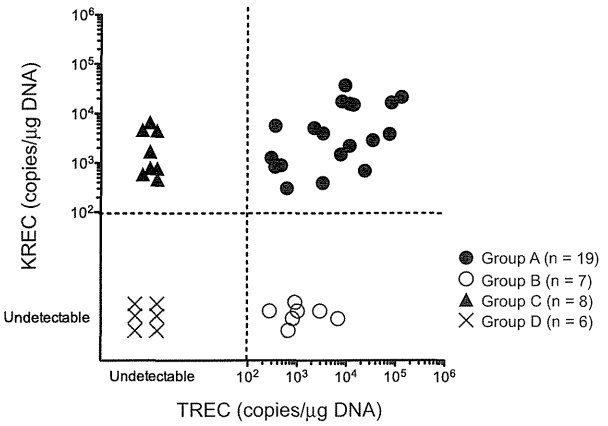

FIG 1. Quantifying TREC and KREC classifies patients with CVID into 4 groups. Patients with CVID were classified as follows: TREC(+)/KREC(+), group A (19 patients); TREC(+)/KREC(−), group B (7 patients); TREC(−)/KREC(+), group C (8 patients); and TREC(−)/KREC(−), group D (6 patients). *Undetectable,* Less than 100 copies/μg DNA.

[*CD40L* or *AICDA* mutated]), (2 with DiGeorge syndrome, and 3 with *FOXP3*, *IKBKG*, or *6p* deletions); and 5 patients with drug-induced hypogammaglobulinemia. The remaining 40 patients with decreased IgG (≥2 SDs below the mean for age), IgM, and/or IgA levels, as well as absent isohemagglutinins, poor response to vaccines, or both were included in this study as patients with CVID and analyzed for TREC/KREC levels, retrospectively.

Ages of patients with CVID ranged from 2 to 52 years (median age, 15.5 years). The sex ratio of the patients was 21 male/19 female patients. Serum IgG, IgA, and IgM levels were 370 ± 33 mg/dL (0-716 mg/dL), 30 ± 7 mg/dL (1-196 mg/dL), and 40 ± 6 mg/dL (2-213 mg/dL), respectively. TREC and KREC quantification was performed by using DNA samples extracted from peripheral blood, as reported previously.[5,6] Clinical symptoms were then assessed retrospectively. The study protocol was approved by the National Defense Medical College Institutional Review Board, and written informed consent was obtained from adult patients or parents of minor patients in accordance with the Declaration of Helsinki.

Based on TREC and KREC copy numbers, the 40 patients with CVID were classified into 4 groups (groups A, B, C, and D; Fig 1). Comparing lymphocyte subsets, $CD3^+$ T-cell numbers were similar among groups A, B, and D but were significantly lower in group C ($P < .05$; group A, 1806 ± 204 cells/μL; group B, 1665 ± 430 cells/μL; group C, 517 ± 124 cells/μL; and group D, 1425 ± 724 cells/μL; $P = .0019$, Tukey multiple comparison test based on 1-way ANOVA). $CD3^+CD4^+CD45RO^+$ memory T-lymphocyte percentages in groups B, C, and D were significantly higher than those in group A ($P < .0001$; group A, 37% ± 16%; group B, 67% ± 13% [$P = .0006$]; group C, 92% ± 8.2% [$P < .0001$]; and group D: 83% ± 14% [$P < .0001$]; see Fig E1 in this article's Online Repository at www.jacionline.org); additionally, the percentages of these cells in groups C and D were higher than in group B ($P = .0115$). These results indicate that group C and D patients have markedly decreased $CD4^+CD45RA^+$ naive T-cell counts than group A patients and that counts in group B are also significantly decreased, although less so than in groups C or D, which is consistent with a report showing lower TREC copy numbers in $CD4^+CD45RO^+$ cells. Some patients in groups B, C, and D exhibited normal $CD4^+CD45RO^+$ percentages, although TREC levels, KREC levels, or both decreased. This discrepancy indicates that TREC/KREC levels could be independent markers to determine the patient's immunologic status in addition to $CD4^+CD45RA^+$; the reasons underlying the discrepancy between $CD4^+CD45RA^+$ and TREC/KREC levels remain unsolved.

$CD19^+$ B-cell numbers in group A were significantly higher ($P < .05$) than those in groups B and D (group A, 269 ± 65 cells/μL; group B, 35 ± 16 cells/μL; group C, 60 ± 11 cells/μL; and group D, 29 ± 16 cells/μL; $P = .0001$). However, B-cell subpopulations, including $CD27^-$, IgD^+CD27^+, and IgD^-CD27^+ cells, were not significantly different among the groups. Standardizing KREC copy numbers for each patient by dividing their $CD19^+$ by their $CD27^+$ percentages revealed the same patient classification as that shown in Fig 1 (data not shown), indicating that the original classification was independent of $CD19^+$ B-cell or $CD27^+$ memory B-cell percentages.

Because TREC and KREC levels decrease with age (see Fig E2 in this article's Online Repository at www.jacionline.org)[5,6] and age distribution was wide in this study, we compared patients' ages among groups at the time of analysis to determine whether classification was associated with age. TREC/KREC-based classification was independent of both age and sex because age distribution was not significantly different among groups ($P > .05$; group A, 12.7 ± 2.3 years [2-30 years]; group B, 23.4 ± 4.2 years [6-39 years]; group C, 21.5 ± 6.1 years [4-52 years]; and group D, 25.5 ± 4.4 years [15-46 years]; data not shown) nor was male/female sex ratio (overall, 21/19; group A, 10/9; group B, 2/5; group C, 5/3; and group D, 4/2; $P = .4916$, χ^2 test; data not shown).

