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Treatment of the X-Linked Lymphoproliferative, Griscelli and Chédiak–Higashi Syndromes by HLH Directed Therapy

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Background. Griscelli syndrome type 2 (GS2), the X-linked lymphoproliferative (XLP) and the Chédiak–Higashi (CHS) syndromes are diseases that all may develop hemophagocytic syndromes. We wanted to investigate whether the treatment protocols for hemophagocytic lymphohistiocytosis (HLH) can also be used for these syndromes. ***Procedure.*** In the HLH-94/HLH-2004 treatment study registries, we evaluated all patients with GS2 (n = 5), XLP (n = 2) or CHS (n = 2) treated between 1994 and 2004. ***Results.*** All patients responded to the therapy, and all are alive but one (suffering from CHS), with a mean follow-up of 5.6 years. All GS2, one XLP and one CHS patient underwent hematopoietic stem cell transplant. Mean follow-up post transplant was 6.0 years. Six of the seven transplanted children achieved non-active disease status at the time for SCT. Neurological sequelae were reported in all, except for the XLP patients. ***Conclusions.*** Our results indicate that HLH treatment can be an effective first line treatment to induce remission in patients with GS2, XLP and CHS that have developed a hemophagocytic syndrome. We suggest that these patients should be included as a separate cohort in the international HLH study. Pediatr Blood Cancer 2009;52:268–272. © 2008 Wiley-Liss, Inc.

Key words: Chédiak–Higashi syndrome; etoposide; Griscelli syndrome; HLH; X-linked lymphoproliferative disease

INTRODUCTION

Familial hemophagocytic lymphohistiocytosis (FHL) is an autosomal recessive disorder with an incidence of 1/50,000 children born [1]. It is characterized by deficient natural killer (NK) and cytotoxic T lymphocyte (CTL) function, leading to an excessive immune response with activation of T cells and macrophages, resulting in hypercytokinemia, fever, hepatosplenomegaly, cytopenia, and hemophagocytosis. Other common findings include hypertriglyceridemia, hypofibrinogenemia, hyperferritinemia, and neurological manifestations [2]. The disease is fatal if untreated. After introduction of the treatment protocol HLH-94, combining immunochemotherapy and hematopoietic stem cell transplantation (HSCT), survival for children with FHL has improved drastically, and now about half of the affected children survive [3].

Griscelli syndrome type 2 (GS2) (OMIM 607624), Chédiak–Higashi syndrome (CHS) (OMIM 214500), and X-linked lymphoproliferative syndrome (XLP) (OMIM 308240) are rare hereditary diseases caused by mutations in the genes *RAB27A*, *LYST*, and *SAP*, respectively [4–6]. The underlying defects result in impaired cytotoxic function, and a predisposition to develop a hemophagocytic syndrome. We wanted to investigate if these syndromes respond to specific hemophagocytic lymphohistiocytosis (HLH) therapy.

PATIENTS AND METHODS

Patients

Among the 447 patients with hemophagocytic syndromes aged ≤16 years registered in and treated according to HLH-94 or HLH-2004 prior to December 31, 2004, nine patients were later identified to have GS2 (patients 1–5), XLP (patients 6 and 7), or CHS (patients 8 and 9) (Table I).

The HLH-94 and HLH-2004 Treatment Protocols

HLH-94 consists of 8 weeks initial therapy with dexamethasone, etoposide, and in selected patients intrathecal methotrexate, aiming at achieving disease remission. The continuation therapy, also including cyclosporine A, aims at maintaining stable resolution until HSCT is performed. The HLH-94 diagnostic criteria were fever, splenomegaly, bicytopenia, hypertriglyceridaemia and/or hypofibrinogenaemia, and hemophagocytosis [3]. HLH-2004 includes revised diagnostic criteria, and an intensified initial treatment with cyclosporine A from treatment day 1 and the addition of corticosteroids with the intrathecal therapy [7].

