Association of Clinical Progression in Classic Kaposi’s Sarcoma With Reduction of Peripheral B Lymphocytes and Partial Increase in Serum Immune Activation Markers

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Objective: To evaluate various immunologic markers in the peripheral blood of patients with early and advanced classic Kaposi’s sarcoma (CKS).

Design: Cross-sectional study.

Setting: A major referral center for skin and venereal diseases.

Patients: Sixty-eight patients with histologically confirmed CKS staged according to a modified version of the Mitsuyasu-Groopman classification in stage I-II (cutaneous involvement only) and stage IV (skin and systemic involvement).

Main Outcome Measures: Concentrations of neopterin and β2-microglobulin, titer of anti–human herpesvirus 8 antibodies, number of natural killer cells, and numbers of total lymphocytes, B lymphocytes, T lymphocytes, and their subsets in peripheral blood.

Results: The median values of β2-microglobulin and neopterin were elevated in patients with CKS in stage IV (median, 3.679 μg/mL [312.72 nmol/L] and 14.0 nmol/L, respectively) compared with patients in stage I-II (median, 2.406 μg/mL [204.51 nmol/L] and 6.5 nmol/L, respectively). A statistically significant reduction in total lymphocyte and B-lymphocyte counts was observed in patients with advanced-stage CKS (1679/µL and 79/µL, respectively) compared with patients in earlier stages of the disease (2142/µL and 224/µL, respectively). The human herpesvirus 8 antibody titer, determined by latent immunofluorescent assay, decreased from stage I-II to stage IV, although not at a statistically significant level (P = .14).

Conclusion: The evolution of CKS from the early stages of the disease to the more advanced may be associated with a partial activation of the immune system and a gradual decrease in the number of total and B lymphocytes.

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Classic Kaposi’s sarcoma (CKS) is an angioproliferative disease of the skin that was originally described by Kaposi in 1872.1 It occurs predominantly in elderly men of Mediterranean or Jewish descent and presents with violaceous plaques and nodules on the lower extremities with a slow tendency to progress.2 Other epidemiologic forms of Kaposi’s sarcoma (KS) are also known, such as the endemic form occurring in children and young adults of central and eastern Africa, the iatrogenic form that develops in patients receiving immunosuppressive agents, and the epidemic form that occurs among persons infected by human immunodeficiency virus 1 (HIV-1).3 The etiologic agent of CKS, as well as of the other forms of KS, was discovered in 1994 by Chang et al4 and is a herpesvirus, known as human herpesvirus 8 (HHV-8) or KS-associated herpesvirus. Despite recent advances in the molecular biology of HHV-8, the exact pathophysiological mechanisms that take place between the infection by HHV-8 and the clinical development of KS remain un-
create an immunologic microenvironment that stimulates the growth of HHV-8 and promotes the development of clinical lesions. In the present study, we examined a number of immunologic factors that are associated with HHV-8 infection and sought to determine their differences between the early, skin-localized, and the advanced stages of CKS. These factors included serum neopterin and β2-microglobulin; the numbers of total peripheral blood lymphocytes, B lymphocytes, T lymphocytes, and T-lymphocyte subsets; the number of natural killer cells; and the antibody titer against HHV-8.

Table 1. Staging of the Studied Population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Stage of Disease</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I-II</td>
<td>IV</td>
</tr>
<tr>
<td>No. (%) of patients</td>
<td>60 (88)</td>
<td>8 (12)</td>
</tr>
<tr>
<td>Sex, No. (%)*</td>
<td>Male 45 (75)</td>
<td>6 (75)</td>
</tr>
<tr>
<td></td>
<td>Female 15 (25)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>Age, y, mean (SD)†</td>
<td>73.4 (10.22)</td>
<td>72.9 (12.01)</td>
</tr>
</tbody>
</table>

*P = .65, stage I-II vs stage IV.
†P = .77, stage I-II vs stage IV.

STATISTICAL ANALYSIS

Differences in average levels of hematological measures by stage (I-II vs IV) were tested by the nonparametric Mann-Whitney test or the 2-tailed, unpaired t test, as appropriate. Spearman correlation coefficient was used to describe relationships between hematological measures.

