We retrospectively reviewed 5 cases of hemophagocytic lymphohistiocytosis (HL) associated with human herpesvirus 8 (HHV-8) reactivation in human immunodeficiency virus (HIV)–infected patients. All patients had clinical and biological features characteristic of HL. Pulmonary symptoms were present in all patients and were frequently life threatening. The mean number of HL episodes was 6. Four patients had HL-associated Kaposi sarcoma, and 3 had multicentric Castleman disease. The mean CD4 cell count was 200 cells/mm³. HIV loads were stable in all patients. All patients had high levels of HHV-8 in peripheral blood mononuclear cells during attacks, and a significant increase in this parameter before the attacks was seen in 3 patients. Although 2 patients died of HL, 3 are still alive and receiving etoposide therapy (mean follow-up, 3 years). HHV-8–related HL is associated with life-threatening symptoms and biological HHV-8 reactivation, and it may be controlled in the long term by etoposide therapy combined with highly active antiretroviral therapy.

Hemophagocytic lymphohistiocytosis (HL; also called “hemophagocytic syndrome” and “macrophage activation syndrome”) is a reactive disorder of the mononuclear phagocytic system that is characterized by benign, generalized histocyte proliferation with marked hemophagocytosis. Both familial and sporadic forms are recognized. Acquired hemophagocytic syndromes are mostly associated with underlying diseases such as immunodeficiency, hematologic malignancies, and autoimmune conditions. This immunohematologic process may also be triggered by infection or medication. Infection-associated hemophagocytic syndrome was originally described in 1979 [1] in a patient with viral disease. Since the initial description, this syndrome has also been documented in patients with bacterial, parasitic, and fungal infections. Viruses in the herpes group, especially Epstein-Barr virus (EBV), are well-known causes of hemophagocytosis. However, the relationship between this syndrome and human herpes virus 8 (HHV-8), the herpes virus associated with Kaposi sarcoma (KS), multicentric Castleman disease (MCD), and primary effusion lymphoma [2], has rarely been reported. We describe 5 HIV-infected patients with HL that was associated with a reactivation of HHV-8 infection.

MATERIALS AND METHODS

We retrospectively reviewed the charts of 5 HIV-infected patients with HHV-8 reactivation–associated HL who received diagnoses at Saint-Louis Hospital, Paris, and René Dubos Hospital, Pontoise, France, during 1997–2002.

Diagnosis of HL. The diagnosis of HL was based

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on clinical, biological, and histological findings, as defined elsewhere [3].

**HHV-8 quantitative load evaluation in PBMCs.** DNA extracted from PBMCs was amplified using real-time quantitative PCR on an ABI PRISM 7700 (Perkin Elmer Applied Biosystems) in the presence of an internal probe within open-reading frame 26, as described elsewhere [4]. This specific application of real-time quantitative PCR yielded an amplification of >85%. Dilutions of known amounts (1–7.5 × 10⁶ copies) of a fragment of KS330g DNA cloned in a plasmid were used for the standard curve. Plotting the cycle threshold values against given copy numbers, we obtained a linear amplification for the standard curve. Predictive analyses were done for all DNA extracts. The HHV-8 load in PBMCs was assessed in all patients and prospectively monitored in 4 of them.

**HHV-8 serological testing.** A latent and lytic immuno-fluorescent assay, as described elsewhere [5], was used for all patient samples.

**RESULTS**

**Clinical features of HL.** All patients presented with fever, asthenia, peripheral lymphadenopathy, splenomegaly, and/or hepatomegaly (table 1). Fever was present for ≥1 week. Severe weight loss (>4 kg) was seen in all patients. Peripheral, mediastinal, and retroperitoneal lymph nodes were enlarged in all patients. Splenomegaly was present in all patients, and hepatomegaly was seen in 3 patients. Pulmonary symptoms were seen in all patients. Four patients presented with respiratory distress syndrome in the absence of a detectable opportunistic infection, and 1 patient complained of cough and exertional dyspnea. Neurological abnormalities, such as asthenia, headache, irritability, and an altered level of consciousness, were seen in 2 patients.