We next evaluated whether any correlation existed between TREC/KREC-based classification and clinical symptoms in each patient group. All patients in the study had been treated with intravenous immunoglobulin (IVIG) substitution at the time of analysis. We found that the cumulative events of complications (opportunistic infections, autoimmune diseases, and malignancies) per 10 patient-years were highest in group D (0.98 events/10 patient-years), followed by group C (0.63 events/10 patient-years), group B (0.30 events/10 patient-years), and group A (0.04 events/10 patient-years), where events in groups D and C were significantly higher than group A (group A vs group D, $P = .0022$; group A vs group C, $P = .0092$; group A vs group B, $P = .0692$; Fig 2). Furthermore, we found similar results when evaluating only patients 19 years old or older for group D (1.01 events/10 patient-years), group C (0.56 events/10 patient-years), group B (0.32 events/10 patient-years), and group A (0.06 events/10 patient-years; group A vs group D, $P = .0074$; group A vs group C, $P = .0407$; group A vs group B, $P = .1492$; data not shown). Categorizing patients by using several different previously reported CVID classifications (focused primarily on separating patients based on levels of circulating B-cell subsets), we found that no classification scheme showed any significant event increases in any particular group (see Fig E3 in this article's Online Repository at www.jacionline.org). Assessing longitudinal cumulative opportunistic infection incidence among the groups, group D and C values were significantly higher than in group A (see Fig E4, A, in this article's Online Repository at www.jacionline.org; $P = .0059$). Autoimmune and malignant diseases ($P = .5168$ and $P = .6900$, respectively) were observed in groups B and D but not in group A (see Fig E4, *B* and *C*). Cumulative events were significantly different between groups ($P = .0313$, log-rank test; group A, 5.3% and 5.3%; group B, 14.3% and

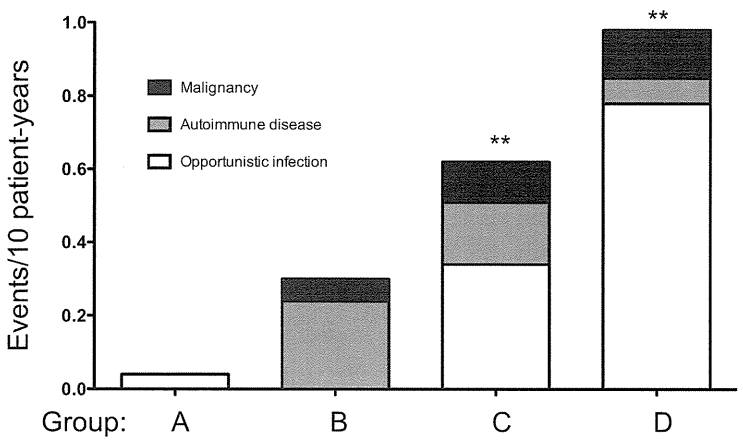

FIG 2. Cumulative incidence of complication events per 10 patient-years differs among groups. Opportunistic infections, autoimmune diseases, and malignancies were evaluated for each patient group. Complication incidences in group D (0.98 events/10 patient-years), group C (0.63 events/10 patient-years), and group B (0.30 events/10 patient-years) were higher than in group A (0.04 events/10 patient-years). Group A versus group D: $^{**}P = .0022$; group A versus C: $^{**}P = .0092$; group A vs group B: $P = .0692$.

57.1%; group C, 27.1% and 63.5%; and group D, 33.3% and 83.3% at 10 and 30 years of age, respectively; see Fig E4, *D*). One patient in group D died of *Pneumocystis jirovecii* pneumonia, and 2 other patients in the same group received hematopoietic stem cell transplantation after complications caused by EBV-related lymphoproliferative disorder.

Assessing these data, TREC/KREC-based classification matches clinical outcomes. Because group D patients exhibited the most frequent complications (opportunistic infections, autoimmune diseases, and malignancies), they could receive a diagnosis of CID based on these symptoms. If they are indeed determined to have CID, then TREC/KREC analysis is helpful to distinguish between CID and CVID. Their TREC(−)/KREC(−) phenotype might relate to defective V(D)J recombination in T- and B-cell development[8] because patients with B-negative SCID (*RAG1*, *RAG2*, *Artemis*, and *LIG4*), as well as patients with ataxia-telangiectasia (AT) and Nijmegen breakage syndrome (NBS; see Fig E5 in this article's Online Repository at www.jacionline.org),[5,6] were also negative for both TREC and KREC; it is intriguing to speculate that an unknown V(D)J recombination gene or genes is responsible. As for treatment, hematopoietic stem cell transplantation should be considered the preferred treatment to "cure" group D patients, as reported in patients with severe CVID/CID, because event-free survival is poor.[9]

In contrast to group D patients, TREC(+)/KREC(+) group A patients treated with IVIG substitution therapy remained healthy. One possible explanation is that these patients harbor defects only in terminal B-cell differentiation, but not in T cells, and represent typical patients with CVID, as originally reported.

Group C patients had a high frequency of both opportunistic infections and malignancies, suggesting that these TREC(−) patients have T-cell defects. Although group C patients had a similar TREC/KREC pattern to patients with SCID with B cells (*IL2RG* and *JAK3*; see Fig E5, *A*), they do not fulfill the European Society for Immunodeficiencies criteria for SCID, and no mutation was identified in the SCID genes estimated from clinical manifestation and lymphocyte subset analysis. However, from our data, they would likely benefit from undergoing similar treatment to patients with SCID or CID to prevent these complications.

