IL-10RA Mutation as a Risk Factor of Severe Influenza-Associated Encephalopathy: A Case Report

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Influenza-associated encephalitis and encephalopathy (IAE) is a severe complication of influenza infection with high morbidity and mortality. We present the case of a patient with *IL-10RA* mutation who developed encephalopathy after influenza infection. A 10-day-old boy developed recurrent fever and anal fistula. Growth failure gradually became apparent. He had been treated with antibiotics and elemental nutrition. However, the patient did not respond to the treatments. At 11 months, he suddenly developed shock with encephalopathy and multiple organ failures. He was then diagnosed with IAE. A cytokine study revealed elevated levels of IL-1 receptor antagonist, IL-2, IL-6, IL-8, IP-10, eotaxin, G-CSF, MCP-1, and IL-10. These cytokines are normally downregulated by IL-10. Genetic testing revealed a *IL-10RA* mutation at the 3′ end of exon 4 (c.537G→A). These findings might reflect an increased risk of severe IAE in patients with *IL-10RA* mutation.

IL-10 is a key anti-inflammatory cytokine, which is produced predominantly by leukocytes, including T cells, B cells, monocytes, macrophages, and dendritic cells, as well as by some epithelial cells.[1,2] Polymorphisms in *IL-10* and its receptors *IL-10RA* and *IL-10RB* have been observed in individuals with several immune-mediated diseases, such as very early onset inflammatory bowel disease (VEOIBD),[3] autoimmune thyroid diseases,[4] and diabetes. *IL-10* polymorphisms are also associated with some infectious diseases, including otitis media, rhinovirus infection, and pulmonary tuberculosis.[5,6] However, whether these variants are associated with severe infections because of influenza has not been reported. We reported the first case of a child with influenza virus-related systemic inflammatory response syndrome.

CASE REPORT

The patient, who was born at the 38th week of gestation, developed chronic diarrhea soon after his birth. His father had chronic nephritis. However, none of his family members had a history of inflammatory bowel diseases. His parents were not consanguineous, although they were from the same geographical region in Japan. He first developed fever at 10 days of life and recurrent fever thereafter, particularly after immunizations. A perianal skin tag developed at 21 days of life, and growth failure gradually became apparent. Ketotifen was used for skin eczema. However, no improvement was observed. At 5 months, he was referred to our hospital. On consultation, he presented with eczema, and anal fistula with rectal stenosis was observed during rectal examination (Fig 1). Bloody diarrhea was also observed. His

abstract

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Dr Ishige participated in the diagnosis of the patient, was involved in the administration of medical care, and drafted the initial manuscript; Dr Igarashi participated in the diagnosis of the patient and was involved in the administration of medical care; Drs Hatori and Tatsuki collected data and conducted the cytokine analysis; Dr Sasahara conducted the patient's *IL-10RA* sequencing and performed the functional study on *IL-10RA*; Drs Takizawa and Arakawa designed the cytokine study and coordinated and supervised data collection; and all authors reviewed and revised manuscript and approved the final manuscript as submitted.

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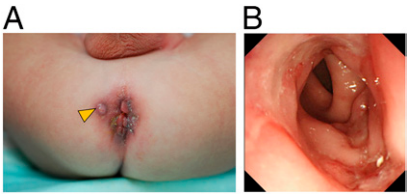

FIGURE 1
Clinical features of the patient. A, Unique perianal manifestation with fistula and skin tag. B, Longitudinal ulcer of the rectum.

height was 1.8 SD below average, and his weight was 3.4 SD below average. Laboratory tests revealed significant leukocytosis (31 000 per mm^3) and mildly elevated C-reactive protein (0.34 mg/dL). Serum immunoglobulin-E level was also elevated for his age (109.1 IU/mL; reference: <20 IU/mL). Because the patient did not respond to elemental nutrition therapy and trimethoprim-sulfamethoxazole administration, we performed sigmoidostomy. However, the patient developed a skin-sigmoidostomy fistula 2 weeks after operation. An extensive immunologic evaluation was conducted, including assessments of immunoglobulin levels, complete blood counts, lymphocyte subsets, and nicotinamide adenine dinucleotide phosphate oxidase function, which has been recommended as an additional laboratory screening procedure for patients with VEOIBD.[7] However, no signs of primary immunodeficiency diseases were observed. Considering perianal fistulas and recurrent fever from the neonatal period, we suspected the patient had monogenic disease involving IL-10 pathways, and a direct sequence analysis on *IL-10*, *IL-10RA*, and *IL-10RB* was conducted by using genomic DNA that is isolated from peripheral blood mononuclear cells from the patient and his parents.