**KS and other HHV-8-associated diseases.** At the time of HL diagnosis, skin examination revealed KS in 4 patients. KS was limited and stable in 1 patient but was diffuse and progressive in the 3 others. Three patients had never received any KS-specific therapy. One had received unsuccessful courses of chemotherapy (bleomycin, daunorubicin, vinblastine, and dauxorubicin). These treatments had been withdrawn ≥6 months before the first episode of HL. KS tumor, immune system, and systemic illness staging are shown in table 1.

MCD was associated with HL in 3 of 5 patients (table 2). No histological features of associated lymphoma were observed. It is noteworthy that plasmablastic microlymphoma in the spleen was present in 3 patients (patients 1–3), as defined by Dupin et al. [7] and Du et al. [8].

**Laboratory features of HL.** Anemia and thrombocytopenia or pancytopenia occurred in all cases (table 1). Thrombocytopenia was constant, and the thrombocyte level was <50 × 10⁹ thrombocytes/L in 2 patients. Severe anemia (hemoglobin level, <70 g/L) was present in all patients. This anemia was usually normocytic and nonregenerative. Four patients had leukopenia (leukocyte count, <3.5 × 10⁹ leukocytes/L), and 3 also had neutropenia (neutrophil count, <1.5 × 10⁹ neutrophils/L). Hepatic cytosis was present in 2 patients. Basal triglyceridemia levels were elevated (>1 mmol/L) in all of these patients during the acute phase of HL. Ferritin concentrations were >1500 μg/L in all patients.

**Histological findings.** An examination of bone marrow samples revealed hemophagocytosis in 4 of 5 patients. For the remaining patient, hemophagocytosis was confirmed in the spleen.

**HIV infection.** The mean CD4 lymphocyte count was 200 cells/mm³ (range, 165–234 cells/mm³) (table 1). The mean HIV load was 7000 copies/mL (range, 50–25,000 copies/mL) and was stable in all patients at the onset of HL.

**HHV-8 infection.** All patients were infected with HHV-8, as demonstrated by positive results of HHV-8 serological tests. One white homosexual man was probably infected via sexual exposure. The 4 other patients originated from Africa, an area in which HHV-8 is endemic. All patients had had their HHV-8 load measured. High HHV-8 levels were observed in PBMCs during the acute phase (median, 5.6 log copies/μg DNA; range, 4.9–6.7 log copies/μg DNA) (figure 1). With use of the same technique, one of us (F.A.) had already reported [4] HHV-8 values in 12 HIV-seropositive men with asymptomatic HHV-8 infection. The median (± SD) value was 3.04 ± 1.34 log copies/μg DNA. In 3 of our patients for whom the HHV-8 load had been obtained every 1–2 months, the level increased a few days before the onset of HL symptoms and, for 4 of them, decreased quickly thereafter (figure 2). For patient 4, the HHV-8 load was evaluated once before HL reached the acute phase. During this phase and just before his death, HHV-8 levels, as confirmed by 2 assessments, significantly increased.

**Searches for other associated infections.** The results of repeated series of blood and urine cultures were negative for bacterial pathogens. The results of serological tests, PCR, or immunofluorescent antigen tests done with all of these patients’ samples made the diagnosis of acute EBV, cytomegalovirus (CMV), herpes simplex virus 1 and 2, or parovirus B19 infection highly improbable. There was no evidence of infection with hepatitis B or C. Blood cultures and fungal antigen tests ruled out cryptococcal infection. In 4 patients who also had an acute respiratory syndrome, findings from the evaluation of a bronchoalveolar lavage specimen ruled out pneumocystosis or any other opportunistic infection. Respiratory failure was attributed to KS (n = 1) or to HL (n = 4). The results of repeated mycobacterial blood cultures (BACTEC), bacteriologic
### Table 1. Clinical and laboratory characteristics of patients at baseline.