RESULTS

Most of the 68 study participants were men (75%); their mean (SD) age was 73.3 (10.35) years. Sixty patients were classified in stage I-II (9 in stage I and 51 in stage II) and 8 in stage IV (Table 1). Among those in stage I-II, 75% were men, with a mean (SD) age of 73.4 (10.22) years. The corresponding figures for those in stage IV were 75% and 72.9 (12.01) years, respectively. Mean age and sex did not differ significantly by clinical stage (P = .77 and .65, respectively). Table 2 summarizes the values of the various immunologic measures studied according to clinical stage. The absolute numbers of total lymphocytes and B lymphocytes were significantly lower in patients in clinical stage IV than in those in stage I-II (P = .04 and P = .05, respectively), while the difference in the corresponding percentages was indicative (P = .08 and P = .07, respectively). A similar inverse relationship with disease severity appeared for the T-lymphocyte subsets, namely CD3, CD4, and CD8 T lymphocytes, but the observed differences were not statistically supported. Conversely, levels of neopterin and β2-microglobulin differed significantly between the 2 groups, with patients in clinical stage IV having higher levels than subjects in stages I-II (P = .05 for both markers). Neopterin and β2-microglobulin levels were highly correlated (Spearman r = 0.72, P < .001). The distribution of B lymphocytes and of neopterin levels by clinical stage is shown in the Figure. The seroprevalence of HHV-8 in our cohort was 95.6%, with 65 of 68 patients testing positive for anti–HHV-8 antibodies. Reciprocal anti–HHV-8

LABORATORY ANALYSIS

Twenty milliliters of peripheral blood was drawn from each patient and placed in EDTA for peripheral blood mononuclear cell extraction. Lymphocyte subpopulations were evaluated by flow cytometry in the National Retrovirus Reference Center (Athens) using the same reagents during the entire study period. Monoclonal antibodies for CD3, CD4, CD8, CD19, and CD16/CD56 were used for estimation of total T lymphocytes, T-helper/inducer lymphocytes, T-suppressor/cytotoxic lymphocytes, B lymphocytes, and natural killer cells, respectively (Becton-Dickinson Co, San Jose, Calif). Whole blood leukocytes and differential count were performed with a hematology analyzer (Sysmex KX4500; Diamond Diagnostics, Holliston, Mass). The absolute number of a lymphocyte subset was determined by multiplying the total lymphocyte count by the fraction of lymphocytes bearing the specific antigen. Neopterin (Immuntest Neopterin; Henning, Berlin, Germany) and β2-microglobulin (β2-microglobulin radioimmunooassay; Abbott Laboratories, Chicago, Ill) were measured in serum by commercially available assays. Antibodies to HHV-8 were measured by means of an immunofluorescence assay as previously described. Briefly, antibodies against the HHV-8 latency-associated nuclear antigens were detected by means of immunofluorescent staining of a latently infected cell line of human primary effusion lymphoma (BCP-1) cells. Serum was diluted at 1:100 for screening, and 12 twofold dilutions were used for titrations. Positive serum produced a distinctive nuclear speckling pattern.
titers tended to be lower for patients in the more severe stage, but the observed difference was not statistically significant (Table 2).

###COMMENT

Several staging systems have been proposed for the classification of KS. In AIDS-associated KS, the proposed staging system incorporates the clinical extent of the tumor, the appraisal of immunologic function, and the assessment of HIV-related systemic illness. In the other epidemiologic forms of KS, 2 staging classifications have mainly been used: (1) the Kriegel et al classification, which takes into account the prevalent type of lesions, the pattern of evolution, and the presence of complications, and (2) the Mitsuyasu-Groopman staging system, which considers the extent of the disease and the involvement or lack of involvement of internal organs. We elected the latter because it allows a better distinction between early (skin-localized) and advanced (disseminated) KS and is, therefore, more likely to show distinct differences in the immunologic profile of patients. One of the limitations of our study was the disproportionately lower number of patients with stage IV disease (8/68 [12%]) compared with those with stage I-II. This can be explained by the indolent course of the disease and the fact that most patients are diagnosed when the disease is confined to the skin. Furthermore, the proportion of patients with systemic involvement in our cohort was comparable to that seen in other series of patients with CKS, ranging from 4% to 10% of patients.

