BRIEF REPORT

Clearance of Human Herpesvirus 8 from Blood and Regression of Leukopenia-Associated Aggressive Classic Kaposi's Sarcoma during Interferon-α Therapy: A Case Report

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A human immunodeficiency virus–negative woman with severe classic Kaposi's sarcoma, idiopathic leukopenia, and massive spread of human herpesvirus 8 (HHV-8) in circulating cells showed stable disease remission in response to systemic interferon-α treatment that was accompanied by increased $CD3^+$ and $CD4^+$ T cell numbers and complete clearance of HHV-8 from the circulation. These results suggest a direct relationship between HHV-8 clearance from blood and regression of Kaposi's sarcoma and are consistent with the in vitro inhibitory effects of interferon-α on HHV-8 infection.

Recent studies have indicated that reactivation of human herpesvirus 8 (HHV-8)/Kaposi's sarcoma (KS)–associated herpesvirus leads to peripheral blood mononuclear cell (PBMC)–associated viremia that is predictive of development of KS in persons at risk [1–3]. This observation has suggested that blocking of HHV-8 infection and its clearance from circulating cells may lead to disease remission. In support of this view, several studies have indicated that clearance of HHV-8 from circulation during highly active antiretroviral therapy (HAART) is associated with regression of KS in HIV-infected patients ([4] and references therein). However, KS regression during HAART is likely related to the control of HIV infection, because HIV-stimulated cytokine production and proliferative and angiogenic effects of the HIV Tat protein are key to the development of AIDS-associated KS [3, 5]. In addition, immune system restoration in patients undergoing HAART may also contribute to the control of tumor growth by activating specific cytotoxic responses against HHV-8 and/or KS spindle cells. In fact, in a recent study, clearance of HHV-8 viremia was found to be poorly correlated with clinical response in patients with AIDS-associated KS who were treated with lipid formulations of doxorubicin, a cytotoxic chemotherapy that did not induce significant changes in plasma HIV RNA levels or CD4 T cell counts [6]. These data indicated that inhibition of HHV-8 infection in circulating cells may not be sufficient to obtain KS regression in the setting of HIV infection and suggested that a better understanding of the role of HHV-8 clearance from blood in KS regression may be obtained by studying HIV-negative patients.

One of the most used therapeutic agents for KS in HIV-negative subjects is recombinant IFN-α, which has been administered locally or systemically to patients with classic KS. In these patients, major clinical improvements and complete KS regression were observed after periods of treatment ranging from 4 to $\geqslant$24 months, without important side effects [7, 8]. In this context, our recent studies have shown that IFN-α inhibits HHV-8 reactivation in primary effusion lymphoma cell lines and reduces HHV-8 load in cultured PBMC from patients with KS or at risk for KS [9]. In contrast to these data, in a recent study, Deichmann et al. [10] failed to detect HHV-8 DNA clearance in PBMC from patients with classic KS who showed major responses to IFN-α administrated subcutaneously. The authors, however, did not determine whether the load of HHV-8 DNA in PBMC from these patients was decreased during therapy [10].

These observations prompted us to study the case of an Italian HIV-negative woman with severe and advanced classic KS who showed KS regression during systemic IFN-α treatment. The patient never traveled to Africa nor had contacts with Africans. At hospital admission, she presented with severe lesions of the right leg and foot and conspicuous edema (figure

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Peripheral blood was obtained and immunological and virological analyses done after informed consent to the study was obtained.