| Patient, sex | Country of origin/race | Pulmonary symptom(s) | KS TIS stage [6] | Hemoglobin level, g/dL | WBC count, $\times10^9$/L | Neutrophil count, $\times10^9$ neutrophils/L | Platelet count, $\times10^9$ platelets/L | Plasma triglyceride level, m mol/L | Ferritin level, $\mu$g/L | Fibrinogen level, g/L | CD4 cell count, cells/mm$^3$ | HIV load during first occurrence of HL, copies/mL | Outcome |
|--------------|------------------------|----------------------|------------------|------------------------|--------------------------|-----------------------------------|---------------------------------|---------------------------------|---------------------|------------------|-----------------|------------------|-----------------|----------------|-----------|
| 1, F         | Zaire/black            | RDS                  | D, E T0 I1 S1    | 4.1                    | 3.3                      | 2.24                              | 138                             | 4.5                             | 4730                | 1.4              | 196             | 25,000           | >8 recurrences of HL; patient lived$^d$ |
| 2, F         | Haiti/black            | RDS                  | L, S T0 I1 S0    | 4.7                    | 1.7                      | 0.94                              | 91                              | 6                               | 22,500              | 3                | 179             | 2730             | >8 recurrences of HL; patient lived$^e$ |
| 3, F         | Burundi/black          | RDS                  | No               | 6.7                    | 2.3                      | 1.3                               | 100                             | 7                               | 25,000              | 1.2              | 224             | ND               | >10 recurrences of HL; patient died |
| 4, M         | France/white           | RDS                  | D, E T1 I1 S1    | 5                      | 1.1                      | 0.44                              | 46                              | 2.2$^f$                       | 3650                | 1.2              | 165             | 205              | 1 episode of HL; patient died |
| 5, F         | Mali/black             | Cough, ED            | D, E T0 I0 S0    | 6.9                    | 9.8                      | 8.0                               | 39                              | 3.80$^g$                      | 1550$^h$            | 2                | 234             | UD               | 1 episode of HL; patient lived$^i$ |

**NOTE.** D, diffuse; E, extensive; ED, exertional dyspnea; HL, hemophagocytic lymphohistiocytosis; L, limited; ND, not done; KS, Kaposi sarcoma; RDS, respiratory distress syndrome; S, stable; TIS, tumor, immune system, and systemic illness; UD, undetectable.

$^a$ Normal range, 0.45–1.5 mmol/L.

$^b$ Normal range, 15–250 $\mu$g/L.

$^c$ Normal range, 2–4 g/L.

$^d$ Three years of follow-up.

$^e$ Four years of follow-up.

$^f$ Level had been 1.1 mmol/L 1 month previously.

$^g$ Level had been 1.3 mmol/L 3 weeks previously.

$^h$ Level had been 235 $\mu$g/L 3 weeks previously.

$^i$ Five months of follow-up.
examinations of patients’ sputum smears, and, for 4 patients, bronchoalveolar lavage specimens were negative. Moreover, cultures of spleen (4 patients), lymph node (1 patient), or bone marrow (5 patients) specimens were negative for bacteria, mycobacteria, parasites, and fungi.

**Outcome.** One patient (patient 4) died during his first occurrence of HL. A second patient (patient 3) developed an acute respiratory distress syndrome during acute-phase HL and died despite receiving intensive care. Two other patients (patients 1 and 2) also developed an acute respiratory distress syndrome that completely regressed with chemotherapy, without the use of any other medication. No infectious or cardiogenic etiologies were found. The respiratory symptoms were attributed to HL.

Three of these 5 patients are still alive 1, 3, and 4 years after the onset of hematologic symptoms. Treatment consisted of etoposide administration that was associated, in 2 patients, with splenectomy. Etoposide maintenance therapy has allowed clinical remission for a mean follow-up period of 3 years in 2 patients (patient 1 had 2 relapses a few days after her course of etoposide was delayed).

