Early-onset inflammatory bowel disease caused by mutations in the X-linked gene IL2RG

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Mutations in the X-linked gene, *IL2RG* cause severe combined immunodeficiency (X-SCID), which is the most severe primary immunodeficiency (PID). In typical X-SCID, the disease is characterized by an almost complete absence of T and NK cells, and nearly normal or high numbers of functionally deficient B cells.[1]. Clinically, patients with X-SCID present in the first few months after birth with severe and persistent infection, usually of the lung and gastrointestinal tract [2]. The disease is often fatal unless treated with allogeneic hematopoietic stem cell transplantation. On the other hand, patients with various PIDs exhibit clinical overlap with inflammatory bowel disease (IBD) as one of their leading symptoms in very early age [3]. SCID patients often suffer enteropathy and failure to thrive, and rarely present with IBD manifestation in the absence of severe infection [4]. However, there have been no previous reports on the pathogenesis of X-SCID with IBD manifestation.

A 6-month-old boy was referred to our hospital with a two-month history of

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intractable diarrhea and recurrent perianal abscess with fever. He had no history of illness until the age of 4 months. He had not received any live vaccines, including Bacille Calmette-Guérin, polio, and rotavirus vaccine. His physical examination was unremarkable without perianal region. At the time of referral, he had persistent chronic diarrhea with bloody stool, and no sign of respiratory infections, but showed positive *Pneumocystis jiroveci* polymerase chain reaction (PCR) results in the gastric fluid. No other pathogenic microbes were detected in blood, respiratory tract secretions, gastric fluid, urine, and stools (Supplementary Material, Table 1). Immunological investigation showed hypogammaglobulinemia (IgG, 10 mg/dL; IgA, 2 mg/dL; IgM, 9 mg/dL; and IgE < 25 IU/mL) and severe lymphopenia (1363/μL) with a marked reduction in $CD3^+$ $CD4^+$ T cells (5%), $CD3^+$ $CD8^+$T cells (0%), and $CD3^-$ $CD56^+$ NK cell cells (1%), but preservation of $CD19^+$ B cells (94%). Colonoscopy revealed multiple longitudinal ulcers from the transverse to the rectum (Figure), and biopsies demonstrated neutrophil infiltration, proliferative inflammatory granulation tissues, and small granuloma in the colon (Supplementary Figure). Immunohistochemistry revealed an abundance of CD3-positive cells in the inflammatory lesion. Conversely, there were no CD56-positive cells (Supplementary Figure).

Treatment with sulfamethoxazole/trimethoprim for *Pneumocystis jiroveci* and cefazolin aimed at the perianal abscess was initiated in addition to immunoglobulin replacement therapy. Fever, perianal abscess, and bloody diarrhea improved after these medical interventions, but

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watery diarrhea became persistent. Fifteen days after the intervention, sigmoidoscopy revealed that most of ulcers in the rectum and sigmoid colon were scarring. One month after referral, an HLA 8/8 matched cord blood was transplanted into the patient after a preparative regimen consisting of fludarabine (30 mg/m^2/day for 5 days) and melphalan (70 mg/m^2/day for 2 days). After starting preparative regimen drugs, water diarrhea gradually ameliorated. Tacrolimus and short-term methotrexate (7 mg/m^2/day on day 1 and 5 mg/m^2/day on days 3 and 6) were used for graft-versus-host disease (GVHD) prophylaxis. The clinical course after cord blood transplantation (CBT) was uneventful and diarrhea completely resolved four months after CBT.

DNA sequencing showed a point mutation in the *IL2RG* gene (*IL2RG* gene (c.536_552delTGAACCACTGTTTGGAG; p.Leu179Argfs*26), representing the T(-)B(+)NK(-) phenotype of SCID. However, low numbers of $CD3^+$ $CD4^+$ T cells had been observed in the peripheral blood (PB) (81–141/μL). A subtle peak in the maternal allele was detected in the PB by microsatellite chimerism using short tandem repeat. DNA isolated from bead-selected CD3 T cells was detected the maternal allele. All T cells expressed HLA-DR^+-activation T lymphocyte marker and a monoclonal peak pattern for T cell receptor (TCR)γ was detected in the PB. Similarly, maternal T cells and TCRγ clonality were confirmed in biopsied gut tissue, which was infiltrated by a large number of T cells.

Some PIDs that affect intestinal immune and epithelial function can lead to IBD-like

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disease. PIDs caused by immune dysregulation such as LPS-responsive beige-like anchor protein (LRBA) deficiency also resemble symptoms of enteropathy presenting in immunocompetent individuals [5,6]. Azizi G et al. showed the efficacy of sirolimus which block the malian target of rapamycin for patients with LRBA deficiency refractory to conventional therapies [6]. Therefore, it is considered that some of the PIDs associated with non-infectious enteropathy are caused by immune abnormalities. Regarding X-SCID, little is known about the mechanism.

Maternal T cells from fetal or perinatal transplacental passage can be engrafted and expanded in hosts with SCID as they are unable to reject circulating maternal T cells due to severe cellular immune dysfunction, and occurs in 40% of all patients with SCID [7]. The majority of SCID patients with maternal T engraftment are asymptomatic, but some have mild symptoms and signs such as skin rash, elevated liver enzyme involved with increased palpable lymph nodes, hepatosplenomegaly, or eosinophilia. In some rare cases, engraftment maternal T cells cause GVHD, such as severe erythematous skin rash or chronic liver disease [7,8]; however, to date, the described manifestations of GVHD due to maternal T cells have been limited to skin and liver. In our X-SCID infant, the patient showed no clinical signs except gastrointestinal symptoms.

Various animal models for IBD have been developed and the T cell transfer model has been widely used. The setting of IBD mice model showed that donor $CD4^+$ T cells induced

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colitis resembling IBD in SCID mice [9]. In general, $CD4^+$ T cells direct appropriate immune responses, maintain immune tolerance, and support differentiation of memory cells. However, $CD4^+$ T cell subsets have also been shown to contribute to chronic intestinal inflammation, accumulating in the mucosa of IBD patients [10]. It was assumed that maternal $CD3^+$ $CD4^+$ T cells expanded in the patient's PB monoclonally and caused colitis, similar to that in IBD model mice. Following antimicrobial treatment, endoscopic findings improved. The colitis may have been associated with infections, although we were unable to detect any pathogenic microbes in the stools.

Since the number of T cells was very small, we were unable to investigate detailed $CD3^+$ $CD4^+$ T cell subsets in the present case. Moreover, we were completely unable to deny the coexistence of autologous T cells. Nonetheless, we showed the presence of maternal cells in the intestinal lesions with significant T cell infiltration despite a few T cell in the PB. Therefore, it is likely that these cells were strongly associated with pathogenesis of early-onset inflammatory bowel disease in our patient.

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Conflicts of interest: The authors declare that they have no conflict of interest.

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FIGURE LEGENDS

Figure. Endoscopic view of a typical ulcer in sigmoid colon (upper part) and transverse colon (lower part).