RESULTS

Patient Characteristics

The five GS2 patients (no. 1–5) were generally younger than the XLP/CHS patients at onset of symptoms. They all presented with neurological disease, as defined by either neurological symptoms (n = 3) and/or pathological cerebrospinal fluid (CSF) (n = 4). One patient (no. 1) had onset of neurological disturbances and language impairment at 3 years of age, and was developmentally delayed with severely retarded speech and spastic asymmetric diplegia when first seen at the treatment centre at age 5 years. His MRI showed widespread loss of white substance. At that time, however, he had normal CSF. Another patient (no. 3) developed seizures only 4 days after diagnosis, followed by hemiparesis and atactic head movements. The third patient (no. 5) presented with eye abduction deficit

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TABLE I. Patient Baseline Characteristics

Patient no.	Disease	Sex	Ethnic origin	Age at onset of symptoms; at diagnosis[a] (months)	Consanguinity	Familial disease	Diagnostic basis	Genetic information	HLH-94 diagnostic criteria	NK cell function	Initial neurological symptoms; CSF abnormal	Initial neuroradiological abnormalities	Infections at diagnosis[a]
1	GS2	M	Afghani	36; 80	Yes	No	Genetics	*RAB27A* mutation	5/5	Reduced	Yes; No	Yes	Influenza A
2	GS2	M	Danish	3; 4	No	No	EM of hair	*RAB27A* mutation	4/5 (no phag)	Reduced	No; Yes	Not done	Parainfluenza
3	GS2	M	Turkish	1; 4	Yes	No	Genetics	*RAB27A* mutation	4/5 (no tg/fib)	Reduced	Yes[c]; Yes	No	Fever responsive to antibiotics
4	GS2	M	Maltese	8; 8	No	Yes[b]	EM of hair	Not done	5/5	Not done	No; Yes	Not done	No
5	GS2	M	Swedish	2;7	No	Yes[b]	Genetics	*RAB27A* mutation	4/5 (no phag)	Reduced	Yes; Yes	No	No
6	XLP	M	Danish	53; 53	No	Yes[b]	Genetics	*SAP* mutation	1/5 (fever only)	Reduced	Yes; Yes	Not done	HHV6
7	XLP	M	Serbian	69; 69	No	No	Genetics	*SAP* mutation	4/5 (no splenomegaly)	Reduced	Yes; Yes	Yes	EBV
8	CHS	F	Danish	60; 60	No	No	EM of hair and skin. Specific granules in leucocytes	Not done	5/5	Not done	No; No	No	No, but debut after respiratory infection
9	CHS	M	Afghani	104; 104	Yes	No	Genetics	*LYST* mutation	5/5	Reduced	No; Yes (but sampled after start of therapy)	Not done	*Staphylococcus epidermidis* septicemia + EBV

EM, electron microscopy; Phag, hemophagocytosis; tg/fib, hypertriglyceridemia/hypofibrinogenemia. [a]Diagnosis of hemophagocytic syndrome; [b]Pat 4: Sister died of liver failure/cytopenia and also had gray hair. Pat 5: Twin sisters later confirmed to have GS2. Pat 6: Brother died of liver failure after EBV-infection; [c]Debuted 2 days after start of therapy.

and facial palsy, and his magnetic resonance imaging (MRI) showed signs of oedema of the brain stem, thalamus and cerebellum. Both males with XLP (patients 6 and 7) had neurological manifestations initially; one (no. 6) had febrile convulsions and the other (no. 7) was somnolent with CT examination showing signs of brain oedema. Both had elevated CSF protein and cell counts. Investigation for infectious agents revealed Epstein–Barr virus (EBV) and human herpes virus 6 (HHV6) in patients 6 and 7, respectively. The patients with Chédiak–Higashi (patients 8 and 9) had no initial neurological manifestations. In one patient (no. 8), the accelerated phase followed a respiratory tract infection. In the other (no. 9), the onset of the accelerated phase coincided with a *Staphylococcus epidermidis* septicaemia and further investigation also showed positive EBV-PCR.

Treatment and Outcome

Seven patients (patients 1–6 and 8) have undergone HSCT (mean follow-up 5.6 years, range 1.5–9.1). Four patients had a matched unrelated (MUD), two a matched related (MRD) and one had a cord blood-, unrelated, one antigen mismatched (MMUD) donor. One patient (no. 3) had active disease at the time of HSCT. One of the XLP patients (no. 7) responded well, is off all therapy and presently in stable remission with a follow-up of 33 months. As of November 2007 eight out of nine patients are reported alive (mean follow-up 6.0 years, range 2.2–10.4). One patient (no. 9) died while on HLH treatment (Table II).