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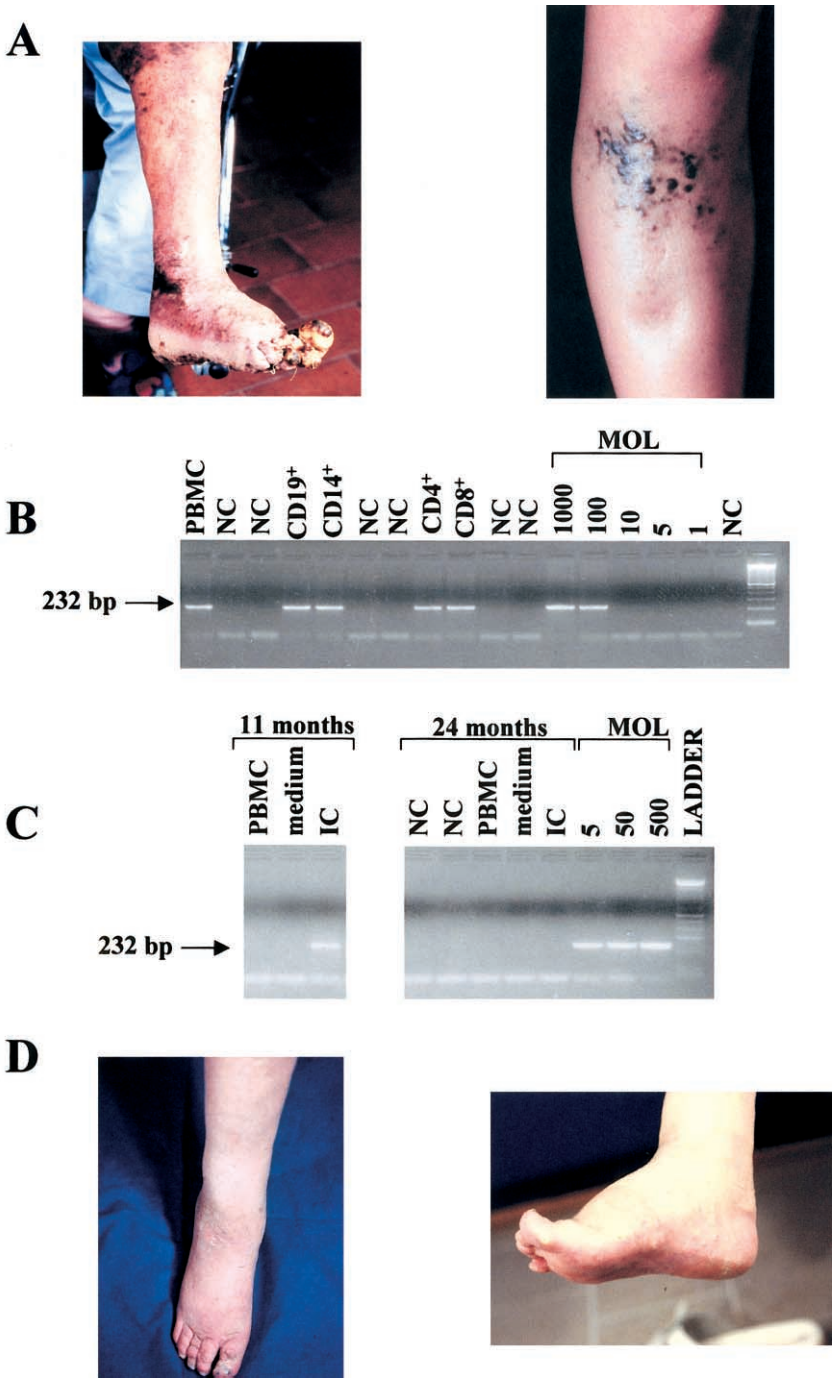

