Correlation between Enzyme-Linked Immunosorbent Assay and Immunofluorescence Assay with Lytic Antigens for Detection of Antibodies to Human Herpesvirus 8

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The purpose of this study was to evaluate, in Kaposi’s sarcoma patients, the correlation between antibody titers to the lytic antigens of human herpesvirus 8, as assessed by immunofluorescence assay, and values obtained by an enzyme immunoassay. The methods showed a stringent correlation, \( r = 0.625 \) (\( P < 0.001 \)).

DNA sequences from human herpesvirus 8 (HHV8) have been found in the lesions as well as in the peripheral blood mononuclear cells (PBMC) of patients with all forms of Kaposi’s sarcoma (KS), including the AIDS-associated KS (AIDS-KS) (6), classical or Mediterranean KS (C-KS), African or endemic KS, and KS developing in organ transplant recipients (T-KS) (3). The virus has subsequently been associated with body cavity-based lymphomas (BCBL) (4, 5) and with some variants of multicentric Castleman’s disease (8). Serologic evidence suggests that the virus is a necessary cofactor for KS, since HHV8 seroconversion or an increase in the lytic antibody titer to HHV8 appears to be critical and highly predictive of KS development in human immunodeficiency virus (HIV)-coinfected patients (10). In addition, assessment of HHV8 serostatus is important in monitoring organ transplant donors and recipients. Particularly, kidney recipients infected by HHV8 prior to transplantation and receiving an organ from a seropositive donor show an exceedingly high risk of KS development, probably due to viral reactivation (15).

Several efforts have been made to develop serologic assays for the detection of antibodies to HHV8, to be employed on a routine and screening scale. Until now, no tests have been recommended for diagnostic use, even if those already available and based on self-made immunofluorescence assays (IFA) or on Western blotting confirmed a stringent association of HHV8 seroprevalence with all forms of KS (1, 2, 9, 10, 12, 13, 14, 17, 19, 20). The majority of the studies performed until now are, however, based on IFA, which is time-consuming and not easy to use in large-scale studies to assess disease reactivation, especially in countries where KS still has a high incidence. There is only one commercially available system, based on an enzyme-linked immunosorbent assay (ELISA), which detects antibodies to the lytic antigens of HHV8 using whole virus as the substrate (7). The aim of our work was to study the antibody pattern to the lytic antigens of HHV8 in KS patients using two different methods, ELISA and IFA. Particularly, IFA antibodies to lytic antigens were compared with the optical densities (OD) obtained by ELISA in order to establish a correlation between the two methods.

A total of 70 subjects were enrolled in the study. Seventeen AIDS-KS patients were studied and staged according to the Krown classification (11). Eight of them were sampled at the time of first clinical diagnosis and during protease inhibitor (PI)-containing highly active antiretroviral therapy (HAART). In four AIDS-KS cases, diagnosis was biopsy confirmed. Sera from the remaining patients were available only during (two cases) or without (seven cases) PI treatment. Thirty-one C-KS patients with a biopsy-confirmed diagnosis as well as four T-KS patients were studied. The T-KS patients developed the disease after a mean time of 8 months following renal transplantation and subsequent immunosuppressive therapy, consisting of cyclosporin and steroids. As a control group, 15 apparently healthy blood donors (BD) born in Rome were studied. Three HHV8-seropositive patients, including the partner of an AIDS-KS patient, were also examined.

HHV8 ELISA. Anti-HHV8 immunoglobulin G (IgG) antibodies were detected by a commercially available assay (Advanced Biotechnologies Incorporated, Columbia, Md.), according to the manufacturer’s instructions. Briefly, serum samples diluted 1:100 were incubated in the antigen-coated microtiter wells for 30 min at 37°C. Antigen was represented by whole virus. The wells were then washed to remove unbound sample components. Peroxidase-conjugated anti-human IgG was then added to the wells and incubated for 30 min at 37°C. The wells were washed again to remove unreacted conjugate. The microtiter wells containing immobilized peroxidase conjugate were incubated with peroxidase substrate for a mean time of 15 min at room temperature without light. Then the reaction was stopped, and the OD of the solution was measured spectrophotometrically at 450 nm. The cutoff point was given at 0.023 OD unit.

