A Syndrome Involving Intrauterine Growth Retardation, Microcephaly, Cerebellar Hypoplasia, B Lymphocyte Deficiency, and Progressive Pancytopenia

Patrick Revy, MSc*; Meinrad Busslinger, PhD‡; Kei Tashiro, MD, PhD§; Fernando Arenzana, MD, PhD‖; Pascal Pillet, MD¶; Alain Fischer, MD, PhD*; and Anne Durandy, MD, PhD*

ABSTRACT. We report a new complex syndrome involving profound failure to thrive with severe intrauterine growth retardation, cerebellar abnormalities, microcephaly, a complete lack of B lymphocyte development, and secondary, progressive marrow aplasia. B cell differentiation was found to be blocked at the pro-B cell stage. Although not strictly proven, a genetic origin is likely, according to similar cases reported in the literature.

Three candidate genes, PAX5, encoding B cell-specific activator protein, a factor involved in B cell lineage commitment, stromal cell-derived factor 1, and CXCR4, encoding a chemokine and its receptor, respectively, were thought to be responsible for this disease, given the similarity between the phenotype of the corresponding knock-out mice and the clinical features of the patient. However, the genomic DNA sequences of these 3 genes were normal, and normal amounts of stromal cell-derived factor 1 and CXCR4 were present.

These data strongly suggest that another molecule is involved in early B cell differentiation, hematopoiesis, and cerebellar development in humans. Pediatrics 2000; 105(3). URL: http://www.pediatrics.org/cgi/content/full/105/3/39; e105(3). URL: http://www.pediatrics.org/cgi/content/full/105/3/39

Inherited primary immunodeficiencies, defined by the World Health Organization scientific group, include either isolated immunodeficiencies or immunodeficiencies associated with other major defects. DiGeorge anomaly, ataxia telangiectasia, cartilage-hair hypoplasia, and Schimke syndrome all involve a T cell defect associated with abnormalities in other organ systems. In contrast, we describe here another syndrome associating a humoral immunodeficiency and profound failure to thrive, severe intrauterine growth retardation, neurological abnormalities including microcephaly, mental retardation and cerebellar abnormalities, and secondary progressive marrow aplasia. The molecular basis of this syndrome, previously described in 4 patients with putative autosomal recessive inheritance, remains unknown.

Three gene products have been shown to be involved in both B cell ontogeny and the development of other cells or tissues including the cerebellum. A mutation in 1 of these genes was sought in this patient. The PAX5 gene encodes the B cell-specific activator protein (BSAP) transcription factor. BSAP is expressed in the midbrain, testis, and all B-lymphoid tissues during mouse ontogeny. It is involved in the regulation of expression of the B cell-specific transmembrane protein, CD19. Pax5 knock-out mice completely lack mature B cells and exhibit severe failure to thrive and cerebellar abnormalities. These features are similar to the patient’s phenotype.

Progressive marrow aplasia is a characteristic of mice deficient for stromal cell-derived factor 1 (SDF-1) or its receptor CXCR4. The stromal-derived factor SDF-1 is a chemokine that was initially described as a key factor for B cell lymphopoiesis and bone marrow myelopoiesis. Its only known receptor is CXCR4, which has also been described as a co-receptor for human immunodeficiency virus. SDF-1 and CXCR4 are also constitutively expressed in a wide variety of nonhematopoietic tissues, including the brain and heart. SDF-1 or CXCR4 knock-out mice have no B cells, secondary marrow aplasia, and other features, including severe failure to thrive and cerebellar and vascular abnormalities. The clinical presentation of our patient was very similar to the phenotype of these deficient mice, except that the patient had no detectable vascular defect.

CASE REPORT

The patient, a girl, was born to nonconsanguineous parents. She suffered from profound severe intrauterine growth retardation and weighed only 620 g when born at 31 weeks of gestation. The observation of microcephaly led to a computed tomography scan of the brain at 2 months old, which demonstrated enlarged sulci and less dense white matter than usual. Apart from the microcephaly, the patient did not present any other detectable malformation. She was discharged at 2 months old but suffered from persistent diarrhea, failure to thrive, and recurrent respiratory and digestive infections.

Agammaglobulinemia was diagnosed at 9 months old. Bone marrow aspiration showed normal differentiation of hematopoietic lineages except for the absence of B (CD19+) cells. She was treated by intravenous immunoglobulin therapy.
At 18 months old, she was pancytopenic (Hb: 9 g/dL; reticulocytes: 64 000/µL; white blood cell count: 1500/µL; polymorphonuclear neutrophils: 1000/µL; lymphocytes: 300/µL; and platelets: 35 000/µL). Bone marrow aspiration showed progressive marrow aplasia. Development of erythroid lineage was poor, myeloid cell development was blocked, rare megacytocytes were present, and erythrophagocytic macrophages were also found. The pancytopenia progressed and the patient received regular erythrocyte and platelet transfusions.