At 11 months, he was admitted to our hospital for a scheduled blood test and induction of enteral nutrition therapy. On the next morning, he developed a high fever (41°C [105.8°F]). Shortly thereafter, he suddenly developed generalized seizures and became unconscious. He showed marked tachycardia, and his blood pressure was unmeasurable; hydration and catecholamine were used to maintain his circulation. Laboratory data revealed marked elevated liver enzymes and ferritin levels, as well as lactic acidosis. Anemia, thrombocytopenia, and abnormal coagulation resembled disseminated intravascular coagulation. His bloody stool worsened, and respiratory failure due to coma required intubation and mechanical ventilation. A brain MRI revealed diffuse cerebral edema, and a flat electroencephalography was also observed. Using a rapid diagnosis reagent kit (Quick Navi-Flu; Otsuka Pharmaceutical Company, Limited, Tokyo, Japan), we observed influenza type A virus antigen in his nasal discharge. Therefore, a diagnosis of influenza-associated enteropathy was made. Multiple organ dysfunction developed, and he died 1 month after influenza infection.

TABLE 1 Serum Cytokines From the Samples Taken Before and After the Onset of Influenza

Cytokines and Chemokines	Before Onset (d 1)	After Onset (d 0)	Reference Values
PDGF	23c855.1	25c835.76	—
IL-1 β	4.37	11.52	<10
IL-1 ra[a]	577.02	2431.44	105–1062
IL-2[a]	28	737.24	—
IL-4	13.03	13.38	<6.0
IL-5	35.97	35.49	<3.9
IL-6[a]	28.12	673.45	<4
IL-7	21.7	19.22	—
IL-8[a]	36.85	1097.07	<2.0
IP-10[a]	1666.01	5119.74	—
IL-9	125.94	86.32	—
IL-12 (p70)	133.81	10.28	—
IL-13	16.95	7.96	—
IL-15	32.73	19.29	—
IL-17	169.35	154.86	—
Eotaxina	202.71	773.77	<11.2
FGF basic	106.52	74.88	<39.0
G-CSF[a]	66.12	5085.07	<2.0
GM-CSF	185.45	154.7	<20.6
IFN-γ	104.93	183.65	—
MCP-1[a]	113.21	638.17	—
MIP-1 α	3.97	3.94	—
MIP-1 β	161.26	143.45	—
RANTES	881.79	881.79	—
TNF-α	67.11	114.89	0.6–2.8
VEGF	284.06	71.41	38.3
IL-10[a]	193.58	704.05	<7.05

Unit: pg/mL. Reference values are for Japanese adults and are only shown when those data are available. —, not applicable.
[a] These cytokines had an increase of more than twofold after the onset of influenza.

His serum cytokine level was assessed by using samples taken a day before and on the day of the onset of influenza (Table 1). A significant increase in serum IL-1 β, IL-1 receptor antagonist, IL-2, IL-6, IL-8, IL-10, and G-CSF levels were observed after influenza virus infection.

After his death, we confirmed a homozygous and heterozygous mutation (c.537G→A) at the 3′ end of exon 4 of *IL-10RA* in the patient and his parents, respectively (Fig 2).[8] The IL-10 (65.1 pg/mL; reference: 0–7.9 pg/mL) and IL-1A (76.2 pg/mL; reference: 0–13.5 pg/mL) levels from the serum sample obtained at 8 months of age were also elevated. On the basis of these results, the patient was diagnosed with VEOIBD along with *IL-10RA* polymorphism.

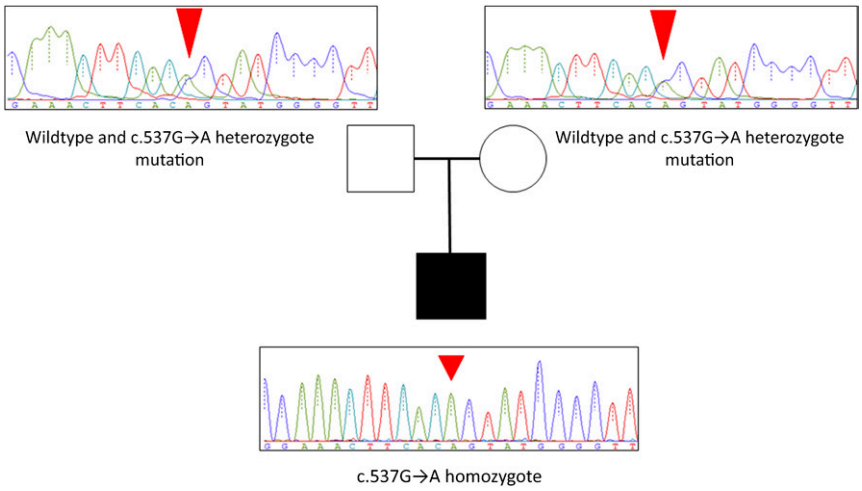

FIGURE 2

IL-10RA mutation in the genomic DNA of the patient and parents. Homozygote c.537G→A mutation at the 3′ end of exon 4 in the *IL-10RA* gene of the patient was confirmed via Sanger sequencing.[7]

DISCUSSION

Influenza causes severe morbidity and mortality. Every year, influenza epidemics are estimated to result in about 3 to 5 million cases of severe illness, and about 290 000 to 650 000 people die of complications because of influenza.[9]

Influenza-associated encephalitis and encephalopathy (IAE) is defined as an altered mental status lasting for >24 hours.[10] The morbidity and mortality of this disease can be relatively severe. Morishima et al[11] identified 148 cases of IAE and reported that 46 (31.3%) of 148 patients died and 41 (27.7%) had a long-term disability.

