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BRIEF COMMUNICATION

Impact of folate therapy on combined immunodeficiency secondary to hereditary folate malabsorption

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Abstract Hereditary folate malabsorption (HFM) is a rare autosomal recessive disorder. Severe folate deficiency in HFM can result in immunodeficiency. We describe a female infant with HFM who acquired severe *Pneumocystis* pneumonia. The objective of the present study was to elucidate her immunological phenotype and to examine the time course of immune recovery following parenteral folate therapy. The patient demonstrated a combined immunodeficiency with an impaired T cell proliferation response, pan-hypogammaglobulinemia, and an imbalanced pro-inflammatory cytokine profile. She had normal white blood cell count, normal lymphocyte subsets, and normal complement levels. Two novel mutations were identified within the *SLC46A1* gene to produce a compound heterozygote. We confirmed full recovery of her immunological and neurophysiological status with parenteral folate replacement. The time course of recovery of her immunological profile varied widely, however. HFM should be recognized as a unique form of immunodeficiency.

1. Introduction

Folates are a family of B vitamin compounds that are interconvertible in a series of intracellular biochemical reactions that play a key role in the synthesis of nucleic acids. Folate deficiency occurs for several reasons: poor intake, excessive requirements, exposure to antifolate drugs, or impaired metabolism pertaining

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to the absorption, transport, and intracellular reactions. Hereditary folate malabsorption (HFM; OMIM 229050) is a rare autosomal recessive disorder, characterized by folate deficiency with decreased intestinal folate absorption and decreased folate transport into the central nervous system [1]. The genetic basis of HFM is loss-of-function mutations of the proton-coupled folate transporter gene (*PCFT*, *SLC46A1*) [2].

HFM patients present very early in life with megaloblastic anemia, failure to thrive and infections, and can develop severe neurodevelopmental defects unless they receive prompt and aggressive parenteral folate therapy [3]. Various immunodeficiencies have been found in association with folate deficiency [4–6]. HFM has been linked with a reversible severe combined immunodeficiency (SCID) phenotype [7]; however, few studies have analyzed sequential reconstitution of the immune system in the setting of parenteral folate therapy.

We describe a female child with severe immunodeficiency secondary to HFM. The objective of the present study was to elucidate her immunological phenotype as well as the time course of immune recovery following folate therapy.

2. Materials and methods

2.1. Patient history

A 3-month-old female developed persistent cough and failure to thrive. She was born to healthy non-consanguineous Japanese parents as their third child. Her two brothers had no remarkable medical history. Her clinical presentation included severe megaloblastic anemia, developmental retardation, and an intractable respiratory tract infection. Laboratory results were remarkable for pan-hypogammaglobulinemia, megaloblastic anemia, thrombocytopenia, and an extremely low serum folate concentration (Table 1). Further investigation revealed bilateral bronchopneumonia and *Pneumocystis jirovecii* was detected by polymerase chain reaction (PCR) in the sputum. Initial treatment consisted of oral folate therapy and trimethoprim/sulfamethoxazole (TMP/SMX), which did not correct her serum folate level or result in clinical improvement. Invasive mechanical ventilation was required due to respiratory failure with severe pulmonary hypertension. She was then treated with parenteral folate therapy (PFT) together with continuous oral TMP/SMX after admission to the intensive care unit. After three days of administration of parenteral folate, the anemia and thrombocytopenia improved as well as the pneumonia. She was provisionally diagnosed with HFM and subjected to gene analysis. Subsequent PFT achieved complete normalization of her hematological parameters. Although the serum folate level had increased to over 10 ng/mL, the cerebrospinal fluid (CSF) level remained significantly low (2.8 ng/mL). Dosing of parenteral folinic acid was increased to 12 mg daily to maintain CSF folate levels in the normal range. She had developed normally by the age of 18 months, without any neurological or respiratory sequelae.

Table 1 Initial laboratory values.

Value	Result	Reference range
White blood cell	6.1	5.0–19.5 × 10^9/L
Absolute neutrophil count	1.6	1.0–8.5 × 10^9/L
Absolute lymphocyte count	3.5	2.5–16.5 × 10^9/L
Hemoglobin	5.9	9.0–14.0 g/dL
Hematocrit	17.5	28–42%
Mean corpuscular volume	92	70–86 fL
Platelets	17	150–400 × 10^9/L
Lactate dehydrogenase	791	170–580 U/L
Iron	205	22–184 μg/dL
Total iron-binding capacity	244	100–400 μg/dL
Folate	<0.5	15–55 ng/mL
Vitamin B12	346	200–835 pg/mL
IgG	97	176–581 mg/dL
IgA	14	4.6–46 mg/dL
IgM	22	24–89 mg/dL
C3	60	64–131 mg/dL
C4	12	8.7–27 mg/dL

2.2. Evaluation of the white blood cell count and immunoglobulins

The cellular and humoral immunities of the patient were evaluated by performing sequential white blood cell counts, absolute lymphocyte counts (ALCs), as well as quantitative determination of immunoglobulins. Levels of immunoglobulins were compared with previously reported reference intervals for age [8].

2.3. Lymphocyte subset analysis

Peripheral blood lymphocyte subsets were analyzed by flow cytometry before and three months after the initiation of PFT. Immunophenotyping of lymphocytes was performed using the following anti-human monoclonal antibodies: anti-CD3 [peridinin chlorophyll protein (PerCP)], anti-CD4 [fluorescein isothiocyanate (FITC)], anti-CD8 [phycoerythrin (PE)], anti-CD19 [FITC] (BD Biosciences Japan, Tokyo, Japan), and anti-CD56 [PE] (Beckman Coulter Japan, Tokyo, Japan). Flow cytometry was performed using a FACScalibur flow cytometer (BD Japan, Tokyo, Japan). Data were processed using CellQuest Pro (version 6.0) software (BD Biosciences Japan, Tokyo, Japan). Subsets were compared with previously reported reference intervals for age [9].

2.4. Lymphocyte proliferation assays

Lymphocyte proliferation in response to phytohemagglutinin (PHA) and concanavalin A (ConA) was also assessed by routine procedures before and 4 weeks after the initiation of PFT. Data are expressed in terms of stimulation indexes (S.I.).

2.5. Measurement of serum cytokine levels

Blood samples were obtained at five time points (TPs): a week before (TP −1), immediately before (TP 0), a week after (TP 1), 2 weeks after (TP 2), and 3 weeks after (TP 3) the initiation of PFT. The sera were separated and kept frozen (−80 °C) until analyzed. Twelve cytokines including tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), interleukin-1 beta (IL-1β), IL-5, IL-6, IL-8, IL-10, monokine induced by IFN gamma (MIG, CXCL9), IFN