Although opportunistic infections were rare in group B patients, autoimmune diseases were often observed. This is consistent with this group being TREC(+)/KREC(−) and the idea that balance between T and B cells is important to prevent autoimmune diseases in patients with CVID.[1] Intriguingly, a group of patients with AT and NBS were also TREC(+)/KREC(−) (see Fig E4, *B*), which is similar to group B patients. Additionally, $CD45RA^{+}CD4^{+}$ naive T-cell numbers were reduced in most group B patients, which is similar to the phenotype exhibited by patients with AT and NBS. This finding raises the possibility that although some group B patients are also T-cell deficient, as well as B-cell deficient, and should be treated similarly to patients with CID, other patients have only B-cell deficiency and are effectively treated with IVIG substitution therapy.

By analyzing a large CVID patient cohort, the overall survival rate of patients with more than 1 complication was worse than that for patients without other complications.[4] Our findings indicate that low TREC levels, KREC levels, or both are useful markers that correlate well with the overall survival rate in patients with CVID. Therefore we conclude that TREC and KREC are useful markers to assess the clinical severity and pathogenesis of each patient with CVID and to distinguish CID from CVID. Thus patient classification based on TREC/KREC levels would provide a helpful tool for deciding on an effective treatment plan for each patient with CVID.

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Homing frequency of human T cells inferred from peripheral blood depletion kinetics after sphingosine-1-phosphate receptor blockade

To the Editor:

Naive and central memory (CM) T cells home through lymph nodes (LNs), whereas T cells with an effector memory (EM) phenotype preferentially screen peripheral tissues in search of cognate antigen.[1] LN entry and egress are distinct and highly regulated processes mediated by an orchestrated interplay of chemokines/chemokine receptors and adhesion molecules.[2] Interaction of peripheral node addressins with L-selectin on T cells allows tethering/rolling along high endothelial venules (HEVs).[2] Interaction of the chemokine receptor CCR7 with its ligands CCL19/CCL21 and CXCR4 with CXCL12 then mediates firm adhesion to HEVs through high-affinity interactions of lymphocyte function–associated antigen 1 and intercellular adhesion molecule 1, permitting transmigration of T cells across the HEV cell layer.[2] Within the LNs, T-cell migration is directed through T-cell zones toward the cortical sinuses.[3] A sphingosine-1-phosphate (S1P) gradient established across the endothelial cells of the cortical sinuses is directing LN egress of T cells through efferent lymph back to the peripheral blood circulation.[4] Acting as a functional antagonist on the S1P receptor, the pharmacologic compound fingolimod, which has shown efficacy in the treatment of multiple sclerosis (MS), blocks this egress.[4,5] As a consequence, in fingolimod-treated subjects naive and CM T cells are trapped in LNs and reduced in the blood circulation.[6]

Here, by studying depletion kinetics of T cells in the blood of *de novo* fingolimod-exposed subjects in combination with *in vitro* migration experiments, homing frequencies and LN access hierarchy between T-cell subsets were derived indirectly. First, we defined the effect of *de novo* fingolimod exposure on the number of circulating $CD4^+$ and $CD8^+$ phenotypic T-cell subsets in patients with MS during a 6-hour observation period (hourly measurements, 1 time before and 6 times after drug exposure) by using flow cytometry (detailed information on patients and methods is provided in the Methods section and Table E1 in this article's Online Repository at www.jacionline.org). In fingolimod-treated subjects, 6 hours after the first drug dose, numbers of $CD4^+$ T-cell subsets with an LN homing phenotype (ie, naive and CM T cells) were significantly reduced (Fig 1, *A* [representative example; absolute cell counts], and Fig 1, *B* [pooled data; proportional change]). Intriguingly, the kinetics of reduction differed between phenotypic naive (CD62 ligand [CD62L]–positive $CD45RA^+$) and CM ($CD62L^+CD45RA^-$) $CD4^+$ T cells. Specifically, compared with baseline measurements, naive $CD4^+$ T-cell counts started to decrease earlier than CM $CD4^+$ T-cell counts (2 vs 5 hours after fingolimod exposure; Fig 1, *B*). In $CD8^+$ T cells, contrasting $CD4^+$ T cells, only naive ($CD62L^+CD45RA^+$) $CD8^+$ T-cell counts decreased significantly (after 3 vs 2 hours in naive $CD4^+$ T cells) after the first dose of fingolimod (Fig 1, *C* [representative example; absolute cell counts], and Fig 1, *D* [pooled data; proportional change]).