Figure 1. Clinical presentation of HIV-uninfected patient with classic Kaposi's sarcoma (KS) and detection of human herpesvirus 8 (HHV-8) DNA by PCR in peripheral blood mononuclear cells (PBMC) and purified cell populations. *A,* Presentation of KS at patient's lower extremities before therapy; advanced nodular coalescent lesions of right foot and legs with extensive necrosis and edema of leg are evident. *B,* Presence of HHV-8 DNA in patient's PBMC before therapy. HHV-8 DNA amplicon of expected size (232 bp) was detected in PBMC and purified cell populations including B cells ($CD19^+$), monocytes ($CD14^+$), and $CD4^+$ and $CD8^+$ T cells. Cell cytometric analysis indicated >80% purification for B cells, >96% for monocytes, >98% for $CD4^+$ T cells ($CD3^+/CD4^+$), and >99% for $CD8^+$ T cells ($CD3^+/CD8^+$) (data not shown). Monocytic cell fraction had highest virus load, as determined by serial dilution PCR (data not shown). Because contaminant CD14-expressing cells were not detected by cell cytometric analysis in purified cell fractions (data not shown), results indicate virus spread to several cell types (although contamination by unidentified cell type with high virus load cannot be ruled out). *C,* PCR amplification of HHV-8 DNA sequences from patient's PBMC before or after culture for 7 days in medium alone (medium) or in presence of the inflammatory cytokines (IC) used to reactivate HHV-8 in PBMC [1]. Hybridization with DNA oligonucleotide probe internal to amplified sequences confirmed specificity of PCR signals (data not shown). All samples were positive for amplification of β-globin DNA sequences (data not shown). NC, negative control reactions lacking template DNA; MOL, positive control reactions with the indicated numbers of molecules of plasmid containing HHV-8 target sequences in presence of salmon sperm DNA; Ladder, 100-bp DNA ladder used as molecular weight marker. *D,* Regression of nodular KS lesions at right foot after 24 months of IFN-α therapy, although residual edema and regressing plaque-like lesions are still visible.

Table 1. Immunologic parameters and NK cell cytotoxic activity of HIV-uninfected patient with classic Kaposi's sarcoma before and during IFN-α therapy.

Therapy (months)	WBCs	Lymphocytes	$CD3^+$	$CD4^+$	$CD8^+$	$CD16^+56^+$	NK activity (% of target cell lysis) at E/T ratio			
							100:1	50:1	25:1	12.5:1
0	2920	508	140	64	78	77	5.8	4.9	3	1.4
11	4060	730	237	138	138	156	ND	ND	ND	ND
24	3280	656	511	308	144	165	13.5	10.6	8.3	5.8

NOTE. Data are absolute numbers per microliter of WBCs, lymphocytes, and lymphocyte subpopulations, including $CD3^+$, $CD4^+$, and $CD8^+$ T cells and NK cells ($CD16^+56^+$). ^{51}Cr release assay against prototypic cell target (K562 cell line) was done to test NK cell cytotoxic activity; results are reported for different effector-to-target cell (E/T) ratios used. Target cells (10^6) were labeled for 1 h at 37°C with 100 μCi of $Na_2{}^{51}CrO_4$ (NEN) and mixed with effector cells in V-shaped microtiter wells (Linbro) to give final E/T ratios ranging from 100:1 to 12.5:1. Spontaneous release of ^{51}Cr by target cells was determined by adding medium alone to targets and maximal release by lysing targets with Nonidet P-40. Percentage of lysis was determined by formula: [(experimental counts per min − spontaneous counts per min)/(maximal counts per min − spontaneous counts per min)] × 100. ND, not done.

1*A*). This patient was of particular interest because of the presence of a profound idiopathic leukopenia mimicking the immune deficiency found in AIDS-associated KS but in the absence of HIV infection. In fact, the patient had low numbers of total WBCs, $CD3^+$ lymphocytes, $CD4^+$ T cells, $CD8^+$ T cells, and NK cells and cytotoxic activity (table 1); however, she was negative for antibodies to HIV-1 and HIV-2 as determined by ELISA (HIV-1/HIV-2 Antibody Capture ELISA Test System; Ortho) or by Western blot analysis (RIBA HIV-1/HIV-2 SIA; Chiron). She was also negative for HIV in plasma as determined by a PCR amplification assay capable of detecting all HIV clades except clade 0 (Amplicor; Roche), for HIV p24 antigenemia (Western blot; Ortho), and for virus isolation with $CD8^+$ T cell–depleted PBMC, done at the time of KS diagnosis and repeated after 6 months (data not shown).

