Elevated Soluble Interleukin-2 Receptor in Childhood Hemophagocytic Histiocytic Syndromes

By Diane M. Komp, James McNamara, and Patrick Buckley

The serum of children with untreated hemophagocytic syndromes contains elevated levels (23,600 to 75,200 U/mL) of soluble interleukin-2 receptor (SIL2R) that returns toward normal with clinical improvement. These levels are as high as levels reported for HTLV-1-associated adult T-cell leukemia (HATL) and hairy cell leukemia (HCL) in adults and some children with poor-prognosis non-T, non-B, acute lymphoblastic leukemia (ALL). Serum SIL2R is a marker of disease activity that has the potential to identify infants at risk for the inherited form of the disease before the disease is clinically expressed.

We wished to determine if SIL2R is elevated in the sera of children with syndromes of hemophagocytic histiocytes believed to be disorders of macrophage activation. Second, we sought to determine if the levels parallel disease activity and response to therapy.

MATERIALS AND METHODS

Patient material. Sera from nine children with hemophagocytic syndromes were studied. Institutional Review Board guidelines for research subjects were observed. Clinical characteristics are summarized in Table 1. The classification system proposed by the Histioyte Society was used. Patient 1 was born by emergency cesarian section and died several hours after birth. The parents are first cousins, and two of their previous children died of FHLH. Patients 2 through 8 were treated with a VP 16-based regimen. Patients 5 and 7 were born to related parents of Sephardic Jewish ancestry. Patient 7 was studied during a relapse from oral VP 16 maintenance therapy. Patient 8 died of intracranial hemorrhage shortly after splenectomy and initiation of VP-16 therapy. Patient 9 presented with fever, splenomegaly, and cytopenias that spontaneously remitted. The first sample was obtained at the time of admission, before the spontaneous remission. The second sample was obtained at the time of a florid relapse 3 weeks later. A third sample was obtained after one course of interferon-α (IFN-α) at the time of fever defervescence but before objective evidence of clinical improvement. Splenectomy was performed in these two patients because of life-threatening hyperplasia and inability to support the effects of this hypersplenism with blood products. Two samples were obtained after splenectomy during the course of IFN-α treatment, and the final sample was obtained at the time of clinical remission and normalization of the bone marrow (BM). None of the patients had any risk factors for HIV infection. Antibody testing for HIV was performed postmortem or by the treating clinician in four cases and was negative in each.

Normal ranges were defined by mean ± 2 SD for age group. Paired acute and convalescent sera from patients with EBV-documented infectious mononucleosis and sera from patients with invasive Pseudomonas infections were also studied. The placenta and cord blood from patient 1 was available for study as part of an autopsy consultation.

Soluble IL2R assay. Assays for SIL2R were performed using a modification of the sandwich enzyme immunoassay described by Rubin et al17 and commercially available (Cell Free) from T Cell Sciences, Cambridge, MA. One hundred microliters of an anti-IL2R murine MoAb was incubated on polylysine microtiter wells. Sample or standard is thereafter bound to the wells and further treated with a horseradish peroxidase-conjugated anti-IL2R murine MoAb directed against a second epitope on the IL2R molecule, binding to the IL2R fixed by the first antibody. After the plates are washed to remove unbound enzyme-conjugated antibody,
O-phenylenediamine is added for color production. After the reaction is stopped with 2N H<sub>2</sub>SO<sub>4</sub>, absorbance is read at 492 nm. A standard curve is created from four IL2R standards prepared from the supernatant of phytohemagglutinin (PHA)-stimulated normal T cells.

**Placental studies.** A portion of the placenta from patient 1 was frozen at −70°C soon after birth. Portions of two normal placentae from uncomplicated cesarian sections were used for comparison. CD25 expression was studied on frozen tissue sections by a modification of the indirect immunoperoxidase technique described by Hsu et al. The presence of the macrophage antigen detected by MoAb EBM-I was also assayed on placental tissue, as recently described.

**HTLV-I antibodies.** Sera were tested for HTLV-1 antibodies with an enzyme-linked immunosorbent assay (ELISA) method (Dupont) that does not cross-react with HIV. It is prepared from an HuT-103-2B lymphocyte cell line infected with HTLV-I. The inactivated virus is bound to the microplate wells. After serum addition and incubation, plates are washed and treated with alkaline phosphatase-conjugated goat anti-human IgG. Paranitrophenylphosphate is used for color production. The reaction is stopped with 3N NaOH and absorbance read at 410 nm.

**RESULTS**

The results of hemophagocytic patients are summarized in Table 2. Values in untreated patients ranged from 23,600 to 75,200 U/mL. Six studies were made of five patients who had clinical responses to VP-16 therapy. Patients measured midtreatment but before they had achieved complete clinical remission (CR) had elevated levels of SIL2R (three patients). Seven of our seven patients with clinically defined active disease had elevated levels. Five of the five patients with longitudinal studies showed a reduction in SIL2R with treatment. Two of five in clinical CR were in the normal range for age.