On the basis of these *ex vivo* depletion kinetics, *in vitro* chemotaxis experiments were performed, as described in the Methods section in this article's Online Repository. In a transwell system spontaneous migration of bulk $CD4^+$ and $CD8^+$ T cells was comparably low in healthy control subjects and untreated patients with MS (and was further decreased in the presence of fingolimod; see Fig E1 in this article's Online Repository at www.jacionline.org). Gradients of CXCL12, CCL19, and CCL21 mediated a clear increase in migration of bulk $CD4^+$ and $CD8^+$ T cells from healthy control subjects and untreated patients with MS, which was not significantly influenced by fingolimod (see Fig E1). Dot plot distribution (as a percentage) of migrated versus nonmigrated, phenotypic naive, CM, EM, and (for $CD8^+$ T cells) CD45RA re-expressing EM cells (EMRA) was then compared between control cells (spontaneous migration) and cells that migrated toward CXCL12, CCL19, or CCL21. An example of CXCL12-mediated changes in the

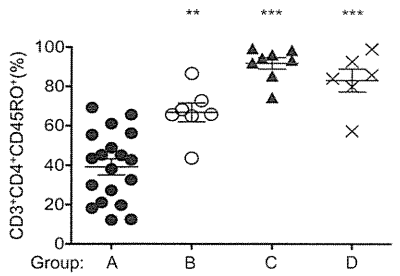
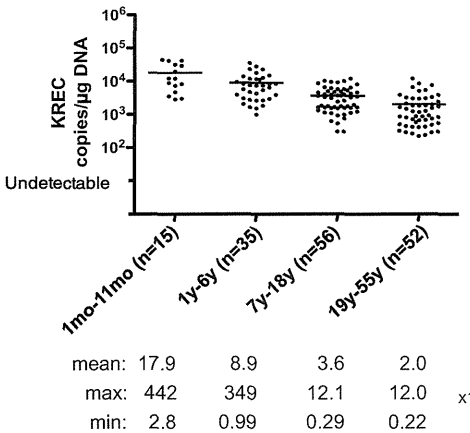


FIG E1. $CD45RO^+CD3^+CD4^+$ T-cell frequency within $CD4^+CD3^+$ lymphocytes was analyzed among groups. $CD45RO^+CD3^+CD4^+$ lymphocyte counts were significantly higher in groups B, C, and D compared with those in group A ($P < .0001$). Group A: 37% ± 16%; group B: 67% ± 13% (**$P < .01$); group C: 92% ± 8.2% (***$P < .001$); and group D: 83% ± 14% (***$P < .001$).



mean:	17.9	8.9	3.6	2.0	
max:	442	349	12.1	12.0	x10^3 copies/µg DNA
min:	2.8	0.99	0.29	0.22	

FIG E2. KREC levels were analyzed in genomic DNA samples extracted from peripheral blood of control subjects at different age groups (n = 158; age range, 1 month to 55 years). KREC levels were significantly higher in infants (17.9 ± 3.9 × 10^3 copies/µg DNA) compared with other children's age groups (8.9 ± 1.3 × 10^3 copies/µg DNA in the 1- to 6-year-old group and 3.6 ± 3.8 × 10^3 copies/µg DNA in the 7- to 18-year-old group) and adults (2.0 ± 3.3 × 10^3 copies/µg DNA; $P < .0001$).