At 24 months old, the child’s growth retardation was profound: 5.9 kg (−4.5 standard deviation [SD]) for weight and 74 cm (−3.5 SD) for height. She was microcephalic: 40 cm (−6 SD) with associated mental retardation. No obvious ataxia was observed, but there was a spastic paresis. Magnetic resonance imaging of the brain demonstrated cerebellar hypoplasia, particularly of the vermis with enlargement of the sulci (Fig 1). The cytogenetic analysis of lymphocytes and fibroblasts showed no anomaly at the resolution of 550 bands used. The mitochondrial DNA studies were also normal. The bone marrow showed poor cell development, with absence of CD34+ cells, although erythrophagocytic macrophages were present. The patient died at 4 years old from a disseminated bacterial infection.

Immunologic Studies

Serum immunoglobulin levels were determined by nephelometry. Peripheral blood mononuclear cells were isolated from freshly drawn heparine-treated blood by Ficoll-Hypaque (Pharmacia Fine Chemicals, Uppsala, Sweden) density gradient centrifugation. T cells were counted with fluorescein isothiocyanate (FITC)-conjugated anti-CD3 monoclonal antibodies (mAbs; IOT3 Immunotech, Marseille, France), CD4+ T cells with FITC-Leu-3a mAb (Becton-Dickinson, Mountain View, CA), and CD8+ T cells with phycoerythrin-Leu-2a mAb (Becton-Dickinson). B cells were enumerated by phycoerythrin-labeled CD19 mAb, and FITC-anti-CD20 mAb from Immunotech. Precursor cells were detected in bone marrow aspiration samples with an FITC-CD34 mAb from Immunotech.

SDF-1 in the serum was measured with the time-resolved fluorimunoassay method (Ikegawa et al, unpublished data). Briefly, 4 assay buffers were prepared: buffer 1 for coating the white C96 maxisorp 96-well microtiterplate (Nunc, Roskilde, Denmark), .15 M phosphate buffer containing ,14 M NaCl (phosphate-buffered saline); buffer 2 for rinsing the plate, .05 M Tris-HCl (pH 7.8) containing .05% Tween 20; buffer 3 for rinsing, .05 M Tris-HCl (pH 7.8); and buffer 4 for dilution of the protein solution, .05 M Tris-HCl (pH 7.8) containing .2% bovine serum albumin, .1% NaN3, and .9% NaCl. Standard solutions (50 µL) of SDF-1 or plasma samples diluted with buffer 4 were pipetted to the coated plate and were incubated at 37°C for 1 hour. After rinsing the plate with buffers 2 and 3, 50 µL of solution of biotinylated anti-human SDF-1 antibodies (R & D System Inc, Minneapolis, MN) diluted 1000-fold with buffer 4 was incubated in the well at 37°C for 1 hour. After the incubation, the plate was rinsed twice with buffer 2 and once with buffer 3, and 50 µL of BHHCT-Eu3+ labeled bovine serum albumin-streptavidin solution (50 µL) was incubated in the well at 37°C for 1 hour. The plate was rinsed 4 times with .05 M Tris-HCl (pH 9.1), containing .05% Tween 20. The plate was subjected to solid-phase fluorometric measurement using 1420 ARVO multilabel counter (Amersham Pharmacia Biotech, Uppsala, Sweden).

The primary fibroblasts of the patient and control subjects were studied for expression of CXCR4 receptor using anti-CXCR4 mAb 6H8 directed against the NH2 terminus part of CXCR424 and a rat FITC antibody directed against murine immunoglobulin (Jackson, West Baltimore, Pike) in a 2-step immunofluorescence assay. Iso-type-matched immunoglobulin G-1 was used as control.

For intracellular immunofluorescence studies, fibroblasts from the patient and control subjects were permeabilized with the fluorescence-activated cell sorter permeabilizing solution from Becton-Dickinson before incubation with the 6H8 mAb.25 Immunofluorescence analysis was performed using a FACStar plus (Becton-Dickinson).

T cell-proliferation was assayed as previously described.21 Peripheral blood mononuclear cells were stimulated with phytohemagglutinin mitogen (Difco, Detroit, MI, final dilution = 1/700) or anti-CD3 mAb (OKT3, Ortho Pharmaceuticals, Raritan, NJ; 50 ng/mL) in 4-day cultures.