Patients with uncomplicated infectious mononucleosis showed elevations of SIL2R that returned toward normal during their convalescence. The highest value was 4,480 U/mL. They were chosen as controls because of the common association of EBV with viral-associated hemophagocytic syndrome. Patients with *Pseudomonas*, a common opportunistic infection in infants with FHLH, had values from 750

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**Table 1. Summary of Clinical Data for Patients With Hemophagocytic Syndromes**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (mo) at Diagnosis</th>
<th>Family History</th>
<th>Infectious Agent</th>
<th>Pathologically Confirmed Tissue</th>
<th>Survival After Diagnosis</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Birth (cord blood)</td>
<td>+</td>
<td>—</td>
<td>BM, liver, spleen, GI, thyroid</td>
<td>Died day 1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>BM, liver</td>
<td>Alive on treatment 1 yr</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>+</td>
<td>—</td>
<td>Bone marrow, CSF</td>
<td>Alive with disease 5½ yr</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>—</td>
<td>CMV</td>
<td>Liver</td>
<td>Alive on treatment 3 mo</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>Sephardic Jew</td>
<td>—</td>
<td>BM, liver</td>
<td>Alive on treatment 24 mo</td>
</tr>
<tr>
<td>6</td>
<td>18.5</td>
<td>—</td>
<td>—</td>
<td>BM, liver, spleen, CSF</td>
<td>Dead of disease</td>
</tr>
<tr>
<td>7</td>
<td>47</td>
<td>+</td>
<td>—</td>
<td>BM, CSF</td>
<td>Alive off treatment 16 mo</td>
</tr>
<tr>
<td>8</td>
<td>108</td>
<td>—</td>
<td>Parvovirus</td>
<td>BM, liver, lymph nodes</td>
<td>Died 1 mo</td>
</tr>
<tr>
<td>9</td>
<td>14 years</td>
<td>—</td>
<td>—</td>
<td>BM, liver, spleen</td>
<td>Alive on treatment 4 mo</td>
</tr>
</tbody>
</table>

**Table 2. Summary of SIL2R Levels in Patients With Hemophagocytic Syndromes**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (mo)</th>
<th>Pretreatment</th>
<th>Midtreatment</th>
<th>Clinical CR</th>
<th>Normal Range For Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Birth (cord blood)</td>
<td>23,600</td>
<td>—</td>
<td>—</td>
<td>267-799</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>—</td>
<td>—</td>
<td>1,000</td>
<td>580-1,712</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>36,420</td>
<td>—</td>
<td>—</td>
<td>322-1,207</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>35,000</td>
<td>—</td>
<td>—</td>
<td>580-1,712</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>26,000*</td>
<td>—</td>
<td>—</td>
<td>341-2,337</td>
</tr>
<tr>
<td>6</td>
<td>18.5</td>
<td>—</td>
<td>14,300</td>
<td>2,420</td>
<td>341-2,337</td>
</tr>
<tr>
<td>7</td>
<td>54</td>
<td>—</td>
<td>3,150</td>
<td>2,050</td>
<td>322-1,207</td>
</tr>
<tr>
<td>8</td>
<td>108</td>
<td>51,260</td>
<td>75,200</td>
<td>—</td>
<td>241-697</td>
</tr>
<tr>
<td>9</td>
<td>14 yr</td>
<td>4,000</td>
<td>35,200</td>
<td>—</td>
<td>241-697</td>
</tr>
</tbody>
</table>

*Sample obtained after one course of chemotherapy.
†Sample obtained immediately after splenectomy.
to 1,250 U/mL, all of which were above the normal range for age (Fig 1).

Frozen sections of the placenta of an infant who died soon after birth were available for study. Traditional histologic examination of bone marrow, spleen, and other viscera showed numerous hemophagocytic histiocytes. The cord blood SIL-2R level of this infant was 23,600 U/mL. Placental fetal villous macrophages stained intensely for CD 25 in comparison to control normal placentas (Fig 2). HTLV-1 antibodies were evaluated in six patients. None of the patients studied had HTLV-1 antibodies documentable by ELISA.

**Discussion**

The levels documented in our cases of childhood hemophagocytic histiocytoses are comparable with or higher than those previously reported in patients with hematologic malignancies. In contrast to adults, SIL2R levels in children are variable. Normal newborn (cord blood) levels are slightly higher (267 to 799 U/mL) than those of normal adults (80 to 600 U/mL) and increase more after birth, peaking between 9½ and 19½ months of age (341 to 2,337 U/mL) and decreasing to adult levels by age 10 years. The levels documented in our cases of childhood hemophagocytic histiocytoses are comparable with or higher than those previously reported in patients with hematologic malignancies. In contrast to adults, SIL2R levels in children are variable. Normal newborn (cord blood) levels are slightly higher (267 to 799 U/mL) than those of normal adults (80 to 600 U/mL) and increase more after birth, peaking between 9½ and 19½ months of age (341 to 2,337 U/mL) and decreasing to adult levels by age 10 years.

