Twenty-two children with hemophagocytic lymphohistiocytosis were treated with a chemotherapy regimen consisting of VP16-213, corticosteroids, and intrathecal methotrexate. A sustained clinical and biologic complete remission was obtained in 15 children and a partial remission in one child; six children died early of opportunistic infection (n = 4) or of disease progression (n = 2). Of the 18 children who were placed in first remission, 10 received maintenance chemotherapy alone, while six underwent bone marrow transplantation (HLA matched in five, HLA mismatched in one). Of the children who received chemotherapy alone, only two are in long-term remission after cessation of treatment. The remaining eight patients relapsed after a mean period of 5.4 months (range 2 to 8 months). Further treatment using the same regimen induced second remissions of short duration; death occurred after a median period of 2.3 months (range 0.5 to 6 months). A total of nine patients received autologous bone marrow transplantation (BMT). Among the six children transplanted in remission, four are in long-term unmaintained remission, 1 to 6 years after BMT. However, the relapse that occurred in one patient 1 year post BMT is difficult to interpret because the donor, the patient’s 5-year-old sister, also developed the disease 1 year later. An HLA-nonidentical BMT resulted in unmaintained remission for 1 year, with autologous hematologic reconstitution followed by disease relapse. HLA-nonidentical BMT failed in three other patients with active disease at time of transplant. The poor long-term results of chemotherapy alone justify the use of related HLA-matched BMT in complete remission.

**Familial Hemophagocytic Lymphohistiocytosis (FHL)** is a rare and lethal disease characterized by the onset, early in life, of high fever, hepatosplenomegaly, neurological symptoms, pancytopenia, low fibrinogen level, and hypertriglyceridemia. The main histopathologic feature is multi-organ infiltration by lymphocytes and histiocytes, including the bone marrow and central nervous system (CNS), with phagocytosis of blood cells. Most reported cases indicate a familial origin suggestive of autosomal recessive inheritance. The distinction between FHL and virus-associated hemophagocytic syndrome (VAHS) is not always easy, although VAHS usually occurs later in childhood. Furthermore, in some cases first manifestations of FHL seem to be triggered by an infection that is often of viral nature. The etiology of the disease is not yet known. Hemophagocytosis and the widespread infiltration by cells of the monocyte-histiocyte lineage appear to reflect a hyperactivation of this lineage, probably due to a defect in a biologic control mechanism. Ambruso et al. were the first to report a long-term remission using VP16-213 (epipodophylotoxin), a cytotoxic drug particularly active on cells of the myelomonocytic lineage. This observation served as a basis for a treatment protocol that gave encouraging preliminary results in a small series of patients. Since then we have reported that bone marrow transplantation (BMT) is able to cure the disease and can be considered as an alternative treatment. We now report the long-term outcome of patients treated with the previously described chemotherapy protocol in comparison with an extended experience of BMT.

**PATIENTS AND METHODS**

**Patients**

In the absence of any specific disease marker, the diagnosis of hemophagocytic lymphohistiocytosis was based on the following criteria: occurrence, before the age of 18 months, of high fever and hepatosplenomegaly associated with biologic abnormalities; pancytopenia with hemophagocytosis in marrow, liver, or cerebrospinal fluid (CSF); low fibrinogen level (≤ 1.5 g/L); and hypertriglyceridemia (> 2.5 nmol/L). Because such a syndrome can be associated with several infectious diseases, extensive microbiologic research was performed on blood, marrow, urine, and CSF samples, including bacterial, fungal, and viral cultures and detection of specific IgG and IgM against cytomegalovirus and Epstein-Barr virus (EBV). Since 1984, EBV DNA has been sought in blood and marrow using Southern blot hybridization. Only patients in whom these investigations were negative were included in this study.

**Treatment Regimen**

**Supportive Care**

In addition to chemotherapy, an intensive supportive care protocol was applied during the early phase of the disease and included fibrinogen infusion, irradiated packed red blood cells and platelet transfusions, restriction of fluid intake, and broad spectrum systemic antibiotic administration. During the maintenance phase, the children received intravenous (IV) Ig every 3 weeks and trimethoprim-sulfamethoxazole orally.