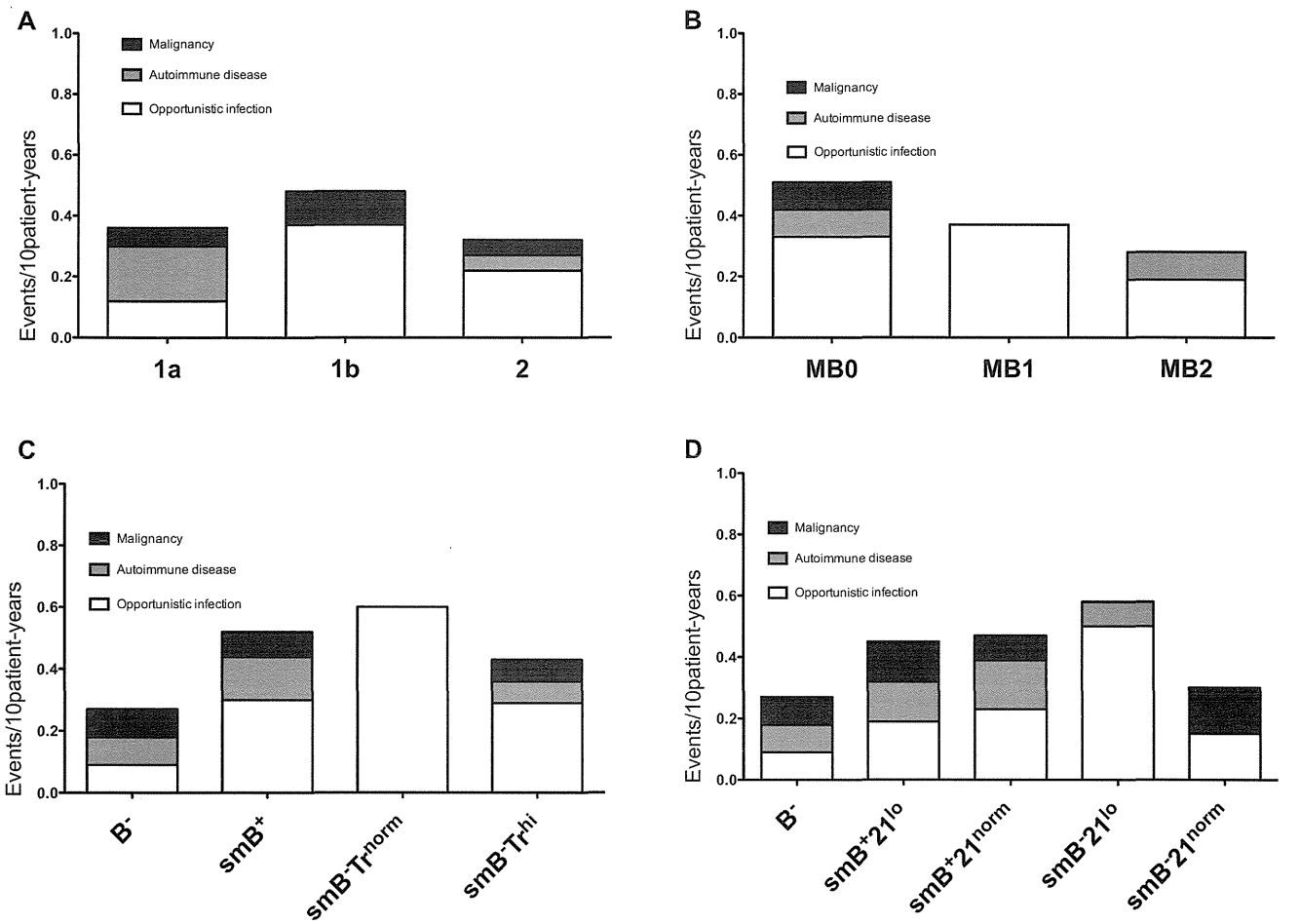


FIG E3. Patients were classified in the following way and analyzed for cumulative incidence of complications: **A,** Freiburg; **B,** Paris; and **C,** EUROclass classifications, according to $CD38^{hi}IgM^{hi}$ transitional B cells (Fig E3, *A-C*) or $CD21^{low}$ B cells **(D)**. Five patients were excluded from the Freiburg and Paris classifications because of decreased B-cell numbers (<1%). Additionally, we excluded 4 patients in the Freiburg classification, 1 patient in the Paris classification, and 4 patients in the EUROclass classification for transitional B cells and 8 in the EUROclass classification for $CD21^{low}$ B cells because of lack of data. The following cumulative events/10 patient-years were found. Freiburg classification: 1a, 0.36; 1b, 0.48; 2, 0.32. Paris classification: MB0, 0.50; MB1, 0.37; MB2, 0.28. EUROclass classification according to transitional B cells: B^-, 0.27; smB^+, 0.52; smB^-Tr^{norm}, 0.60; smB^-Tr^{high}, 0.43. EUROclass classification according to $CD21^{lo}$ B cells: B^-, 0.27; smB^+21^{lo}, 0.45; smB^+21^{norm}, 0.47; smB^-21^{lo}, 0.58; smB^-21^{norm}, 0.30. No classification showed any significantly increased events in any particular group according to calculated *P* values, as follows—Freiburg classification: 1a vs 2 = .898, 1b vs 2 = .479, 1a vs 1b = .838; Paris classification: MB0 vs MB2 = .179, MB1 vs MB2 = .654, MB0 vs MB1 = .764; EUROclass classification according to transitional B cells: B^- vs smB^+ = .298, smB^-Tr^{norm} vs smB^+ = .809, smB^-Tr^{hi} vs smB^+ = .702, smB^-Tr^{hi} vs smB^-Tr^{norm} = .641, smB^-Tr^{norm} vs B^- = .329, smB^-Tr^{hi} vs B^- = .508; EUROclass classification according to $CD21^{lo}$ B cells: B^- vs smB^+21^{norm} = .443, smB^+21^{lo} vs smB^+21^{norm} = .930, smB^-21^{lo} vs smB^+21^{norm} = .695, smB^-21^{norm} vs smB^+21^{norm} = .575, B^- vs smB^-21^{norm} = .926, smB^+21^{lo} vs smB^-21^{norm} = .609, smB^-21^{lo} vs smB^-21^{norm} = .399, B^- vs smB^+21^{lo} = 0.474, B^- vs smB^-21^{lo} = 0.270, smB^+21^{lo} vs smB^-21^{lo} = 0.618.