The identification of HHV-8 as the causative agent of KS has greatly enhanced our understanding of the pathogenetic events that lead to the development of this tumor. Viral oncogenesis, cytokine-induced growth, and immunosuppression are considered the 3 key features of KS development, although the exact series of events that characterize the evolution of HHV-8 infection is still largely unknown. Although immunosuppression has

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###Table 2. Peripheral Lymphocytes and Their Subsets, NK Cells, Neopterin, β₂-Microglobulin, and Reciprocal Anti–HHV-8 Titers by Clinical Stage*

<table>
<thead>
<tr>
<th>Variable†</th>
<th>I-II</th>
<th>IV</th>
<th>P Value‡</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cells/µL (1531-2780)</td>
<td>2142 (1771-2538)</td>
<td>1679 (1461-2088)</td>
<td>.04</td>
<td>2112 (1725-2440)</td>
</tr>
<tr>
<td>%</td>
<td>35 (28-40)</td>
<td>25 (21-35)</td>
<td>.08</td>
<td>33 (26-40)</td>
</tr>
<tr>
<td>CD3 cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cells/µL (656-3130)</td>
<td>1402 (1226-1676)</td>
<td>1075 (951-1198)</td>
<td>.08</td>
<td>1369 (1194-1667)</td>
</tr>
<tr>
<td>%</td>
<td>68.5 (60.4-74.0)</td>
<td>66.6 (63.5-67.7)</td>
<td>.41</td>
<td>68.1 (60.7-73.5)</td>
</tr>
<tr>
<td>CD4 cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cells/µL (622-1359)</td>
<td>839 (638-982)</td>
<td>498 (335-960)</td>
<td>.25</td>
<td>839 (628-973)</td>
</tr>
<tr>
<td>%</td>
<td>39.0 (31.3-50.0)</td>
<td>35.5 (21.8-43.9)</td>
<td>.35</td>
<td>39.0 (31.1-48.5)</td>
</tr>
<tr>
<td>CD8 cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cells/µL (414-952)</td>
<td>689 (491-920)</td>
<td>493 (480-777)</td>
<td>.43</td>
<td>684 (487-832)</td>
</tr>
<tr>
<td>%</td>
<td>33.0 (24.0-40.2)</td>
<td>35.2 (34.2-37.2)</td>
<td>.77</td>
<td>33.7 (24.1-40.1)</td>
</tr>
<tr>
<td>CD4/CD8 (ratio)</td>
<td>1.19 (0.84-1.75)</td>
<td>1.04 (0.59-1.25)</td>
<td>.40</td>
<td>1.18 (0.83-1.71)</td>
</tr>
<tr>
<td>B lymphocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cells/µL (117-432)</td>
<td>224 (152-296)</td>
<td>79 (54-137)</td>
<td>.05</td>
<td>220 (134-296)</td>
</tr>
<tr>
<td>%</td>
<td>10.5 (7.0-14.0)</td>
<td>5.0 (5.0-5.0)</td>
<td>.07</td>
<td>10.0 (7.0-14.0)</td>
</tr>
<tr>
<td>NK cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cells/µL (120-617)</td>
<td>304 (247-573)</td>
<td>440 (248-442)</td>
<td>.70</td>
<td>309 (248-518)</td>
</tr>
<tr>
<td>%</td>
<td>17.5 (12.0-23.5)</td>
<td>23.0 (16.0-24.0)</td>
<td>.59</td>
<td>18.0 (12.0-24.0)</td>
</tr>
<tr>
<td>Neopterin, nmol/L (mean ± SD, 5.4 ± 2.3)</td>
<td>6.5 (5.3-12.1)</td>
<td>14.0 (8.6-29.6)</td>
<td>.05</td>
<td>7.2 (5.3-13.0)</td>
</tr>
<tr>
<td>β₂-Microglobulin, µg/mL (0.900-3.000)</td>
<td>2.406 (1.810-3.420)</td>
<td>3.679 (2.367-9.913)</td>
<td>.05</td>
<td>2.452 (1.885-3.573)</td>
</tr>
<tr>
<td>Anti–HHV-8 titers</td>
<td>102 400 (25 600&gt;400 000)</td>
<td>16 000 (6400-102 400)</td>
<td>.14</td>
<td>102 400 (16 000&gt;400 000)</td>
</tr>
</tbody>
</table>

