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 16 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 allogeneic bone marrow transplantation (BMT). Among the six children transplanted in remission, four are in long-term unmaintained remission, 1 to 6 years after HLA-matched 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. Ambrosio 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 mmol/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 aracynite.

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 lgs, 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.15

Natural killer activity was measured against K562 cells, as previously described.15

**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 moderate macrophage activation syndrome during measles 2 years after BMT.*

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (mo)</th>
<th>Clinical and Biologic Status at BMT</th>
<th>HLA Compatibility Donor (MLR)</th>
<th>Engraftment Markers</th>
<th>NK Activity After BMT</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (ref 9)</td>
<td>27</td>
<td>First, partial remission (splenectomy)</td>
<td>Identical/(-) sibling</td>
<td>Ig allotypes</td>
<td>Normal</td>
<td>Alive and well without treatment 6½ y after BMT.</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>First, complete remission</td>
<td>Identical/(-) sibling</td>
<td>RFLP = mixed chimerism</td>
<td>Negative</td>
<td>Relapse and death 1 y after BMT.*</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>First, complete remission</td>
<td>Identical/(-) sibling</td>
<td>Karyotyping</td>
<td>Normal</td>
<td>Alive and well without treatment 2 y after BMT.</td>
</tr>
<tr>
<td>4</td>
<td>22</td>
<td>First, complete remission</td>
<td>Identical/(-) sibling</td>
<td>Erythrocyte phenotype</td>
<td>Normal</td>
<td>Alive and well without treatment 1½ y after BMT.</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>First, complete remission</td>
<td>Identical/(-) sibling</td>
<td>Not informative</td>
<td>Normal</td>
<td>Alive and well without treatment 1 y after BMT.</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>Partial remission after relapse</td>
<td>2 ag/(+) mismatched parent</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Early relapse, death day 30 after BMT.</td>
</tr>
<tr>
<td>7</td>
<td>13</td>
<td>Partial remission after relapse</td>
<td>2 ag/(+) mismatched parent</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Early relapse, death day 42 after BMT.</td>
</tr>
<tr>
<td>8</td>
<td>22</td>
<td>Partial remission after relapse</td>
<td>1 Haplotype/(+) parent</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Early relapse and death day 65 after BMT.</td>
</tr>
<tr>
<td>9</td>
<td>13</td>
<td>First, complete remission</td>
<td>1 ag/(+) mismatched parent</td>
<td>No evidence on HLA study</td>
<td>Negative</td>
<td>Relapse and death 15 mo after BMT.</td>
</tr>
</tbody>
</table>

Abbreviations: HLA, human leukocyte antigen; MLR, mixed lymphocyte reactivity; ag, antigen.

*The donor developed the disease at the age of 5 years (18 months after transplantation).
TREATMENT OF FHL

The liver or CSF.

went cranial irradiation.

weeks. Donor chimerism was demonstrated in every case
disease. This patient has been described in detail else-

therapy alone relapsed after a mean period of 5.4 months (2

children a second remission was achieved but was of short

The same chemotherapy protocol failed to achieve a second

These relapses were localized to the CNS in four children.

chemotherapy (Table 1).

first complete or partial remission, while the other three

were transplanted in

years, 6 months after cessation of therapy,

were obtained in two children using chemotherapy alone,

and early recovery of natural killer (NK) activity was

and observed. One child died of relapse 1 year after HLA-
demonstrated by RFLP methods on T and B lymphocytes and

surprisingly, the donor, the 5-year-

old HLA-identical sister who was healthy at the time of

transplantation, subsequently developed the disease.

HLA-nonidentical BMT was attempted in four patients.

One child was treated in complete remission, which per-
sisted 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.7,8

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 obser-

vation. The first is based on the presence of a disease

sanctuary in the

liver or CSF.

disease, hemophagocytosis was observed in

spleenomegaly, and mild biologic symptoms related to the
disease. This patient has been described in detail else-

where.9 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 under-

went cranial irradiation.

Eight of 10 patients who received maintenance chemother-

apy 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

sequelea 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 presented 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.13 Intrathecal meth-

otrexate and cranial irradiation have been proposed as

specific CNS therapy, but the efficacy of this radiochemo-

therapy 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 second-

ary effects of combined chemotherapy.14 The most likely

explanation for treatment failure is related to the pathophys-

iology 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 pre-

liminary results suggesting that such a syndrome can be

improved or cured by the same regimen.15

Allogeneic BMT appears to be a logical approach to the

treatment of hemophagocytic lymphohistiocytosis,9 regard-

less of whether excessive macrophage activation is intrinsic

or secondary to T-cell activation.1,5,6 Moreover, the clinical

and biologic syndrome is reminiscent of the acute phase of

Chédiak-Higashi syndrome, a disease which is curable by

BMT.10

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

Table 2. Clinical and Biologic Status of 22 Children With

Hemophagocytic Lymphohistiocytosis at Diagnosis

<table>
<thead>
<tr>
<th>Sex</th>
<th>9 M, 13 F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age at first symptoms</td>
<td>5 mo (1-15)</td>
</tr>
<tr>
<td>Consanguinity</td>
<td>4</td>
</tr>
<tr>
<td>Family history*</td>
<td>10</td>
</tr>
<tr>
<td>Initial clinical presentation</td>
<td></td>
</tr>
<tr>
<td>Hepatomegaly, splenomegaly</td>
<td>22</td>
</tr>
<tr>
<td>High fever</td>
<td>22</td>
</tr>
<tr>
<td>Neurologic symptoms</td>
<td>18</td>
</tr>
<tr>
<td>Cutaneous rash</td>
<td>16</td>
</tr>
<tr>
<td>Initial biologic presentation</td>
<td></td>
</tr>
<tr>
<td>Pancytopenia</td>
<td>22</td>
</tr>
<tr>
<td>Low fibrinogen level</td>
<td>22</td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>22</td>
</tr>
<tr>
<td>Hyponatremia</td>
<td>20</td>
</tr>
<tr>
<td>CSF pleocytosis</td>
<td>19</td>
</tr>
<tr>
<td>Hemophagocytosis on bone marrow aspiration</td>
<td>20†</td>
</tr>
</tbody>
</table>

*The 22 children stem from 20 distinct families.
†For the two remaining children, hemophagocytosis was observed in
the liver or CSF.
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 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

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