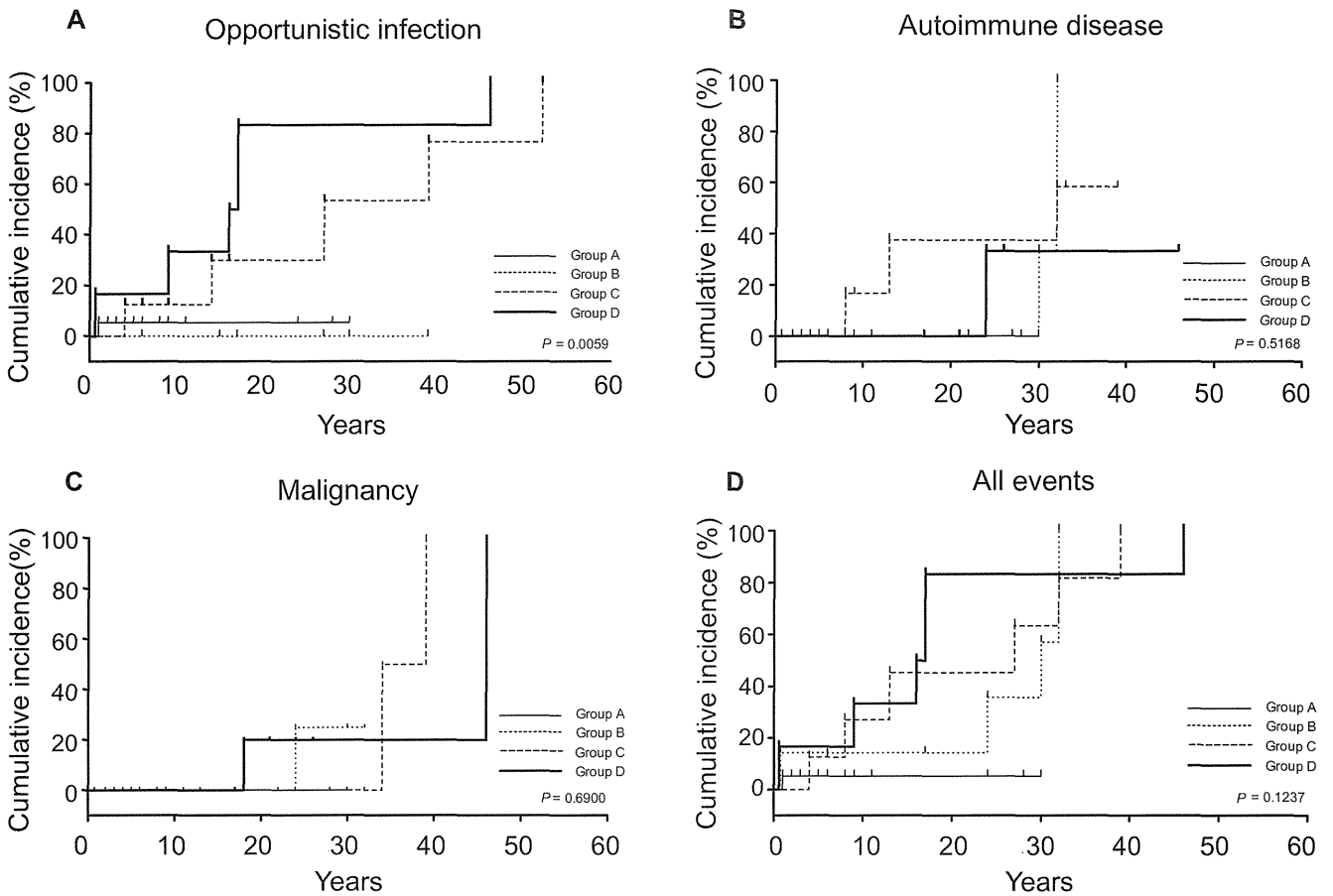


FIG E4. Comparing longitudinal cumulative incidence of complication events among groups. Cumulative incidence was estimated separately and longitudinally by using the Kaplan-Meier method and statistically compared between groups by using the log-rank test. The cumulative incidence of opportunistic infections **(A)**, autoimmune diseases **(B)**, malignancies **(C)**, and all events **(D)** is shown.

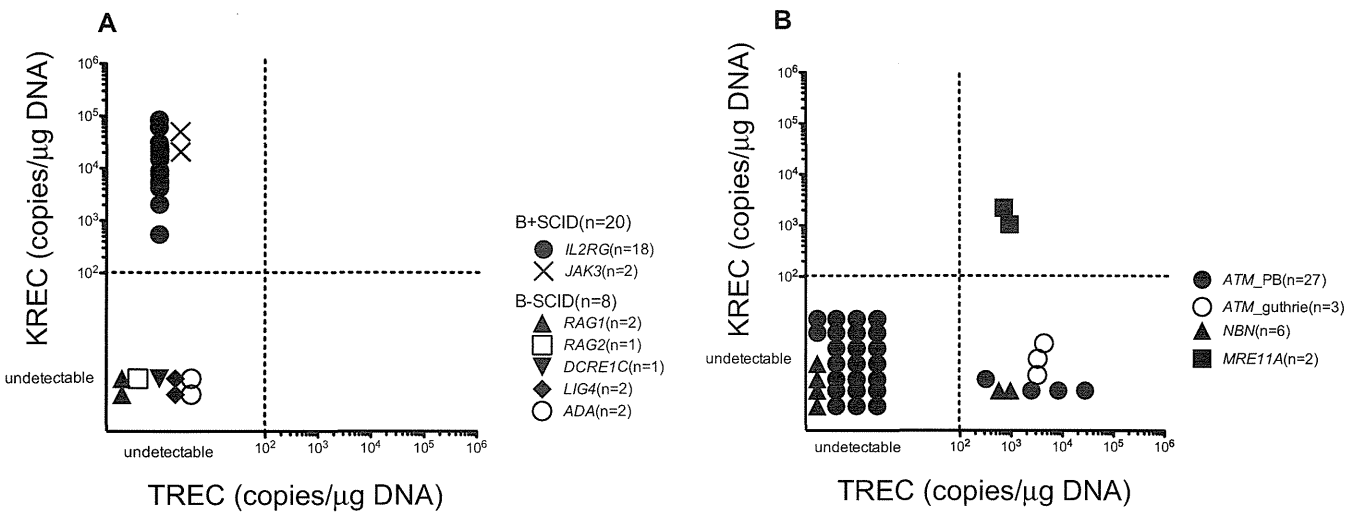


FIG E5. TREC and KREC quantification classifies patients with SCID, AT, NBS, or ataxia-telangiectasia–like disease *(ATLD)* into 4 groups. **A,** Patients with B^+SCID (n = 20) were classified as group C, and patients with B^-SCID (n = 8) were classified as group D; these patients were included in the previous studies.[5,6] **B,** Although most patients with AT (n = 23) and patients with NBS (n = 4) were classified as group D, TRECs were detected in peripheral blood samples (n = 4 in patients with AT and n = 2 in patients with NBS) and neonatal Guthrie cards (n = 3) of some patients with AT, who were classified as group B. Patients with ATLD with *MRE11A* mutations were classified as group A.

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Neonatal Herpes Encephalitis Caused by a Virologically Confirmed Acyclovir-Resistant Herpes Simplex Virus 1 Strain

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A neonate with herpes simplex virus 1 encephalitis was treated with intravenous acyclovir. During the course of therapy, the infection became intractable to the treatment and a mutation in the viral thymidine kinase gene (nucleotide G375T, amino acid Q125H) developed. This mutation was demonstrated *in vitro* to confer acyclovir resistance.

