Distinct Profiles of Antibodies to Kaposi Sarcoma–Associated Herpesvirus Antigens in Patients with Kaposi Sarcoma, Multicentric Castleman Disease, and Primary Effusion Lymphoma

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Antibody responses against lytic and latent Kaposi sarcoma (KS)–associated herpesvirus antigens were investigated in patients with KS, multicentric Castleman disease (MCD), and primary effusion lymphoma. Antibodies against the lytic antigen K8.1 were 5-fold higher in patients with MCD than those with KS, whereas antibodies to the sum of latent antigens v-cyclin and LANA were 27-fold higher in patients with KS, compared with patients with MCD (P < .001). The sum of anti-v-cyclin and anti-LANA antibody titers discriminated patients with KS from those with MCD and KS with 93% sensitivity and 83% specificity. These results suggest that antibody responses to lytic and latent KS-associated herpesvirus antigens differ in these diseases.

Kaposi sarcoma–associated herpesvirus (KSHV), also called human herpesvirus 8, is the causative agent of all forms of Kaposi sarcoma (KS) [1, 2]. In KS, most proliferating tumor spindle cells are infected with KSHV and express KSHV proteins [1, 2]. KSHV has a 170.5-kb genome encoding ~90 gene products and containing a number of pirated genes involved in cell proliferation, angiogenesis, and evasion of the immune system [3]. KSHV is also the causative agent of 2 rare B cell lymphoproliferative disorders, primary effusion lymphoma (PEL) and multicentric Castleman disease (MCD), that occur primarily in patients infected with human immunodeficiency virus. PEL is characterized by its tendency to develop in body cavities, such as pleural or peritoneal spaces [4–6]. MCD is characterized clinically by fevers, wasting, hypoalbuminemia, and cytopenias that result from overproduction of virus-encoded and human-encoded cytokines, especially interleukin-6 (IL-6) [7, 8].

Like other herpesviruses, KSHV has 2 phases of gene expression, latent and lytic. In KS, a majority of KSHV-infected spindle cells express only latent genes, whereas a small percentage express lytic genes [9–11]. By contrast, in MCD a substantial percentage of cells express lytic KSHV genes, including a virally encoded IL-6. The majority of PEL cells express KSHV latent genes but, in addition, can show limited expression of certain lytic genes [9–11].

Luciferase immunoprecipitation systems (LIPS) is a powerful new method to quantitatively measure antibody responses to a wide range of infectious agents. LIPS screening of a panel of KSHV antigens identified v-cyclin as a useful diagnostic antigen that, along with 3 other antigens, provided high sensitivity and specificity for the differentiation between patients with KS and blood bank donor controls [12]. Given the differential expression of KSHV lytic and latent proteins in patients with KS, PEL, and MCD, we hypothesized that different antibody profiles to KSHV antigens detected by LIPS might distinguish these diseases.

Methods. Serum samples were from patients or volunteers under institutional review board–approved protocols at the National Institutes of Health Clinical Center, the National Institute of Allergy and Infectious Diseases, and the National Cancer Institute. Serum samples from 35 patients with KS, 14 with both MCD and KS (MCD+/KS+), 6 with MCD but not KS (MCD+/KS−), and 5 with PEL, all generally obtained at the time of diagnosis, and samples from 34 KSHV-uninfected controls were tested. The median CD4 counts for patients with KS (270 cells/mm3 [interquartile range (IQR), 137–530 cells/mm3]), MCD+/KS+ patients (294 cells/mm3 [IQR, 185–620 cells/mm3]), and MCD+/KS− patients (359 cells/mm3 [IQR, 123–580 cells/mm3]) were not statistically different between the paired groups. The patients with PEL had the lowest median CD4
counts (111 cells/mm² [IQR, 46–368 cells/mm²]), but the counts were only statistically lower than those from MCD+/KS+ patients.

The pREN2-KSHV antigen constructs for K8.1, ORF65, v-cyclin, and LANA have been described elsewhere [12]. The LIPS assay was performed at room temperature, and all light unit (LU) data were obtained from the average of 2 separate experiments and corrected for background protein A/G bead binding [12]. GraphPad Prism software (GraphPad Software) was used for statistical analyses. Antibody titers from serum samples from uninfected control patients, MCD+/KS+ patients, MCD+/KS− patients, and patients with PEL are reported as the geometric mean titer (GMT) ± 95% confidence interval (CI). The nonparametric Mann-Whitney U test was used for comparison of antibody titers in different groups. P values were not corrected for multiple comparisons.

