	100	100
130	33	29	53
120	25	29	67
110	58	43	80
93	42	72	73
82	75	57	73
78	50	14	60
76	58	57	67
67	50	57	67
62	58	72	86
58	42	72	47
54	25	29	40
52	58	86	53
48	42	43	67
44	8	14	20
35	0	29	7
33	0	43	7
30	17	14	7
28	50	43	100
26	50	43	100
24	17	14	13
Values			
ELISA-1*	10^3–10^5	10^3–10^5	10^3–10^4
ELISA-2	0·02–1·2	0·037–1·5	0·26–1·04
Mean % CD4†	26	5	32–60
Mean abs. CD4‡	623	39	340–1610
Mean CD4:CD8	0·63	0·11	0·8–3·1

*ELISA-1 values represent the range of serum dilutions read as positive in test for CMV nuclear antigens. ELISA-2 values represent the range of optical density units recorded at a serum dilution of 1:250.

†T cell values for controls represent the range of normal values observed at the National Bacteriology Laboratory, Solna, Sweden.

‡Mean absolute CD4 numbers, expressed as the number/mm^3.

DISCUSSION

The present study deomonstrates a selective loss in humoral and cellular immunoreactivity to different CMV antigens in HIV-infected individuals compared with HIV-non-infected CMV$^+$ individuals.

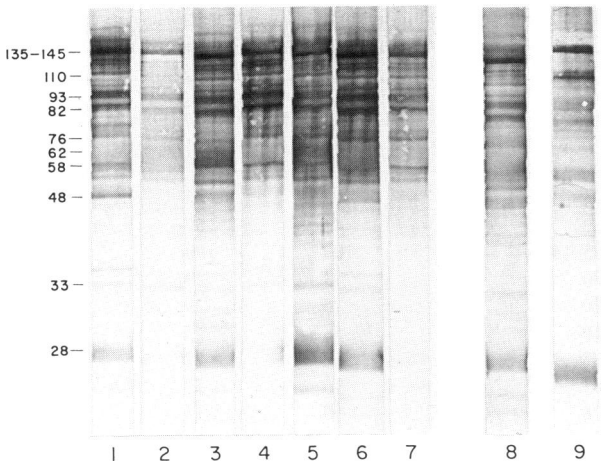

Fig. 4. Serological responses in cytomegalovirus (CMV) immunoblots. An absence of reactivity to an antigen of 26–28 kD was observed in 10 out of 19 HIV$^+$ serum samples (e.g. lanes 2, 4, 7) but not in any of 15 HIV$^-$, CMV$^+$ sera (e.g., lanes 8 and 9). Lanes 1–7; HIV$^+$ individuals with progressively higher absolute CD4 values. Lanes 8 and 9; healthy HIV$^-$, CMV$^+$ controls. All individuals shown were seropositive by ELISA-1 (titres 10 000–100 000) and ELISA-2 (optical densities > 0·2), the normal range of T cell values for healthy individuals in Sweden in shown in Table 2. Further analysis of lanes:

	1	2	3	4	5	6	7	8	9
Absolute CD4	6	20	140	370	670	700	820	ND	ND
CD4:CD8 ratio	0·01	0·04	0·15	0·46	0·31	0·77	1·39	ND	ND
ELISA-1	10^5	5×10^4	10^5	10^4	10^5	10^4	10^4	10^4	10^4
ELISA-2	0·795	0·363	1·106	0·620	1·198	0·793	0·567	1·044	0·264
Serum IgG	15·7	13·8	26·0	17·8	30·0	15·0	17·6	16·8	8·2
Serum IgM	2·6	1·3	0	4·2	3·9	2·1	0·9	1·8	1·7

ND, not determined.

Previous studies of cellular immune responses to human CMV have examined responses to either whole antigen preparations or purified major constituents of the virus—the 64-kD and 72-kD matrix proteins (Forman *et al.*, 1985; Rodgers *et al.*, 1987). However, by screening the whole range of virion molecules separable by SDS–PAGE and blotted onto nitrocellulose, we have succeeded in identifying additional antigen(s) of 130–165 kD that stimulate the T cells of HIV$^-$ responder individuals. According to the analyses of Gibson (1983), the most likely virion constituent representing this antigen would be the major capsid protein of approximately 153 kD. The basic phosphoprotein (135–150 kD) could also be a candidate. However, all individuals, including AIDS patients, tested in our study had high seroreactivity to this determinant, as observed by many investigators (Jahn *et al.*, 1987), suggesting that T cell reactivity to the basic phosphoprotein is lost long before seroreactivity. Seroreactivity to the major capsid protein is unusual (Chee *et al.*, 1989).