IFA. Antibodies to lytic antigens of HHV8 were detected using an IFA based on the BCBL-1 cell line (obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH, from M. McGrath and D. Ganem). The BCBL-1 cells were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), an-
tibiotics (100 IU of penicillin and 100 μg of streptomycin per ml), and 5 \times 10^5 M 2-mercaptoethanol. For IFA to antilytic antibodies, smears were prepared by sedimenting BCBL-1 cells after treatment with 20 ng of tetradecanoyl phorbol ester acetate (Sigma) per ml for 48 h. Ten microliters of a 4 \times 10^4-cell/ml cell suspension was smeared on slides, air dried at room temperature, and fixed with a methanol-acetone solution (1:1; vol/vol) at −20°C for 10 min. For IFA, fixed smears were pre-blocked by incubation with phosphate-buffered saline (PBS) supplemented with 3% FCS for 30 min in a humidified chamber and then incubated in two steps of 45 min each at 37°C with the test serum diluted 1:10 (in PBS supplemented with 1% glycine and 2% FCS) and with goat fluorescein isothiocyanate-conjugated anti-human Ig antibodies. Titrations were done with twofold serial dilutions. Samples reactive at 1:20 were considered positive. All microscopic examinations were done under code in a blinded fashion. Linear correlation was used to study the correlation between IFA antibody titrations and ELISA OD in all groups of patients. Student’s t test in paired analysis was used to test HHV8 lytic antigens as well as the ELISA OD values in HHV8-seropositive patients without KS or the AIDS-KS partner had detectable antibodies by both methods (data not shown).

The demographic features of the study groups are reported in Table 1. Eight AIDS-KS patients were examined for HHV8 serology at baseline and during HAART, whereas for two patients blood samples were only available during HAART (4 months of therapy). One patient was treated with nucleoside reverse transcriptase inhibitors (NRTI) plus chemotherapy, which was based on bleomycin and vincristine, and never underwent HAART.

![Image](56x98 to 291x251)

**Figure 1.** Correlation between values obtained by ELISA and IFA applied to the detection of antibodies to HHV8 in the populations studied. ELISA values are expressed as OD, whereas IFA values are given as log_{10} antibody titers.

![Graph](Image 324 to 368)

**TABLE 1.** Demographic features of the study groups

| Feature | C-KS (n = 31) | AIDS-KS (n = 17) | T-KS (n = 4) | BD (n = 15) |
|---------|--------------|-----------------|-------------|-------------|
| Age, yr (range) | 70 ± 16.73 (29–86) | 43.81 ± 9.35 (28–57) | 51.66 ± 2.08 (50–54) | 29.8 ± 1.6 (27–32) |
| Sex (M/F) | 26/5 | 15/2 | 4/0 | 11/5 |
| Risk factor | None | 14 homosexuals, 2 heterosexuals, 1 intravenous drug user | None | None |
| Previous therapy | Steroids (1/31) | NRTI | HAART (8/17), NRTI (9/17) | Steroids, cyclosporin |
| Concomitant therapy | None (30/31), chemotherapy (1/31) | | Steroids, cyclosporin | None |

*a* Therapy prior to KS onset.

*b* Therapy performed at the time of sampling.

**TABLE 2.** Distribution of IFA antibody titers and ELISA OD in KS patients and BD

| Group | Mean IFA titer ± SD | Mean ELISA OD ± SD |
|-------|---------------------|---------------------|
| C-KS (n = 31) | 216.17 ± 246.06 | 0.805 ± 0.432 |
| AIDS-KS (n = 17) | 286.15 ± 398.89 | 0.621 ± 0.530 |
| AIDS-KS pre-HAART (n = 8) | 95 ± 55.45 | 0.393 ± 0.416 |
| AIDS-KS during HAART (n = 8) | 360 ± 414.59 | 0.719 ± 0.586 |
| T-KS (n = 4) | 480 ± 538.64 | 1.05 ± 0.345 |
| BD (n = 15) | <10 | <0.023 |
T-KS subjects showed the highest titers to HHV8 with both methods (mean IFA titers of 1:480 and mean OD of 1.05). The analysis of variance did not show any significant difference among the groups of patients. Probably the small number of T-KS patients enrolled in the study (four subjects) did not allow statistical significance for these apparently intriguing results. Among the groups of patients, the highest titers to HHV8 were observed in the C-KS patient group. Probably the small number of T-KS subjects showed the highest titers to HHV8 with both methods and, conversely, all but three KS patients were seropositive (one C-KS and two AIDS-KS). However, the last (AIDS-KS), with OD levels lower than the cutoff value, showed a weakly positive IFA titer of 1:20. The remaining HHV8-seropositive C-KS patient was under chemotherapy at the sampling time.

Herpesvirus replication may be inhibited under chemotherapy. As our previous report has in fact shown, by the use of PCR, that C-KS patients undergoing chemotherapy can clear virus from peripheral blood mononuclear cells (18). The ELISA alternative to the IFA assay, although less sensitive, could be particularly useful for monitoring viral reactivation, considering that IFA is largely operator and training dependent. The data obtained must be always validated by other assays and compared with the clinical and therapeutic findings for the subjects being tested.

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