Genetic Studies

Sequence of PAX5, SDF1, and CXCR4

Genomic DNA was extracted from bone marrow samples with proteinase K, sodium dodecyl sulfate, and a series of phenolchloroform extractions. The 10 exons of PAX5, the 4 exons of SDF-1 gene, or the 2 exons of CXCR4 and their adjacent intronic regions were amplified by polymerase chain reaction (40 cycles each at 94°C for 1 minute, 57°C for 1 minute, and 72°C for 1 minute) using intron-specific primers (Vorechovsky et al, unpublished data).22 Polymerase chain reaction products were separated by electrophoresis, extracted by the GeneClean III procedure (Bio 101, Vista, CA) and directly sequenced with the Automatic DNA sequencer from Perkin-Elmer.

RESULTS

Immunologic Data

The immunologic data are presented in Table 1. The first immunologic studies were performed at 9 months old. A profound pan-hypogammaglobulinemia was noticed. There was mild lymphopenia, with absence of B cells (as shown by anti-CD19 and anti-CD20 mAb staining). In contrast, T cell counts were only slightly lower than normal.

At 24 months old, serum immunoglobulin M and immunoglobulin A levels were very low (immunoglobulin G levels could not be assessed because of immunoglobulin therapy). No B cells were detected in the blood and no B cell precursors (CD19+ ) were detected in the cells collected by marrow aspiration. The T cell count was also low because of the profound lymphopenia. The T cells could be activated, however, by nonspecific activators, such as phytohemagglutinin mitogen and CD3 mAb.

Genetic Analysis

The failure to thrive together with an absence of B cells and cerebellar hypoplasia suggested a mutation in the PAX5 gene, because all these abnormalities are
present in Pax5 knock-out mice. However, the sequences of the 10 PAX5 exons and adjacent intronic regions were normal. PAX5 expression could not be studied because the cells that normally express this gene were absent from bone marrow (CD34+/CD19− cells) and blood (CD19− cells).

The secondary progressive marrow aplasia suggested the possible involvement of SDF-1 or its receptor CXCR4 in the pathogenesis of this syndrome. SDF-1 and CXCR4 knock-out mice have been reported with failure to thrive and to have defects in B cell development, secondary marrow aplasia, and cerebellar abnormalities. SDF-1 levels in the patient’s serum were within normal range (Fig 2A). The CXCR4 receptor was not detectable by membrane immunofluorescence on fibroblasts from either the patient or control subjects but was detected in both with the same intensity in the cytoplasm as shown by

**TABLE 1. Immunological Data**

<table>
<thead>
<tr>
<th>Immunological Study</th>
<th>Patient 9 Months</th>
<th>Patient 24 Months</th>
<th>Age-Matched Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocyte count (cells/μL)</td>
<td>2023</td>
<td>250</td>
<td>4000–9000</td>
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<tr>
<td>Blood B lymphocytes (%)</td>
<td>0</td>
<td>0</td>
<td>7–15</td>
</tr>
<tr>
<td>CD19</td>
<td>0</td>
<td>0</td>
<td>7–15</td>
</tr>
<tr>
<td>CD20</td>
<td>0</td>
<td>0</td>
<td>7–15</td>
</tr>
<tr>
<td>Marrow B lymphocytes (%)</td>
<td>0</td>
<td>0</td>
<td>25–47</td>
</tr>
<tr>
<td>CD19</td>
<td>0</td>
<td>0</td>
<td>20–30</td>
</tr>
<tr>
<td>CD20</td>
<td>0</td>
<td>0</td>
<td>25–47</td>
</tr>
<tr>
<td>Serum immunoglobulin levels (mg/mL)</td>
<td>0.27</td>
<td>0.12</td>
<td>0.4–9</td>
</tr>
<tr>
<td>Immunoglobulin M</td>
<td>0.20</td>
<td>NE</td>
<td>4.2–10.1</td>
</tr>
<tr>
<td>Immunoglobulin G</td>
<td>0.04</td>
<td>0.04</td>
<td>1.6–8</td>
</tr>
<tr>
<td>Blood T lymphocytes (%)</td>
<td>96</td>
<td>97</td>
<td>65–80</td>
</tr>
<tr>
<td>CD3</td>
<td>68</td>
<td>64</td>
<td>40–50</td>
</tr>
<tr>
<td>CD4</td>
<td>27</td>
<td>32</td>
<td>25–35</td>
</tr>
<tr>
<td>CD8</td>
<td>ND</td>
<td>.2</td>
<td>2</td>
</tr>
<tr>
<td>T cell proliferation (cpm × 10^3)</td>
<td>ND</td>
<td>125</td>
<td>&gt;80</td>
</tr>
<tr>
<td>Unstimulated</td>
<td>ND</td>
<td>110</td>
<td>&gt;40</td>
</tr>
<tr>
<td>Phytohemagglutinin mitogen</td>
<td>ND</td>
<td>125</td>
<td>&gt;80</td>
</tr>
<tr>
<td>Anti-CD3 mAb</td>
<td>ND</td>
<td>110</td>
<td>&gt;40</td>
</tr>
</tbody>
</table>