The two or three antigenic fractions identified by the methodology used here may not be the only T cell stimulating CMV antigens since the fractions used should represent mainly structural proteins. In a murine model, immunization with non-structural immediate early phosphoprotein pp89 alone, as well as virions, protected mice from a lethal CMV infection (Jonjic *et al.*, 1988). The protection induced by both vaccines was mediated by CD8$^+$ T cells while the whole virion also induced protective neutralizing antibodies.

Studies (Giorgi *et al.*, 1987; Wahren *et al.*, 1987; Ballet *et al.*, 1988; Krowka *et al.*, 1988; Torseth, Berman & Merigan, 1988) of T cell responses to CMV and other antigens by HIV$^+$ individuals without AIDS have generally failed to show significant differences when compared with HIV$^-$ controls, which our findings also support in regard to CMV-infected fibroblasts. However, when responses to fractions separated by molecular weight were measured, a loss of responsiveness to the major CMV antigens even early after HIV infection was observed. Notably, the cellular response to the high molecular weight antigen(s) was absent in five out of the six HIV$^+$ asymptomatic patients who responded to the whole CMV antigen. In addition, there was a decreased response to the fraction containing antigens of 55–65 kD. Thus, the repertoire of T cell responses to CMV in HIV-infected individuals was altered.

Serological probing of immunoblots of CMV antigens has been carried out previously by several investigators. Our findings are in agreement with the results in normal individuals of Gold *et al.* (1988), but also identified a loss of reactivity to certain bands, the most striking of which is loss of reactivity to a band of 26–28 kD in approximately half of HIV$^+$ patients. That this result is related to HIV infection is indirectly supported by the findings of Gold *et al.* (1988) who did not see loss of reactivity to this band in HIV$^+$ homosexual men. The observations of Landini *et al.* (1988, 1989) are also in agreement in that HIV$^-$ controls all reacted with the 28-kD antigen whereas reactivity was lost in some HIV$^+$ individuals. In a separate survey of Ethiopian sera in our laboratory, 36 out of 37 CMV$^+$ individuals had antibody reactivity to the 26–28 kD band (unpublished results).

Loss of reactivity to the 28-kD antigen by HIV$^+$ subjects was observed in our analysis despite high serum immunoglobulin levels and ELISA seropositivity. The 28-kD band is most likely the result of antibody reactivity to a structural protein of CMV (Meyer *et al.*, 1988).

Whether loss of reactivity to the 26–28-kD antigen is not only a correlate of HIV infection but also of rapid progression to clinical disease analogous to loss of antibody to HIV p24 core protein needs to be evaluated in larger groups of patients. Loss of p24 antibody activity is a relatively late phenomenon in HIV infection (Forster *et al.*, 1987; de Wolf *et al.*, 1988) whereas in our study six out of 12 asymptomatic as well as four out of seven ARC/AIDS patients but none of the CMV$^+$ controls lacked reactivity to CMV p28. This suggests that the p28 loss may be an early event in HIV infection.

It is not clear whether the loss of reactivity to certain CMV antigens is secondary to the increased CMV replication that is associated with HIV infection (Drew, 1988) or due to other effects of HIV. Nevertheless, diminished reactivity to CMV antigens may be important for the pathogenesis of HIV infection. CMV reactivation is generally considered to be associated with failing immunity. Reactivation of CMV, as evidenced by increased viraemia, is a frequent occurrence in the course of HIV infection (Fiala *et al.*, 1986). CMV may transactivate the HIV genome and thereby reactivate HIV infection (Ho *et al.*, 1990). Thus, loss of immune reactivity to CMV, which according to our results may be a relatively early event during HIV infection, may lead to reactivation of CMV

and secondarily induce reactivation of HIV and progressive HIV disease.

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