**Results and discussion.** Using the LIPS assay and antigens from both the lytic and latent phase of the KSHV life cycle, we compared antibody responses among the patient cohorts. The GMTs of antibodies to K8.1 early lytic antigen were 145,900 LU (95% CI, 58,490–363,700) in the 35 patients with KS, 684,700 LU (95% CI, 390,100–1,202,000) in the 14 MCD+/KS− patients, 1,020,000 LU (95% CI, 437,500–2,378,000) in the 6 MCD+/KS+ patients, and 1,092,000 LU (95% CI, 366,600–3,252,000) in the 5 patients with PEL (Figure 1A). Statistical analysis using the Mann-Whitney U test revealed that patients with KS had significantly lower anti-K8.1 antibody titers than MCD+/KS− patients (P = .009), MCD+/KS+ patients (P = .008), or the patients with PEL (P = .013). Also, the difference between the anti-K8.1 antibody titers in patients with KS versus the combined MCD+/KS− and MCD+/KS+ patient groups was highly significant (P < .001).

The GMT of the late lytic antigen, ORF65, in the 35 patients with KS was 34,380 LU (95% CI, 12,570–94,050 LU) and was significantly lower (P < .006) than the GMT of 1,010,000 LU (95% CI, 317,300–3,214,000 LU) for the patients with PEL (data not shown). The MCD+/KS− and MCD+/KS+ patients showed variable antibody titers and were not significantly different from the patients with KS (data not shown).

Antibody titers against 2 latent antigens, v-cyclin and LANA (ORF73), were also evaluated. The anti-v-cyclin GMT in the patients with KS was 225,900 LU (95% CI, 128,600–396,900 LU), which was markedly higher than the GMT of 10,840 LU (95% CI, 1964–59,840 LU) in the MCD+/KS− patients (P < .001) or 6751 LU (95% CI, 1446–31,530 LU) for the MCD+/KS+ patients (P < .001). As shown in Figure 1B, the difference between the anti-v-cyclin antibody titers in the KS group versus the combined MCD+/KS− and MCD+/KS+ patient groups was also highly significant (P < .001). The GMT for the patients with PEL was 51,563 LU (95% CI, 2943–903,400 LU), which was lower than the GMT for the patients with KS (P = .055). Using a cut-off value on the basis of results for the uninfected control subjects, 91.4% (32 of 35) of the KS, 71.4% (10 of 14) of the MCD+/KS−, 40% (2 of 5) of the MCD+/KS+, and 80% (4 of 5) of the PEL patients were seropositive for anti-v-cyclin antibodies.

As shown in Figure 1C, antibodies against the latent protein, LANA, in the patients with KS showed a GMT of 14,940 LU (95% CI, 6520–34,250 LU). Lower levels of anti-LANA antibodies were found in the MCD+/KS− patients with a GMT of 907 LU (95% CI, 292–2823 LU) and the MCD+/KS+ patients with a GMT of 465 LU (95% CI, 177–1218 LU) (Figure 1C). Significant differences were found between the anti-LANA antibodies in patients with KS versus the MCD+/KS− (P < .001) and MCD+/KS+ patients (P = .002). Additionally, the difference between the anti-LANA antibody titers in patients with KS versus the combined MCD+/KS− and MCD+/KS+ patient groups was highly significant (P < .001). The patients with PEL had high anti-LANA antibody titers, which were not significantly different from those in the patients with KS (P = .87). Using a cut-off derived from the uninfected control subjects (Figure 1C), 63% (22 of 35) of patients with KS had positive results for the anti-LANA antibodies, compared with 80% (4 of 5) of the PEL, 21% (3 of 14) of the MCD+/KS−, and 0% (0 of 6) of the MCD+/KS+ patients. These results suggest that the relative absence of anti-LANA antibodies is a common feature of MCD, compared with KS or PEL.