ND indicates not done; NE, not evaluable (attributable to Ig substitution).

![Fig 2. A, Serum SDF-1 concentration in the patient and control individuals. Serum samples were prepared from heparnized blood of the patient and of 3 control subjects. Data shown are representative of triplicate measurements in separate experiments. B, Intracytoplasmic CXCR4 expression in the fibroblasts of the patient and a control individual. Data shown are representative of 2 independent experiments. A solid line indicates control isotype-matched mAb; a broken line, 6H8 mAb.]
in intracellular immunofluorescence staining (Fig 2B). Moreover, the various exons and flanking intronic regions of the SDF-1 and CXCR4 genes were sequenced and found to be normal.

**DISCUSSION**

We describe a complex syndrome associating prenatal growth retardation, failure to thrive, absence of mature B cells, progressive marrow aplasia, microcephaly, and cerebellar abnormalities. This syndrome, previously described by Hoyeraad et al., Hreidarsson et al., and Berthet et al., is not attributable to a classified immunodeficiency. This association is not observed in usual B cell deficiencies, including autosomal recessive agammaglobulinemia. The lack of karyotype abnormality excluded ataxia-telangiectasia and other immunodeficiencies associated with chromosomal abnormalities.

In this case, the immunodeficiency mostly affected B cell development; there was an absence of B cells in blood and bone marrow and a severe hypogammaglobulinemia. The detection of a low level of serum immunoglobulins is similar to observations in X-linked agammaglobulinemia, suggesting the escape of a few B cells from the genetic defect. In vitro T cell activation was normal, despite a T cell lymphopenia. Moreover, this patient did not present with the opportunistic infections commonly observed in cellular immunodeficiencies. In contrast, the patient reported by Hreidarsson et al. developed a disseminated Candida albicans infection, suggesting a T cell/monocyte cell defect, and the patient described by Berthet et al. presented with T cell immunodeficiency with dissociated and progressively impaired cytokine responses. However, it is unknown whether this cellular abnormality was primary or secondary to infections. In this case, we cannot strictly exclude an acquired immunodeficiency, secondary, for example, to an antenatal infection. However, there was no evidence of maternal prenatal infection, and the phenotype of the patient was very similar to that of the previously published cases, in whom an autosomal recessive inheritance pattern was strongly suspected because of the consanguinity of the parents. Indeed, growth retardation with prenatal onset, lack of B cells, progressive marrow aplasia, and neurological manifestations are features common to all patients (Table 2). The combination of failure to thrive, arrest of B cell maturation at the pro-B cell stage, and cerebellar abnormalities was strongly reminiscent of the features described in Pax5 knock-out mice. Therefore, we studied the Pax5 gene in this patient. We could not investigate the presence of BSAP mRNA or protein attributable to the absence of BSAP expressing cells like B cells and B cell precursors. Therefore, we sequenced the 10 exons and flanking intronic regions of the Pax5 gene on genomic DNA. No abnormality was detected, indicating that the structural Pax5 gene is not mutated in this patient. However, we cannot exclude the possibility of a regulatory defect as mutations in the B cell-specific promoter or enhancer of Pax5 in 1 of its transactive regulatory proteins or in the 8-kb 3’ noncoding region of Pax5, could affect Pax5 transcription or mRNA stability.

In contrast with Pax5 knock-out mice, the patient studied here and the other patients described in previous studies developed a severe marrow aplasia. This feature was similar to the phenotype of SDF-1 or CXCR4 knock-out mice. However, defects in the SDF-1 or CXCR4 genes were excluded because their sequences were found to be normal and the proteins were normally expressed.

This case, and those previously reported, clearly define a complex syndrome involving a lack of B cells, secondary progressive marrow aplasia, intrauterine growth retardation, and cerebellar abnormalities. Our findings strongly suggest that an unknown molecule plays a major role in the early differentiation of B cell progenitors, postnatal hematopoiesis, and cerebellar development in humans.

**ACKNOWLEDGMENTS**

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