Abbreviations: HHV-8, human herpesvirus 8; NK, natural killer.
SI conversion factor: To convert β₂-microglobulin to nanomoles per liter, multiply by 85.
*Data are given as median values (interquartile ranges).
†Reference ranges are given in parentheses.
‡Patients in stage I-II vs those in stage IV.
been considered a predisposing factor for AIDS-associated KS and iatrogenic KS,\textsuperscript{18} its role in the pathogenesis of CKS is less well defined. In a previous case-control study of 91 patients with CKS,\textsuperscript{19} our group showed that patients with CKS have significantly lower total leukocyte, total lymphocyte, and CD4 T-lymphocyte counts than healthy controls. Similar results were found in 2 Italian studies, suggesting the presence of a subtle form of immunosuppression in patients with CKS.\textsuperscript{20,21} In the present study, we found a statistically significant reduction of the absolute numbers of total and B lymphocytes in the advanced vs early stages of the disease. Similar trends were observed for T-lymphocyte subsets, although the differences did not reach the nominal level of statistical significance.

Our findings conform to certain aspects of the current pathogenetic model of KS, according to which peripheral lymphocytes, particularly B cells, are the major latent reservoirs of the virus.\textsuperscript{22} Like the Epstein-Barr virus, HHV-8 has a tropism for CD19\textsuperscript{+} B lymphocytes and is frequently located within these cells, although there are recent reports showing the presence of the virus within circulating CD34\textsuperscript{+} and CD8\textsuperscript{+} T cells.\textsuperscript{23} It is also possible that reactivation from latency occurs in peripheral B cells, leading to “seeding” of the virus within dermal capillaries and lymphatics.\textsuperscript{24} In a study of patients with AIDS-associated KS, low B-cell counts were associated with a statistically significantly increased risk of KS development (rate ratio, 1.98; 95% confidence interval, 1.32-2.97 for B-cell count <39/µL), whereas higher B-cell counts appeared to have a protective effect against the formation of KS lesions (rate ratio, 0.77; 95% confidence interval, 0.62-0.95; P = 0.02 for B-cell count >76/µL).\textsuperscript{25} The reason for the reduction of B-lymphocyte numbers in our study is not clear; it could result from a direct lytic action of the virus, although there is experimental evidence that only a small proportion of B cells in peripheral blood are actually infected in KS. Alternatively, the B-cell lymphopenia could be a secondary effect of the overall immune dysregulation that characterizes HHV-8 infection and KS. Nevertheless, our findings support the existing evidence that lymphocytes are specific targets of HHV-8.\textsuperscript{24} It is also possible that quantitative assessment of peripheral B lymphocytes in patients with KS may have a certain prognostic value, similar to that shown for HHV-8 viral load in plasma or peripheral blood mononuclear cells.\textsuperscript{26-29} However, this hypothesis needs to be explored in future prospective studies comparing the changes in HHV-8 viral load and B-lymphocyte count in the various stages of KS.

Interestingly, the titer of anti-HHV-8 antibodies was lower in patients with advanced forms of CKS, although the difference was not statistically significant. It is generally accepted that the anti-HHV-8 antibody titers increase as the disease progresses, due to HHV-8 reactivation and increased viral load.\textsuperscript{30} However, little is known about the variation of antibody titers through the different stages of KS. Studies using serologic testing for antibodies against lytic and latent HHV-8 antigens in patients with AIDS-associated KS have shown a higher antibody titer against lytic-phase HHV-8 antigens (ORF65) in patients with extensive skin or mucosal-visceral involvement than in those with limited skin disease. In contrast, the antibody titer against HHV-8 latent-phase proteins did not show significant variation in relation to tumor burden.\textsuperscript{31} In a recent study of men seropositive for both HHV-8 and HIV, patients with high titers of anti-HHV-8 antibodies were more likely to have KS, but less likely to have new KS lesions or HHV-8 DNA in peripheral blood mononuclear cells or oral fluids.\textsuperscript{32} These findings suggest that high HHV-8 antibody titers exert a protective role against viral replication and KS development. It is possible that the observed reduction of antibody titers in later stages of KS is linked to the reduction of B lymphocytes, the main source of antibodies, and abrogates the protective effect of these antibodies against HHV-8 replication and further lesion development.