**DISCUSSION**

Criteria for the diagnosis of HL, as proposed by the Histiocyte Society, include clinical, laboratory, and histopathological features [3]. Persistent fever and organomegaly, such as splenomegaly and hepatomegaly, are the most common clinical signs. The most prominent laboratory abnormalities are cytopenia, hypertriglyceridemia, hypofibrinogenemia, and a marked elevation in the ferritin level. Activated macrophages engulfing erythrocytes, leukocytes, platelets, or their precursors are the usual histopathological features seen during the acute phase of HL [9], usually in bone marrow, spleen, liver, or lymph nodes [10]. In our 5 patients, the diagnosis of HL was based on the association of clinical, biological, and histopathological criteria mentioned previously.
Figure 2. Evolution of human herpesvirus 8 (HHV-8) load and episodes of hemophagocytic lymphohistiocytosis (HL). Black arrows, episode of HL; gray arrows, introduction of etoposide therapy.
It is likely that HHV-8 was the main trigger of HL in our 5 patients. At least 4 points enable us to draw this conclusion: (1) other infectious agents known to be involved in the pathogenesis of HL [7] were ruled out; (2) there was no argument in favor of HIV-triggered HL, because our patients' HIV infections had evolved for >5 years and their HIV loads and CD4 cell counts were stable; (3) other known triggers of HL, such as lymphoma [11–13] and use of medication (i.e., nonsteroidal anti-inflammatory drugs [14]), were absent; and (4) as has been described for the exacerbation of MCD [4, 15], the HHV-8 load in PBMCs strongly correlated with clinical and biological features of HL. This virus load markedly increased at the time of or before the acute phase HL and decreased afterward. Therefore, we can hypothesize that HHV-8 was a trigger for hemophagocytosis, as has been postulated for other herpesviruses, such as EBV. Among HHV-8–related neoplastic diseases seen in our patients, KS is, as a rule, not directly associated with HL and therefore can probably be considered to be associated with rather than a cause of HL. The link between HL and MCD, which frequently occurs in HIV-infected patients with plasmablastic microlymphomas, is more complex. Indeed, overlapping clinical and biological features of MCD (such as a deterioration in the clinical status, notable loss of weight, fever, multiple peripheral lymphadenopathies, hepatomegaly, splenomegaly, pulmonary functional complaints, cytopenia, and biological inflammatory syndrome) and HL may make diagnosis difficult and may lead to an underestimation of the number of cases of HL. Moreover, HHV-8–associated MCD is characterized by a high level of lytic infection of follicular B cells [16]. Such a reactivation of HHV-8 infection could thus be directly involved in the acute phase HL.

So far, only 3 cases of HHV-8–associated hemophagocytosis have been reported [17–19], and they were associated with either HIV-related [17, 18] or posttransplantation [18] KS. All patients responded favorably to foscavir therapy. Moreover, it is likely that some cases of presumed HIV-induced HL might be related to HHV-8 infection. Indeed, some cases have been reported in patients with extensive KS or in black or homosexual patients potentially infected with HHV-8 [20–26]. In another case report, the patient’s clinical status improved while receiving treatment with zidovudine and foscavir, the latter of which is known to be effective in vitro in inhibiting HHV-8 reactivation [27].

The physiopathology of virus-induced HL remains unclear [28]. The excessive activation of monocytes during the acute phase of HL may be due to stimulation by high levels of activating cytokines. High levels of IFN-γ [29, 30], soluble IL-2 receptor [29], IL-6 [29], TNF-α and -β [31], IL-1 [32], and IL-18 [33] have been demonstrated. It is of interest that HHV-8 encodes several homologues of cytokines, including a functionally active IL-6–like viral protein (vIL-6) [34]. vIL-6 and human IL-6 share similar biological properties and are involved in the pathogenesis of MCD [16, 35]. Direct HHV-8 infection of monocytes or paracrine effects from HHV-8 reactivation in other reservoirs, such as B cells, could be involved in the pathophysiology of HL. The trigger of HHV-8 reactivation remains to be determined.

In conclusion, physicians should be aware that HL may be associated with the reactivation of HHV-8 infection and that HHV-8 serological characteristics and virus load in PBMCs should be assessed when a patient has a diagnosis of HL. If available, the HHV-8 load should be measured, to clarify the potential association between increased virus load and episodes of HL. In 2 of our cases, etoposide therapy associated with HAART was dramatically effective. The potential interest in antiviral drugs, such as foscarnet, cidofovir, and IFN, or in monoclonal anti-CD20 antibodies [36] should be evaluated in the course of HHV-8–associated HL.

References