Among the patients with GS2, one (no. 4) reached non-active disease status already after initial therapy and underwent MRD-HSCT. He remained well up to 5 years after HSCT, when he developed marked ataxia, dysarthria and choreatic movements, but no signs of systemic reactivation, no headache and no fever. Repeated CSF exams showed normal protein and cell counts, and viral investigations were negative. His EEG was normal. MRI showed white matter lesions that later decreased in number, and different causes were considered including acute disseminated encephalomyelitis, viral encephalitis, or isolated CNS-activation of GS2. Four patients with GS2 (no. 1–3 and 5) received continuation therapy, which resulted in non-active disease (NAD) status in three (no. 1, 2, and 5) within 6 months. The male with severe, long-standing neurological impairment (no. 1) significantly improved during treatment and underwent HSCT after 9 months of HLH-94 therapy. Two patients (nos. 2 and 3) had CNS-relapses during therapy. Both were successfully transplanted; one (no. 3) had active disease at the time of transplant. Neurological sequelae have been reported in all GS2 patients at the last follow-up.

The patients with XLP both improved on initial therapy and received continuation therapy. At 6 months, both had reached non-active disease status, and one (no. 6) had a MUD transplant. He is alive without sequelae. The other male (no. 7) did not have a suitable donor, and was taken off all therapy after 1 year of treatment. He is doing well almost 3 years after having stopped all therapy.

One of the patients with CHS (no. 8) had complete resolution after initial therapy and underwent MRD-HSCT. She was doing well up to 9 years post HSCT, with complete donor chimerism, but she recently developed some learning difficulties. She has pain in her left foot, the cause of which is unknown. She is followed orthopedically, and has required arthrodesis. The other CHS patient (no. 9) first improved, but had no complete resolution and then suffered EBV-encephalitis, septicaemia and renal insufficiency. He

TABLE II. Treatment and Outcome

Patient no.	Disease	Treatment	CNS relapse	Off therapy status[a]	Status at HSCT	HSCT	Conditioning and GvH prophylaxis[b]	Sequelae	Impaired ability to play	Follow-up post HSCT (years)	Total follow-up (years)
1	GS2	HLH-94	No	No	NAD	MUD	B, C, A	Slight hemiplegia, moderate but stable developmental delay	Mild-moderate	3.9	4.7
2	GS2	HLH-94	Yes, during therapy	No	NAD	MUD	B, C, A	Hemiparesis, language problems	Moderate-severe	1.6	2.8
3	GS2	HLH-94	Yes, during therapy	No	AD	MUD	B, C. A, VP	Delayed psychomotor development	No	9.1	10.4
4	GS2	HLH-94	Suspected, 5 years post HSCT	No	NAD	MRD	B, C, A, VP	Dysartric, markedly atactic, chorea	Moderate-severe	8.3	8.5
5	GS2	HLH-94	No	No	NAD	MMUD	B, C, A, VP	Facial paresis, concentration difficulties	Mild-moderate	6.0	6.2
6	XLP	HLH-2004	No	No	NAD	MUD	B, C, A	No	No	1.5	2.2
7	XLP	HLH-94	No	Yes		No		No	No		3.8
8	CHS	HLH-94	No	No	NAD	MRD	B, C, V P	Learning difficulties, pain from foot. Hypothyreosis	No	9.0	9.2
9	CHS	HLH-2004	No	N.A	N.A	No	NA	NA	NA	NA	NA

MUD, matched unrelated donor; MRD, matched related donor; MMUD, mismatched unrelated donor; AD, active disease; NAD, non-active disease; B, busulfan; C, cyclophosphamide; A, antithymocyte globulin; VP, etoposide; NA, not applicable. [a]Off all HLH therapy for $\geq$1 year, with no HSCT and no disease reactivation. [b]All HSCT patients received cyclosporine A and methotrexate as Graft versus Host (GvH) prophylaxis.

died from multiorgan failure and subdural hemorrhage 72 days after start of therapy.

DISCUSSION

Overall, HLH therapy for GS2, XLP, and CHS was effective in inducing remission of the accelerated phase and/or kept patients alive while a suitable transplant donor was pursued in eight of the nine patients. Unfortunately, neurological sequelae were common in the survivors. Among the patients with GS2, all presented with signs of CNS disease, and two developed CNS reactivations during treatment. They all had varying degrees of neurological impairment at follow-up. The surviving patient with CHS has learning difficulties on long-term follow-up, a symptom consistent with progressive neurological dysfunctions previously described in long-term CHS survivors [8]. One of the patients with XLP and a hemophagocytic syndrome triggered by an EBV-infection responded promptly to HLH treatment and has had no relapse although no HSCT was performed. Very few non-HSCT XLP long-term survivors have been previously reported [9,10], and it is still too early to tell whether a HSCT will eventually be necessary.