CASE REPORT

A 13-day-old boy was admitted to National Defense Medical College Hospital due to lethargy and failure to thrive. He was born at 39 weeks 5 days of gestation and 2,558 g birth weight to a healthy 35-year-old mother (gravida 2, para 2). Group B streptococcus (GBS) was detected from the mother's vagina in the third trimester, but the baby's bacterial culture tests performed at birth, including throat, skin, and blood analyses, were negative for GBS. The mother did not have a history of genital herpes. Her herpes simplex virus 1 (HSV-1) and HSV-2 serostatus was not examined, and her history of acyclovir (ACV) use was not clear. Furthermore, the genital swab culture examination for HSV was not performed. On admission, physical examination of the boy revealed skin blisters on the forehead and upper lip. A swab from the blister showed positive and negative reactions for the specific antigens of HSV-1 and HSV-2, respectively, in a direct immunofluorescent antibody assay (Denka Seiken Co. Ltd., Tokyo, Japan) performed by a qualified clinical laboratory (SRL Inc., Tokyo, Japan). A serum sample collected on admission showed positive and negative reactions in the enzyme-linked immunosorbent assay for detection of anti-HSV IgM and IgG antibody (SRL Inc.), respectively. A lumbar puncture revealed pleocytosis (547 cells/μl) and an elevated protein level (168 mg/dl) in the cerebrospinal fluid (CSF). The CSF was also positive for HSV-1 DNA, which was determined by a previously reported method (1) in PCR testing (SRL Inc.). The boy was diagnosed as having neonatal herpes encephalitis (NHE), and intravenous high-dose ACV (60 mg/kg/day) treatment was initiated. His general status improved with resolution of the skin lesions within a few days of the beginning of treatment. However, the viral load in the CSF determined by TaqMan-based quantitative real-time PCR (SRL Inc.), which dropped temporarily, increased again after 4 weeks from the initiation of ACV treatment (Fig. 1A) without obvious deterioration in clinical symptoms. Because the standard dose of ACV was given and drugs which have antagonistic effects for ACV were not used, we assumed that an ACV-resistant HSV-1 strain had developed. The ACV concentration in the CSF was not measured. Foscarnet, an antiviral drug recommended for treatment of ACV-resistant HSV infections (2), was not immediately available. Therefore, vidarabine (15 mg/kg/day) was added to the therapeutic regimen from the fifth week of the treatment course. Subsequently, HSV-DNA in the CSF decreased to a level that was finally undetectable; hence, the antiviral drug treatment was terminated. Because virus isolation from the CSF of the patient was unsuccessful, as is common in herpes encephalitis cases (3), we could not perform a plaque reduction assay to test the *in vitro* inhibition concentration of ACV. Neuroimaging showed residual necrotic changes of the bilateral temporal lobes. Unfortunately, neurodevelopmental sequelae remained in this patient.

To reveal the mechanism of the clinical ACV resistance, sequencing analysis of the viral thymidine kinase (*vTK*) gene was conducted using the CSF samples collected at two different time points. As denoted by the arrows in Fig. 1A, sample 1 and sample 2 were collected before the initiation of and at the 5th week of ACV treatment, respectively. Full-length *vTK* genes were successfully amplified from both samples by a previously reported nested PCR method (4). By direct sequencing, one nucleotide substitution, G375T, leading to a Q125H amino acid substitution was detected. CSF sample 2 contained a mixture of *vTK* genes with and without this mutation (Fig. 1B). To examine whether or not this mutation induced HSV-1 ACV resistance, further analysis was conducted.

The analysis was performed according to a method developed by our group (4). The concept for the novel assay system is to assess the sensitivity of the HSV-1 to ACV and other *vTK*-associated drugs by measuring the replication capacity of the *vTK*-deficient and highly ACV-resistant HSV-1 TAR strain (5) in 293T cells expressed with the recombinant *vTK* protein of the HSV-1 strain of interest. In this study, *vTK* expression plasmid vectors were constructed using pTARGET (Promega, Madison, WI). A *vTK* expression plasmid without the G375T mutation, which was inserted with the *vTK* PCR product from sample 1, was constructed

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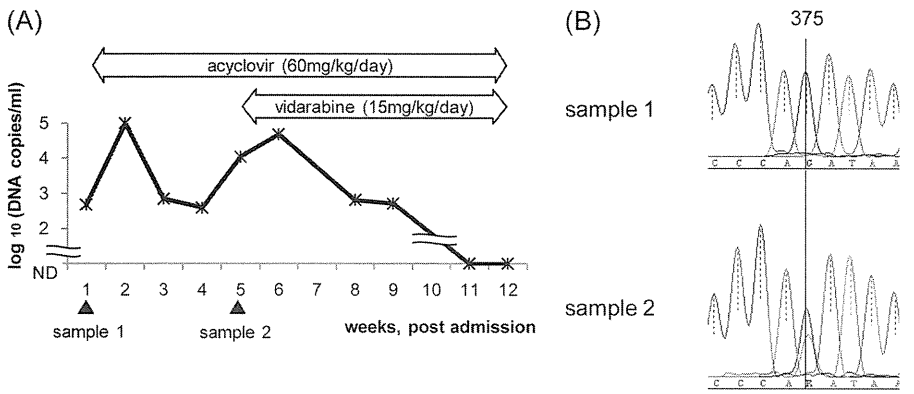