Little is known about the origin of soluble IL2R in vivo, much less the developmental aspects of its expression and regulation in fetal life and early childhood. Whether the elevated levels of SIL2R in young normal children reflect lymphocytic activation in response to childhood immunizations, normal experience with other antigens in the environment, or developmental maturation of the mononuclear-hemophagocytic lineage cells in not clear. According to a recent report, CD 25-identifiable material cannot normally be demonstrated in placental tissue despite the presence of IL-2. Studies in our laboratory using a sensitive immunoperoxidase technique, suggest however, that low levels of IL2R are expressed by normal villous macrophages.

Although the hemophagocytic histiocytoses are presumed pathologically to be benign, our observation of markedly elevated levels of SIL2R are more typical of proliferation in a host-uncontrolled fashion. Deeper showed that stimulation of normal resting mononuclear cells produces modest expression of IL2R with decreasing production in maintained cultures despite addition of IL-2; he postulated that in the normal cell there is a programmed decline of receptor gene transcription that is bypassed by HTLV-1 infection. HTLV-1–infected cells, however, are IL-2 independent in their IL2R production.

Heretofore, the highest levels of SIL2R have been reported in HTLV-1–associated T cell leukemia (HATL) (~1,200 to 70,000 U/mL), hairy cell leukemia (HCL) (13,370 to 48,090 U/mL), and non-T, non-B acute lymphoblastic leukemia (ALL) in children. Values for hemophagocytic patients were far above the range observed in our patients with acute infectious mononucleosis or pseudomonas infection or reported in HIV infections. High levels of the soluble receptor have been reported in the serum of children with non-Hodgkin's lymphomas and ALL traditionally assumed not to be HTLV-related. Children with advanced Burkitt's and other undifferentiated lymphomas are reported to have serum SIL2R levels in the range of 329 to 5335 U/mL.

Although serum triglycerides are frequently elevated in affected children, no marker is yet available to identify affected infants in families at risk of FHLH before clinical expression of the disease. Prospective studies are indicated to determine if serum SIL2R or placental macrophage IL2R will predict affected infants earlier than clinical symptomatology, serum triglycerides, or other markers of disease activity. At birth, the placenta provides noninvasive access to tissue macrophages. Nelson postulated that elevated SIL2R may act as a "blocking factor," capable of inhibiting normal immune response by binding to IL-2. Patients with FHLH are subject to opportunistic infection and may exhibit defects of both cellular and humoral immunity. These defects can be temporarily reversed by blood exchange transfusion. Elevated SIL2R may be involved in the secondary immunodeficiency of these patients. Patients with HCL have a similar severe defect of natural killer (NK) cell activity. In HCL patients, IL2R has been postulated to absorb IL-2, making it unavailable to stimulate NK cell activity.

Until the past few years, treatment for the hemophagocytic syndromes, especially the familial forms, has been ineffective. Recent advances with allogeneic bone marrow transplantation and VP-16–based chemotherapy raise enough hope for familial cases to justify aggressive early identification of potentially affected children.

![Fig 1. Comparison of SIL2R levels in patients with hemophagocytic syndromes (HS), untreated, on treatment, and in complete clinical remission (CR) with infectious mononucleosis (IM) and Pseudomonas infections.](image-url)
REFERENCES

Fig 2. Frozen section immunoperoxidase technique with hematoxylin counterstain. (A) Immunoperoxidase stain on normal third trimester placenta incubated with MoAb directed against IL-2R (CD25). Staining of villous macrophages (arrows) was too weak to be demonstrable. Arrowheads show knots of syncytiotrophoblast (magnification ×200). (B) Placenta from patient 1 incubated using MoAb monoclonal antibody directed against IL2R. Black-stained cells indicated by arrows are intravillous macrophages strongly positive for IL2R; arrowheads indicate syncytiotrophoblast knots. (magnification ×400). (C) Placenta from patient 1 stained with pan-macrophage MoAb (EBM-1). The darkly stained macrophages are morphologically similar to the IL2R+ cells in B. (magnification ×400).

Finally, further elucidation of the cytokine-related abnormalities may shed light on the basic etiology and pathogenesis of these disorders. Studies of IL-2 and other cytokines are in progress in our laboratory. Since SIL2R is normal or near-normal in CR, we do not expect that we can detect asymptomatic carriers by measuring SIL2R. However, this phenomenon may suggest more fruitful paths of genetic research than have been previously available. The molecular genetics of IL2R are the subject of intense investigation.

Such studies in familial cases may help elucidate the underlying genetic defects.

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