GS2, CHS, and XLP share many features with FHL. All syndromes may develop a hemophagocytic syndrome, characterized by decreased cytotoxic activity and a hyperinflammatory state. This accelerated phase is often triggered by viral infections. HSCT is the only known cure of the haematological and immunological abnormalities [11].

FHL-causing mutations have been described in three genes: *PRF1* (FHL2; OMIM 603553), encoding perforin [12], *UNC13D* (FHL3; OMIM 608898), encoding Munc 13-4 [13], and *STX11* (FHL4; OMIM 603552), encoding syntaxin-11 [14]. In addition, FHL1 has been mapped to chromosome 9q [15]. Perforin, the major cytolytic enzyme of the cytotoxic granulae, is crucial for cytotoxic lymphocyte function. Munc13-4 is involved in the priming of cytotoxic granulae prior to membrane fusion and exocytosis, and syntaxin-11 is also important for degranulation [16].

XLP is reported in 1–3/1,000,000 men born, but that may be an underestimation [17]. Patients with XLP have dysgammaglobulinaemia and defective NK cell function, the latter leading to deficient tumor surveillance with increased risk of developing lymphoproliferative diseases, such as lymphomas. The hallmark of the disease is fulminant infectious mononucleosis (FIM), which may develop into a hemophagocytic syndrome. The mean age for developing FIM is less than 5 years, and without treatment a majority of patients die within 1–2 months [18].

XLP is caused by mutations in the genes encoding the signaling lymphocytic activation molecule (SLAM)-associated protein (*SAP*). *SAP* is expressed in NK and T cells and has relevance for the assembly of lytic synapses in CTLs, and in polarizing cytotoxic mediators at the point of contact with target cells [17].

The incidence of GS2 and CHS is unknown. Clinically, these syndromes are characterized by immunodeficiency combined with albinism. In an accelerated phase, these patients will develop hemophagocytic syndromes with cytopenia, fever, hepatosplenomegaly, hypogammaglobulinaemia and sometimes neurological symptoms. The mean age for developing hemophagocytic syndrome in GS2 is 1.5 years [2,19]. Both syndromes have a high mortality and require HSCT for hematological cure [20,21].

GS2 is an autosomal recessive disease and caused by mutations in the *RAB27A* gene. The encoded protein, Rab27a, is highly expressed in secretory cells, and found both in immune cells and melanocytes. The protein Rab27a interacts with other proteins necessary for late endosomal membrane fusion, such as Munc13-4, and thereby plays an essential role in cytotoxic granule exocytosis [22]. CHS, which is also autosomal recessive, is caused by mutations in the lysosomal trafficking regulator gene (*LYST*) causing defective fusion or fission of lysosomal granulae, thus leading to impaired granule exocytosis and decreased NK cell and CTL function [11].

In diseases with extensive cell proliferation, an apoptosis-inducing treatment regimen is appealing. Etoposide has previously been suggested as part of the treatment regimen for XLP [10][23], CHS [24], and GS2 [25][26]. Here we show that treatment with the HLH-94/HLH-2004 protocols can be an effective first line remission-inducing treatment for these syndromes, if a hemophagocytic syndrome has developed. Addition of anti-CD20 monoclonal antibody therapy should be considered in EBV-associated syndromes [9][27].

One important question is whether hematological cure also results in freedom from neurological symptoms. Unfortunately, according to long-term follow-up of other patients with CHS it appears as some neurological signs and symptoms may progress despite HSCT, most likely as a result of a long-term progression of the lysosomal defect in neurons and glial cells [8]. In GS2 on the other hand, expression of Rab27A has not been detected in brain tissue and this subset of patients is reported not to have primary abnormal neurological features [4,28]. Independently, early diagnosis and treatment is most likely beneficial in both CHS and GS2 in order to reduce the neurological complications developing in association with the hemophagocytic syndrome itself, in line with a recent report on a large number of patients with CNS-HLH [29].

GS2, CHS, and XLP are diseases with similar immunological manifestations and a predisposition for a fulminant hemophagocytic syndrome. Treatment delay increases the risk of fatal outcome and may increase the risk for neurological complications. Our report indicates that there is no need to initially differentiate between FHL, GS2, CHS, and XLP since HLH therapy may be initiated even if genetic results are pending. To accumulate further knowledge, all patients administered HLH therapy ought to be registered in the international HLH treatment registries.

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