It has been postulated that a disturbance of the immune system, characterized by immunactivation, is a contributory factor in the development of KS.\textsuperscript{8,33} Helper T cell (Th) 1–type cytokines, in particular interleukin 1, interleukin 6, tumor necrosis factor–α and –β, and interferon γ, have been found to be increased, both locally and systemically, in patients with AIDS-associated KS and CKS and in individuals at risk for KS, such as homosexual and bisexual men, HIV-1–infected individuals, and elderly men of Mediterranean origin.\textsuperscript{34,35} In our study, we examined the levels of serum neopterin and β2-microglobulin, which are soluble markers of cytokine activity, particularly of the endogenous interferon system. Neopterin is a product of macrophages stimulated primarily by interferon γ,\textsuperscript{36} while β2-microglobulin is produced by lymphocytes and is expressed on their surface as part of the major histocompatibility class I molecules.\textsuperscript{37} Measurements of these markers have been used in evaluation of the course of infectious and inflammatory processes, such as renal graft rejection, rheumatoid arthritis, and multiple sclerosis.\textsuperscript{38-40} In earlier studies of HIV infection, elevated serum levels of neopterin and β2-microglobulin were found to be markers of progression to AIDS, independent of CD4 cell counts.\textsuperscript{41,42} In addition, patients with AIDS-associated KS tended to have higher serum levels of these 2 markers compared with other patients with AIDS.\textsuperscript{43,44} Few studies have been published about neopterin and β2-microglobulin in CKS. An Italian study reported that urinary excretion of neopterin was higher in patients with CKS than in healthy control subjects.\textsuperscript{20} In a Greek study, both neopterin and β2-microglobulin levels were found to be elevated in patients with CKS vs controls.\textsuperscript{19} In the present study, we show that these markers tend to be higher in more advanced disease stages, supporting the role of immune activation, and particularly of interferon γ, in the progression of KS. Although variations of interferon γ levels, either serum or lesional, have not been precisely determined in the different clinical phases of KS, it has been hypothesized that the progression of KS is associated with hyperactivation of a Th1 response. This is supported by clinical observations showing that the administration of interleukin 2 in combination with recombinant interferon induced progression of KS, possibly through an increase in gamma interferon levels.\textsuperscript{44} In another patient with AIDS who developed KS and visceral leiomyosarcoma, the administration of gamma inter-
feron together with antileishmanial therapy led to KS progression.45

In summary, we have shown that the advanced forms of CKS are associated with a reduction in total peripheral blood lymphocytes and B lymphocytes and an increase in serologic immune activation markers. Although our study has several limitations (small power due to the limited number of patients; cross-sectional rather than longitudinal type of study), the observed differences in the specific immune markers may be of value for the prognosis of the disease and may serve as useful additions to a staging classification of KS that takes into account both clinical and immunologic measures. Future studies involving a larger series of prospectively observed patients are needed to confirm these findings and to elucidate the role of humoral immunity in the pathogenesis of KS.

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REFERENCES


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News and Notes

First Congress of the International Dermoscopy Society. Naples, Italy, April 27 to 29, 2006. The recently founded International Dermoscopy Society organizes a meeting designed for all colleagues interested in the diagnosis and management of pigmented skin lesions. Special emphasis is given on guidelines for management, standardization of reports, and, particularly, on the development of machine vision in dermoscopy. In addition, seminars in discussion format and half-day workshops with special emphasis on pertinent issues in dermoscopy will be conducted.

The detailed program is presented on the Web site: http://www.dermoscopy-ids.org.

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