Autoimmunity Including Intestinal Behçet Disease Bearing the KRAS Mutation in Lymphocytes: A Case Report

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abstract

We experienced the case of a 3-year-old male with a very rare combination of autoimmunity, including immune thrombocytopenia, recurrent Henoch-Schönlein purpura and intestinal Behçet disease. Exome sequencing of the patient’s peripheral blood mononuclear cells identified a KRAS G13C mutation. Interestingly, the KRAS G13C mutation was observed in T and B lymphocytes, as well as natural killer cells, but not granulocytes. Our case was completely phenotypically different from RASopathies and did not meet the criteria for Ras-associated lymphoproliferative disease or juvenile myelomonocytic leukemia. This is the first reported case in which the KRAS mutation existed only in the lymphoid lineage. Based on the findings of our case and the current literature, it is clear that the RAS mutation in lymphoid cells is tightly linked with various autoimmune symptoms. The presence of the RAS mutation in lymphocytes should be reconsidered as a pathogenesis in cases of autoimmunity.

Only a limited genetic association has been identified in cases of autoimmunity disorders. One of the well-characterized autoimmune disorders caused by a gene mutation is autoimmune lymphoproliferative syndrome (ALPS).¹ Most genetic mutations of ALPS involve molecules of the FAS-dependent apoptosis signaling pathway. Patients with several congenital syndromes, such as Noonan syndrome and Kabuki syndrome, are also known to be susceptible to developing autoimmune disorders.²,³ Noonan syndrome develops as a result of germline mutations of the PTPN11, SOS1, RAF1, KRAS, and NRAS genes, which are involved in the RAS-mitogen-activated protein kinase signaling pathway.⁴ Dysregulation of T lymphocytes in this pathway appears to play an important role in the development of autoimmunity in general and systemic lupus erythematosus in particular.⁵ Mutations at codons 12 and 13 of the KRAS and NRAS genes have been described in cases of Ras-associated lymphoproliferative disease (RALD).⁶–⁸ RALD features autoimmune cytopenia and lymphoproliferation secondary to a T-cell apoptosis defect, similar to ALPS. Approximately 20% to 25% of patients with juvenile myelomonocytic leukemia (JMML) have RAS mutations at codons 12, 13, and 61,⁶ and 65% of cases of JMML are known to involve immunologic abnormalities, including autoimmunity.⁷ The association between RAS mutations and autoimmunity is distinct in both germline and somatic mutations. We herein present a case of a novel

important role in the development of autoimmunity in general and systemic lupus erythematosus in particular.¹ Mutations at codons 12 and 13 of the KRAS and NRAS genes have been described in cases of Ras-associated lymphoproliferative disease (RALD).⁶–⁸ RALD features autoimmune cytopenia and lymphoproliferation secondary to a T-cell apoptosis defect, similar to ALPS. Approximately 20% to 25% of patients with juvenile myelomonocytic leukemia (JMML) have RAS mutations at codons 12, 13, and 61,⁶ and 65% of cases of JMML are known to involve immunologic abnormalities, including autoimmunity.⁷ The association between RAS mutations and autoimmunity is distinct in both germline and somatic mutations. We herein present a case of a novel