**Chemotherapy**

Patients were treated according to a previously published regimen. Briefly, it consisted of VP16-213 (etoposide) infused over 3 consecutive days at a daily dose of 200 to 300 mg/m². The 3-day course was repeated once or twice until the disappearance of clinical symptoms. Thereafter, VP16-213 was administered IV (200 mg/m²) weekly, and then at a progressively decreasing frequency until it reached one infusion a month. After at least 1 year of...
monthly infusions, the patients received oral VP16-213 (150 mg/m²) three times a week. Steroids were first given IV (3 mg/kg of methyl-prednisolone) and then orally, and progressively tapered over 3 months. A minimum of six intrathecal methotrexate injections (4 to 10 mg according to age) were given over a 2- to 3-month period. Until 1985, patients also underwent cranial irradiation (12 Gy) after the age of 12 months. Complete remission was defined as the sustained absence of clinical and biologic manifestations of the disease that persisted when VP16-213 was administered once every 2 weeks. Relapses were treated with the same protocol.

**BMT**

**HLA-compatible grafts.** The conditioning regimen consisted of 300 mg/m² VP16-213 on days −12, −11, and −10; 4 mg/kg busulfan on days −9 to −6; and 50 mg/kg cyclophosphamide on days −5 to −2. The first patient (number 1 in Table 1) also received 2 g/m² aracytine.9

Graft-versus-host disease (GVHD) prophylaxis consisted of the association of methotrexate (days 1, 3, 6, and 11) and cyclosporine for a total of 6 months and T-cell depletion by E-rosetting for the first patient.

**HLA-incompatible grafts.** Patients were treated with the same conditioning regimen reinforced by the infusion of 0.2 mg/kg/d of a monoclonal anti-LFA-1 antibody (25-3 murine IgGl specific for the chain of LFA-1/CDlla antigen) from day −3 to day +6.11

Prevention of GVHD was based on graft T-cell depletion by E-rosetting (patients 6 and 7, Table 1) or by treatment with Campath 1 antibody (Dr G. Hale, Cambridge, UK) plus human complement and administration of cyclosporine for 2 months (patients 8 and 9, Table 1).

All patients underwent gut decontamination, received weekly IV Igs, and were placed in a sterile isolator (Isoconcept, Paris, France).

**Methods**

**Chimerism**

After BMT, chimerism was studied by karyotyping in case of sex mismatch, Ig allotyping, erythrocyte phenotyping, or HLA typing after HLA-mismatched BMT. In one case (number 2, Table 1), chimerism was studied by means of restriction fragment length polymorphism (RFLP) on DNA extracted from T lymphocytes, B lymphocytes, and polymorphonuclear cells.12

Natural killer activity was measured against K562 cells, as previously described.13

**RESULTS**

Twenty-two children from 20 families met the criteria of familial hemophagocytic lymphohistiocytosis and were treated in our institution between 1983 and 1989. The main clinical and biologic features are given in Table 2. All of the children first received chemotherapy. Four patients died at an early stage from infection (respiratory syncytial virus, Pneumocystis carinii, adenovirus, or mucormycosis infection) with no evidence of chemotherapy resistance. Two other patients did not achieve remission and died from disease progression. Remission was obtained in the remaining 16 patients after a mean period of 3.4 months (range 2 to 6 months). The remission was complete in 15 children and partial in one, with persistence of hepatomegaly,
splenomegaly, and mild biologic symptoms related to the disease. This patient has been described in detail elsewhere. The mean dose of VP16-213 required was 2.05 g/m² (range 0.6 to 5.2). The patients received a mean of seven intrathecal injections (range 3 to 15). Four patients underwent cranial irradiation.

Eight of 10 patients who received maintenance chemotherapy alone relapsed after a mean period of 5.4 months (2 to 8 months). Seven patients were still under intermittent IV VP16-213 therapy, and one received oral VP16-213 after cessation of IV VP16-213 and steroids 6 months previously. These relapses were localized to the CNS in four children. The same chemotherapy protocol failed to achieve a second complete remission in four children, whereas in four other children a second remission was achieved but was of short duration in two. All of these patients died within 6 months of relapse. However, for two of these children, severe CNS sequelae led to premature cessation of treatment, rapid systemic relapse, and death. Only two children having received chemotherapy alone are alive and well 3 years, 6 months and 4 years, 6 months after cessation of therapy, respectively (patients 1 and 3, ref 8). These patients cannot be distinguished from the other 20 in terms of clinical and biologic presentation of the disease but there was no family history or consanguinity.