The titer data were also analyzed to determine whether any single antibody or antibody combination might distinguish patients with KS from those with MCD and KS or MCD alone. In part because the anti-LANA and anti-v-cyclin antibody titers in the patients with KS tracked each other poorly (r = 0.03), even greater antibody titer differences were observed between the patient groups with use of the sum of the anti-v-cyclin and anti-LANA antibodies. Specifically, the sum of the anti–v-cyclin and anti-LANA antibodies was 350,700 LU (95% CI, 223,700–550,000 LU) in the 35 patients with KS, 15,880 LU (95% CI, 3746–67,330 LU) in the 14 MCD+/KS− patients, and 7686 LU (95% CI, 1783–33,130 LU) in the 6 MCD+/KS+ patients (Figure 1D). As shown in Figure 1D, significant differences were found between the sum of the anti–v-cyclin and anti-LANA antibodies in patients with KS versus the MCD+/KS− (P < .001) and MCD+/KS+ patients (P < .001). Also, the difference between the sum of the antibody titers in patients with KS versus the combined MCD+/KS− and MCD+/KS+ patient groups was highly significant (P < .001). With receiver operator characteristics, the most informative approach to optimally separate the patients with KS from the MCD+/KS+ patients involved the use of the sum of these latent antigens with a cut-off of 70,000 LU; patients with KS were discriminated from MCD+/KS− patients with 100% sensitivity and 91% specificity (Figure 1D). Although this 70,000 LU cut-off was less useful in discriminating patients
Figure 1. Anti-K8.1, anti–v-cyclin, and anti-LANA antibody titers in uninfected control subjects and patients with Kaposi sarcoma (KS), multicentric Castleman disease (MCD), and primary effusion lymphoma (PEL). Shown are results from 34 uninfected controls (CTRL), 35 KS, 14 MCD+/KS+, 6 MCD+/KS−, and 5 PEL patients. Each symbol represents a serum sample from an individual patient. The geometric mean antibody titer and 95% confidence interval for anti-K8.1 lytic antibodies (A), anti–v-cyclin latent antibody (B), and anti-LANA antibody (C) titers (in light units [LU]) are plotted on the y-axis with a log10 scale. The dashed line represents the cut-off level for determining seropositivity and is derived from the mean plus 5 standard deviations of the antibody titer of the 34 uninfected controls. All of the uninfected controls had negative results for all KHSV antibodies and were below the established cut-off. D, Sum of anti–v-cyclin and anti-LANA antibody titers. The solid line represents the optimum cut-off (70,000 LU) for discriminating patients with the KS from the MCD+/KS+ patients, whereas a higher cut-off (165,000 LU), indicated by the dotted line, optimally separated the patients with KS from the MCD+/KS− patients. All P values were calculated using the Mann-Whitney U test.

By profiling antibodies against several latent and lytic human herpesvirus 8 antigens, significant differences in antibody titers were observed between the patients with KS, MCD, and PEL. One of the most obvious differences was that antibody titers against the early lytic human herpesvirus 8 antigen, K8.1, were markedly higher in the patients with PEL and MCD, compared with the patients with KS. The findings of higher anti-K8.1 antibody levels in patients with MCD are consistent with studies showing that a substantial percentage of MCD cells express lytic KSHV genes, including a virally encoded IL-6. Similarly, the higher antibody titers against the ORF65 lytic protein in patients with PEL, compared with patients with KS, may also reflect the greater KSHV load and expression of lytic antigens in patients with PEL [9, 13]. It is not clear why antibodies to another lytic antigen, ORF65, were not significantly higher in patients with MCD than in those with KS. It is possible that it relates to ORF65 being a late lytic antigen. Alternatively, it is possible that this occurs, in part, because patients with MCD have some subtle defects in specific antibody production, per-
haps related to cytokine dysregulation that blunts what would otherwise be an increase.

In contrast to the anti-K8.1 antibody profile, markedly higher levels of antibody to v-cyclin, a latent KSHV gene, were found in the patients with KS and PEL, compared with those with MCD. Anti-LANA antibody titers also were also markedly higher in the patients with KS, compared those with MCD. Together these results are consistent with immunohistochemical studies demonstrating that KS spindle cells express large amounts of latent human herpesvirus 8 proteins, compared with MCD cells [9, 10, 14]. At present, we have no definitive explanation for why MCD+/KS+ patients have lower antibody titers to v-cyclin and LANA, compared with patients with KS alone. Relatively greater human immunodeficiency virus–induced immunosuppression in the patients with MCD does not seem to be the cause, because the CD4 counts were similar in the KS, MCD+/KS+, and MCD+/KS− groups. As noted above, it is possible that patients with MCD have a blunting of specific antibody responses to KSHV antigens, perhaps related to local cytokine dysregulation.

Because elevated anti-latent antibody titers are a common feature found in patients with KS, compared with those with MCD, the sum of the anti–v-cyclin and anti-LANA antibody titers was the most useful approach for optimally separating patients with KS from MCD+/KS+ and MCD+/KS− patients. Using this approach with a cut-off value of 165,000 LU discriminated patients with KS from MCD+/KS+ patients with 93% sensitivity and 83% specificity. This and other KSHV antibody tests may be useful in identifying patients with KS who may also have developed MCD, a disease that can be difficult to diagnose. Possible explanations for the higher antibody responses against latent proteins in patients with KS, compared with MCD+/KS+ and MCD+/KS− patients, may include greater expression of KSHV latent antigens in patients with KS [9, 10] or, possibly, blunting of KSHV-specific antibody formation in patients with MCD, as postulated above. In summary, antibody responses to latent and lytic KSHV proteins are different between KS, MCD+/KS+, MCD+/KS−, and PEL patients and likely reflect altered protein expression and/or immune recognition differences among patients with these diseases.

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References