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**CLINICAL CASE**

A 3-year old Japanese boy presented with abdominal pain, purpura on the lower limbs, and arthralgia of the bilateral ankles. No hepatosplenomegaly or lymphadenopathy was observed, and a blood examination revealed no abnormalities. The patient received oral prednisolone under a disease-free status after steroid cessation. However, 2 months after steroid termination, the purpura on the patient’s hip and extremities reappeared. A blood test demonstrated mild anemia and thrombocytopenia (hemoglobin level, 8.5 g/dL; platelet count, $22 \times 10^9/L$; white blood cell count, $8.4 \times 10^9/L$ [monocytes: 4%; blasts: 0%; and reticulocytes, 1.2%]). The patient’s serum bilirubin (0.6 mg/dL), haptoglobin (134 mg/dL), and lactate dehydrogenase (281 IU/L) levels were within normal limits. Direct Coombs and antplatelet antibody tests were negative, and the patient’s platelet-associated immunoglobulin (Ig) G level was not elevated (14 ng/10^7 cells). The etiology of the patient’s anemia was identified as iron-deficiency (mean corpuscular volume = 62.7 fL, mean corpuscular hemoglobin = 18.1 pg, serum iron = 17 µg/dL, unsaturated iron-binding capacity = 347 µg/dL, and ferritin = 11.8 ng/mL). A bone marrow analysis revealed normal cellularity without increased blasts or myelomonocyes. Morphologically, no dysplasia was observed, whereas more megakaryocytes with fewer attached platelets were detected. Based on these findings, a diagnosis of ITP was made, and oral prednisolone therapy (2 mg/kg per day) was started. The prednisolone therapy gradually increased the platelet count, which subsequently normalized. During the process of tapering the prednisolone dose, HSP recurrence (abdominal pain, arthralgia, and purpura) was noted in association with thrombocytopenia (platelet count, $32 \times 10^9/L$). The patient also had a high-grade fever with increased serum C-reactive protein and procalcitonin, which suggested complications with bacterial infection. The administration of antibiotics and cyclosporine (3 mg/kg per day) with an increased prednisolone dose (2 mg/kg per day) resolved these symptoms and the thrombocytopenia. A skin biopsy was performed to conduct a further evaluation of the purpura, which revealed cutaneous vasculitis (Fig 1 A and B). Mutation analysis of 2 ALPS-causative genes (FAS and FASL) and several of autoinflammatory syndrome-associated genes (LPIN2, NOD2, PSTPIP1, NLRP3, MEFV, TNFRSF1A, and MVK) was performed. No pathogenic mutations were identified, although a heterozygous variation in exon 2 of the MEFV gene (p. Gly304Arg) was found (rs75977701). Colchicine was administered due to suspicions of atypical familial Mediterranean fever; however, it was not effective. Colonoscopy was also performed to assess the patient’s recurrent abdominal pain, revealing an oval-shaped large ulcer in the terminal ileum in addition to many sites of ulceration in the large intestine (Fig 1C). Based on the endoscopic findings of the gut, Behçet colitis was suspected. Treatment with an antitumor necrosis factor (TNF) monoclonal antibody (adalimumab) was administered, with which he achieved a good response. Therapy with adalimumab was provided every other week and continued for more than 13 months while the prednisolone dose was gradually tapered to maintain a symptom-free condition.

**RESULTS:**

We experienced a case exhibiting various autoimmune disorders. The percentage of double negative T cells (2.75% of TCRαβ+ T lymphocytes; 2.18% of total lymphocytes) met the diagnostic criteria for ALPS (ie, more than 2.5% of TCRαβ+ lymphocytes and 1.5% of total lymphocytes, respectively). The patient’s serum levels of IgG (1018 mg/dL), IgM (63 mg/dL), IgA (81 mg/dL), vitamin B12 (309 pg/mL; normal range, 233–914), soluble FASL (449.82 pg/mL; normal range, 451.6 ± 35.7), and interleukin (IL)-10 (6.49 pg/mL; normal range, 8.6 ± 9.6) were not elevated. However, there was a modest elevation in the patient’s IL-18 level (520.0 pg/mL; normal range, 126.0 ± 44.5). The relative proportion of lymphocyte subpopulation was within the normal range (T cells [CD3+CD19−], 73.1%; B cells [CD3−CD19+) 14.7%]. Finally, the diagnosis of ALPS was denied, based on the findings of FAS-dependent apoptosis and mutational screening of FAS and FAS-L were negative. We also conducted genomic sequencing of several candidate genes of autoinflammatory syndrome. However, these approaches failed to identify causative genes. Eventually, exome sequencing identified the oncogenic KRAS mutation (c. 37G>T, p. Gly13Cys). Germline KRAS mutations are known to be involved in the pathogenesis of cardio-facial-cutaneous syndrome and Noonan syndrome. Our case is phenotypically different from them. JMML and RALD
are also known to involve KRAS somatic mutations. To explore RAS activity in lymphocytes, the apoptosis after IL-2 depletion was investigated. The apoptosis fraction of activated T lymphocytes 72 hours after IL-2 withdrawal was 34.2%, whereas that of a healthy volunteer was 45.3%. The expression of proapoptotic Bcl-2-interacting mediator of cell death (Bim) protein in activated T lymphocytes was reduced in comparison with that observed in the healthy volunteer control. These phenomena were compatible with RALD; however, the phenotypic variation without lymphadenopathy or hepatosplenomegaly in the current case did not meet the concept of RALD. The criteria of JMML were not completely fulfilled, because our patient did not show any sign of monocytosis, increased hemoglobin F, GM-CSF hypersensitivity, and the percentage of double negative T cells. These heterogeneities may be induced by which hematopoietic lineage acquired RAS mutation (ie, lymphoid lineage, myeloid lineage, or multiple hematopoietic lineage) and the degree of the gain-of-function including the presence of somatic mosaics.