Nine patients were treated with allogeneic BMT after chemotherapy (Table 1). Six patients were transplanted in first complete or partial remission, while the other three were treated after relapse and in partial remission.

Among the five recipients of HLA-identical marrow, four are well without treatment 1, 1½, 2, and 6½ years after BMT. The oldest patient developed a mild and transient hemophagocytic syndrome during a bout of measles 4 years after BMT, which included hepatomegaly, thrombocytopenia, and neutropenia and resolved spontaneously in 2 weeks. Donor chimerism was demonstrated in every case and early recovery of natural killer (NK) activity was observed. One child died of relapse 1 year after HLA-identical BMT. In this case, mixed chimerism was demonstrated by RFLP methods on T and B lymphocytes and polymorphonuclear cells. Surprisingly, the donor, the 5-year-old HLA-identical sister who was healthy at the time of transplantation, subsequently developed the disease.

HMA-nonidentical BMT was attempted in four patients. One child was treated in complete remission, which persisted for 1 year after BMT without therapy. However, engraftment was not documented by HLA typing or by NK activity recovery. The disease relapsed 1 year after BMT. The other three patients were in the active phase of the disease at the time of BMT and died rapidly of disease progression.

DISCUSSION

Sustained remission of hemophagocytic lymphohistiocytosis was first achieved with the use of VP16-213-etoposide combined with steroids and intrathecal methotrexate. However, most of the children we treated with this protocol eventually relapsed and the relapses were less sensitive to this regimen. Two explanations can account for this observation. The first is based on the presence of a disease sanctuary in the CNS. CNS involvement is nearly always present at the onset of the disease and CNS relapse is very frequent, often leading to systemic relapse. VP16-213 poorly crosses the blood-brain barrier. Intrathecal methotrexate and cranial irradiation have been proposed as specific CNS therapy, but the efficacy of this radiochemotherapy regimen is not established and it can lead to severe brain damage. Intensification of systemic chemotherapy is controversial in infants less than 1 year of age at diagnosis. The morbidity due to infections in our study was high. Another group has also reported severe and lethal secondary effects of combined chemotherapy. The most likely explanation for treatment failure is related to the pathophysiology of the disease itself. Clearly, the underlying genetic susceptibility to macrophage activation is poorly accessible to chemotherapy. Long-term treatment-free remissions were obtained in two children using chemotherapy alone, but it should be stressed that the genetic origin of the disease could not be proved because there was no family history for these two children. In addition, the negativity of microbiologic tests does not exclude a virus-associated hemophagocytic syndrome; indeed, we have obtained preliminary results suggesting that such a syndrome can be improved or cured by the same regimen.

Allogeneic BMT appears to be a logical approach to the treatment of hemophagocytic lymphohistiocytosis, regardless of whether excessive macrophage activation is intrinsic or secondary to T-cell activation. Moreover, the clinical and biologic syndrome is reminiscent of the acute phase of Chédiak-Higashi syndrome, a disease which is curable by BMT. In our study, HLA-matched BMT was successful in most of the cases, remission being maintained without therapy for a number of years. Clinical relapse occurred in one
patient a year after HLA-matched BMT, despite engraftment. This may be explained by the fact that the donor developed the same disease 6 months later. As there is no known marker predictive of FHL, and all parameters, including triglyceride serum levels and NK cell activity, are normal before the onset of the disease (unpublished observations), it would seem prudent to avoid donors aged less than 5 years.

HLA-nonidentical BMT was not successful. However, it is worth noting that in our series three of the four children were transplanted while the disease was active and resistant to VP16-213. HLA-nonidentical BMT and BMT from matched unrelated donors are experimental approaches that appear to be effective in about 50% of children with various immunodeficiencies or osteopetrosis. Therefore, we feel that the very poor results of chemotherapy alone may justify, in the absence of an HLA-matched related donor, the continued experimental use of other chemotherapy protocols and HLA-mismatched BMT or unrelated HLA-matched BMT after remission has been achieved.

REFERENCES

Treatment of hemophagocytic lymphohistiocytosis with chemotherapy and bone marrow transplantation: a single-center study of 22 cases

S Blanche, M Caniglia, D Girault, J Landman, C Griscelli and A Fischer