In addition, the patient was negative for other infections known to be associated with leukopenia, including parvovirus B19 or active cytomegalovirus infection (data not shown). By contrast, PBMC and purified B lymphocytes, $CD8^+$ and $CD4^+$ T cells, and monocytes from the patient were all positive for HHV-8 by PCR (figure 1*B*), indicating virus spreading in most circulating cell types. IgG antibodies directed against HHV-8 antigens expressed during latent infection (anti-latent antibodies) or active viral replication (anti-lytic antibodies) [9] were remarkably high (1:640 and 1:320, respectively).

The patient was treated with recombinant IFN α-2b at the dose of 3 million IU subcutaneously 3 times a week. The clinical condition started to improve after 8 months of therapy, with edema and lesion regression. After 11 months of therapy, the patient's leukocytes were increased to 4060/μL, $CD3^+$ T cells to 237/μL, and NK cells to 156/μL (table 1). At this time, HHV-8 anti-lytic antibody titers were decreased to 1:320, freshly isolated PBMC were HHV-8–negative by PCR (figure 1*C*), and the patient's clinical condition continued to improve. However, PBMC were still HHV-8–positive by PCR after culture of the cells with the inflammatory cytokines previously shown to reactivate and to increase HHV-8 load to detectable levels [1] (figure 1*C*). These data indicated that clinical response was accompanied by an improvement of the immune status of the patient and by a decrease of HHV-8 load in blood, but HHV-8 was still recovered from PBMC.

During continued therapy, leukocyte numbers continued to rise and HHV-8 anti-lytic antibody titers further declined (1:160). After 24 months, numbers of $CD3^+$ T cells were 511/μL and of NK cells were 165/μL, and the NK cell cytotoxic activity was restored to normal levels (table 1). At the same time, PBMC became HHV-8–negative by PCR after culture with inflammatory cytokines (figure 1*C*). By contrast, HHV-8 anti-latent antibody titers did not show significant variations, suggesting that a reservoir of latently HHV-8–infected cells was present even after complete clearance of HHV-8 from the circulation. At this time, nodular lesions disappeared, although a slight edema and regressing plaque-like lesions were still visible (figure 1*D*). Thereafter, because the patient showed a stable KS resolution, therapy was withdrawn. However, KS relapsed. IFN-α administration was therefore resumed at the same doses, and a prompt clinical response with stable KS regression was again observed. The patient is now under follow-up and is still in good clinical conditions, without notable side effects.

In this patient, clinical improvement and stable KS regression in response to IFN-α was associated with HHV-8 clearance from blood. Such a decrease in virus load was likely due to inhibition of HHV-8 reactivation and replication, as indicated by our previous work showing that IFN-α inhibits HHV-8 replication in primary effusion lymphoma cell lines and decreases HHV-8 load in PBMC from patients with KS [9]. Of note, virus clearance was accompanied by increased $CD4^+$ T cell numbers and improved NK cell cytotoxic activity, which, in fact, is known to be potentiated by IFN-α. These antiviral and immunomodulatory actions of IFN-α were probably responsible for KS regression in our patient, as indicated by disease relapse on withdrawal of therapy, and may also explain the increase in $CD3^+$ and $CD4^+$ T cells that accompanied the clinical improvement in her KS.

Although the mechanisms underlying the efficacy of IFN-α therapy in KS may be several, including inhibition of angiogenesis and increased susceptibility of KS spindle cells to NK cell lysis [9], our data indicate that the therapeutic control of HHV-8 reactivation and replication in circulating cells from our patient was likely key to KS remission. Whether restoration of normal cytotoxic immune responses had or might have effects on KS regression independently from the control of HIV or HHV-8 infection (i.e., by controlling tumor growth) requires further studies. However, inhibition of tumor growth by IFN-α generally requires much higher doses of the drug. Nevertheless, our data indicate that HHV-8 infection may likely represent a key component of the multiple factors responsible of KS development that need to be targeted for reducing the incidence and inducing regression of KS.

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