This case provides 2 novel clinical findings. First, the RAS mutation in lymphocytes may exist among patients with common autoimmunity, such as chronic ITP and recurrent HSP. It is not surprising that the dysregulated gain-of-function effect on Ras signaling participates in autoimmunity, because Ras is an essential protein for normal T lymphocyte function. Actually, 52% of 42 patients with RASopathies were shown to have autoantibodies against

**TABLE 1 A GM-CSF Sensitivity Assay**

<table>
<thead>
<tr>
<th>Cytokine(−)</th>
<th>Patient</th>
<th>JMML Case</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM-CSF 0.01 ng/mL</td>
<td>4/5</td>
<td>16/20</td>
<td>0–1</td>
</tr>
<tr>
<td>GM-CSF 0.1 ng/mL</td>
<td>9/9</td>
<td>27/26</td>
<td>0–8</td>
</tr>
<tr>
<td>GM-CSF 1 ng/mL</td>
<td>14/15</td>
<td>42/62</td>
<td>1–14</td>
</tr>
<tr>
<td>GM-CSF 10 ng/mL</td>
<td>21/23</td>
<td>56/65</td>
<td>3–26</td>
</tr>
<tr>
<td>GM-CSF 100 ng/mL</td>
<td>40/44</td>
<td>86/194</td>
<td>6–31</td>
</tr>
</tbody>
</table>

The numbers indicate the count of the colony forming unit-granulocyte macrophage colonies.
various tissues. We investigated platelet autoantibodies that could be measured with commercially available products; however, they were negative due to low sensitivity. It remains possible that high sensitivity examinations for autoantibodies against the critical domains of GPIb/IX and GPIIb/IIIa could be positive in the present patient. HSP vasculitis involves a deposition of galactose-deficient glycosylation of IgA1 with IgG immune complexes, which also suggests an autoimmune mechanism. Second, the RAS mutation induced inflammatory bowel disease (IBD). An inflammatory infiltrate composed mainly of neutrophils and lymphocytes occurs in the intestinal lesions of patients with BD; however, we could not confirm this because we were not able to obtain an adequate biopsy specimen. IL-17-producing T cells are able to mediate inflammation by promoting the recruitment of neutrophils mainly by the G-CSF pathway and may have a key role in IBD pathogenesis; however, anti-IL17A neutralizing antibody treatment has been demonstrated to be unsuccessful in patients with Crohn disease. T cells have been shown to be key players in driving intestinal inflammation; however, a complex and unsolved innate and adaptive cytokine network exists. Very early onset IBD (VEOIBD) occurs in children with a diagnosis before 6 years of age, as in our case. More than 50 genes have been identified as candidate genes of VEOIBD, and VEOIBD reveals a low response rate to conventional antiinflammatory and immunomodulatory therapies. Therefore, obtaining a genetic diagnosis of VEOIBD is essential for selecting appropriate treatment, including allogeneic hematopoietic stem cell transplantation. Thus far, anti-TNF-α monoclonal antibody treatment has led to an excellent outcome in our patient. However, curative stem cell transplantation should be considered when resistance to conservative therapies, including biological response modifiers, appears. We experienced a case of RALD accompanied by Crohn disease (unpublished observation). Hence, RAS mutations should be considered as a causative gene of VEOIBD.

Recently, Lenzarotti et al described a case of the KRASG13C mutation firstly leading to RALD and subsequently evolving from an indolent to aggressive form of leukemia. RALD and JMML are not distinct entities, but rather reflect a continuum with additional genetic or epigenetic events contributing to the clinical phenotype and evolution of the disease process. Although our case did not meet the criteria for JMML or RALD, the features are the same as those of JMML and RALD from the view of KRAS oncogenic mutations. Therefore, close monitoring of our patient is recommended to detect such evolution early. In addition, further investigations are necessary to clarify which factors determine the lineage specificity of RAS mutations to address the unsolved question as to why the RAS mutation is identified only in the lymphoid lineage.

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ABBREVIATIONS

ALPS: autoimmune lymphoproliferative syndrome
BD: Behçet disease
GM-CSF: granulocyte–macrophage colony-stimulating factor
HSP: Henoch-Schönlein purpura
IBD: inflammatory bowel disease
Ig: immunoglobulin
IL: interleukin
ITP: immune thrombocytopenia
JMML: juvenile myelomonocytic leukemia
RALD: RAS-associated lymphoproliferative disease
TNF: tumor necrosis factor
VEOIBD: very early onset inflammatory bowel disease

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