Authors of previous studies have hypothesized that there is an association between genetic background and the severity of and mortality due to influenza infections. On the basis of the computerized genealogical database linked to the disease data of Utah (Utah Population Database), Albright et al[12] reported that the relative risk of death from influenza is significantly higher in individuals who have both close and distant relatives who died because of influenza. Studies revealed that distant relatives have a higher risk of dying compared with those of their spouses, which reveals that genetic background is significantly more important than environmental factors.[12] Kubota et al[13] reported that carnitine palmitoyltransferase II variants are associated with the severity of acute encephalopathy. Hidaka et al[14] reported a case of *TLR3* gene missense mutation in a patient with encephalitis. These mutations may partly cause IAE. However, not all patients have these mutations.

IL-10 is an important anti-inflammatory cytokine in humans,[15] and is secreted by immune cells, including monocytes, macrophages, T cells, B cells, and granulocytes. IL-10 inhibits the expression of IL-1 α, IL-1 β, IL-6, IL-12, IL-18, G-CSF, TNF cytokines, and IL-10 itself.[1] Regarding the chemokines, production of MCP1, MCP5, MIP-1 α, MIP-1 β, MIP-3 α, MIP-3 β, RANTES, MDC, IL-8, IP-10, and MIP-2 are inhibited by activated monocytes.[1] Of these cytokines and chemokines, IL-1, IL-2, IL-6, G-CSF, TNF, IL-10, MCP-1, IL-8, and IP-10 were confirmed to be elevated after the onset of influenza in our patient. Because these samples were taken on 2 consecutive days, we presumed that the observed changes were predominantly affected by the influenza infection but not by changes in the condition of VEOIBD. The mutation c.537G→A, which was observed in this patient, is the cause of VEOIBD, which develops 18-base deletion of the exon 4 of the IL-10RA protein and causes the impaired phosphorylation of STAT3 in peripheral blood mononuclear cell samples.[8,16] The changes in serum cytokines after influenza infection in our patient were highly affected by *IL-10RA* gene mutation.

IL-10 plays a critical role in limiting inflammation during encephalitis. Wilson et al[17] reported that IL-10 can downregulate the proinflammatory properties of astrocytes in knockout mice, thus leading to decreased cytokine production after toxoplasma infection. Cheeran et al[18] reported that cytomegalovirus brain infection is lethal in IL-10–deficient mice, and IFN-γ and IL-6 messenger RNA levels from whole brain homogenates were significantly elevated in IL-10–deficient mice, as compared with wild-type mice. IL-10 controls excess brain inflammation during encephalitis. Regarding studies of humans, Yang et al[19] reported that *IL-10* 1082 G/A polymorphism is associated with enterovirus 71 encephalitis. Polymorphism at this locus is also associated with mortality and the severity of the systemic inflammatory response syndrome that is associated with pneumonia.[20] Moreover, Kawada et al[21] reported that the transcription of IL-6, IL-10, and TNF-α is significantly upregulated in patients with IAE, as compared with patients in whom influenza infection has not had neurologic implications.

In addition, polymorphisms in *IL-10* are associated with the response to influenza vaccination. Individuals with *IL-10* 1082 G/A polymorphism are reported to have significantly lower risks of developing adverse responses when compared with individuals with the A/A genotype.[22] An allele in this locus is associated with a higher susceptibility to sepsis, and a G allele is associated with

a higher mortality in relation to sepsis.[23]

In the current study, the first confirmed case of *IL-10RA* mutation in a patient with IAE was reported. On the basis of the cytokine assay results, we assumed that the patient's *IL-10RA* mutation was highly associated with the development of encephalopathy and poor outcome after influenza infection. Therefore, strict precautions against influenza infection should be considered in patients with VEOIBD with *IL-10*, *IL-10RA*, or *IL-10RB* mutations. Because patients with VEOIBD with *IL-10*, *IL-10RA* or *IL-10RB* mutations are known to have severe perianal diseases, we recommend examining these mutations for patients with perianal abscesses or fistula.[24] In addition, considering the association between *IL-10* polymorphisms and influenza vaccination, authors of future studies should consider these polymorphisms in patients with IAE even if no intestinal manifestations are observed.

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ABBREVIATIONS

IAE: influenza-associated encephalitis and encephalopathy
VEOIBD: very early onset inflammatory bowel disease

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