FIG 1 (A) Sequential changes in the HSV-1 DNA level in the CSF determined by quantitative real-time PCR. Arrows below the chart represent the time points of the CSF sample collection for sequencing analysis. ND, not detected. (B) Chromatogram of the *vTK* genes amplified from sample 1 and sample 2. A nucleotide substitution (G375T) was found in sample 2 (lower panel).

and designated *vTK*-375G. Subsequently, a G375T-mutant *vTK* expression plasmid was constructed by site-directed mutagenesis using the following primers, reagents, and PCR conditions and designated *vTK*-375T. Primers 5′-ATATAACAATGGGCATGCC TTATGCCand 5′-GGGCGCTTGTCATTACCAC were designed for the inverse PCR (the underlined T is the targeted nucleotide), and *vTK*-375G was used as the template. The reaction was performed using a PrimeSTAR GXL DNA polymerase kit (TaKaRa Bio, Otsu, Japan), and the amplification conditions included an initial denaturation step of 2 min at 94°C, followed by 10 cycles of 10 s at 98°C, 15 s at 55°C, and 7 min at 68°C. Digestion and self-ligation were performed with a KOD mutagenesis kit (Toyobo, Osaka, Japan). G375T substitution without other nucleotide changes was confirmed by sequencing analysis. The expression plasmid for the ACV-sensitive HSV-1 *vTK* TAS strain (5) was constructed as a positive control and designated *vTK*-TAS. Empty pTARGET served as a negative control. In the final stage of the assay, the titers of the replicated TAR were determined by the standard plaque assays. Then, $\Delta\log_{10}$ PFU values were calculated as follows: $\Delta\log_{10}$ PFU = $\log_{10}$ (PFU per milliliter of the replicated TAR at each ACV concentration) − $\log_{10}$ (PFU per milliliter of the replicated TAR at the ACV concentration of 0 μg/ml). This value represents the inhibitory effect of ACV on TAR replication, which is brought about by the transfection. Thus, the higher the value is, the lower the activity of the expressed *vTK*. In this way, the *vTK*-related resistance of HSV-1 can be judged from the $\Delta\log_{10}$ PFU values. The sensitivities to ganciclovir (GCV; Sigma-Aldrich Chemical Company, St. Louis, MO), penciclovir (PCV; Wako), and brivudine (BVDU; Sigma-Aldrich) were also tested in the same way.

TAR replication in 293T cells transfected with a negative control was not affected by any concentrations of any antiviral compounds (Fig. 2). When ACV was used, $\Delta\log_{10}$ PFU values elicited by *vTK*-375G transfection were at almost the same level as those elicited by *vTK*-TAS transfection, indicating that HSV-1 in sample 1 and TAS had nearly equal levels of sensitivity to ACV. However, $\Delta\log_{10}$ PFU values elicited by *vTK*-375T transfection were significantly higher than those elicited by *vTK*-375G transfection (Welch's *t* test; $P = 0.004$, <0.001, and $= 0.045$, at ACV concentrations of 0.4, 4, and 40 μg/ml, respectively), indicating that the HSV-1 with the G375T mutation in the *vTK* gene had acquired ACV resistance (Fig. 2A). When GCV, PCV, and BVDU were used, transfection of *vTK*-375G, *vTK*-375T, and *vTK*-TAS resulted in almost the same level of $\Delta\log_{10}$ PFU values (Fig. 2B to D). The HSV-1 G375T mutant was therefore considered to be sensitive to these drugs.

To our knowledge, this is the first report of a patient with ACV-resistant neonatal HSV-1 disease. Neonatal HSV infection is estimated to occur in 1 in every 3,500 to 5,000 deliveries (6). Approximately 30% of the patients are diagnosed as having NHE (7). Although the introduction of ACV has significantly improved the prognosis, NHE is still a severe disease with a mortality rate of 6%, and 70% of the survivors suffer from moderate-to-severe neurological abnormalities (7, 8). ACV-resistant HSV mainly threatens immunocompromised patients, and the prevalence among them is reported to range from 3.5% to 10%. In immunocompetent individuals, the prevalence of ACV-resistant HSV is far lower, ranging from 0.1% to 0.7% (2). Neonatal ACV-resistant HSV infections are quite rare, and all the cases previously described have been caused by ACV-resistant HSV-2 (9–11).

The present study also showed for the first time that a Q125H amino acid substitution in the *vTK* polypeptide induces ACV resistance. Using a method previously described (4), it was confirmed that the Q125H mutation was not a part of natural polymorphism. Q125 of HSV-1 TK has been shown to be located above the nucleotide binding pocket in the three-dimensional (3D) structure of the *vTK* protein (12). Several studies have shown that substitution of Q125 to other amino acids changes *vTK* activity; Q125E and Q125L are associated with resistance to ACV, and Q125N leads to hypersensitivity to ACV (13, 14). Interestingly, the Q125H mutation did not induce cross-resistance to GCV, PCV, and BVDU, suggesting that these drugs may be effective with respect to this specific mutant.

This study showed also for the first time a central nervous system infection caused by a virologically confirmed ACV-resistant HSV-1 strain. There is one report of a possibly ACV-resistant HSV-1 encephalitis adult patient (15). In that report, virus isolation from the CSF failed, but an amino acid substitution of R41H found in the *vTK* polypeptide was suspected to be responsible for the ACV resistance, although it has not been virologically confirmed whether the mutation confers ACV resistance. The method

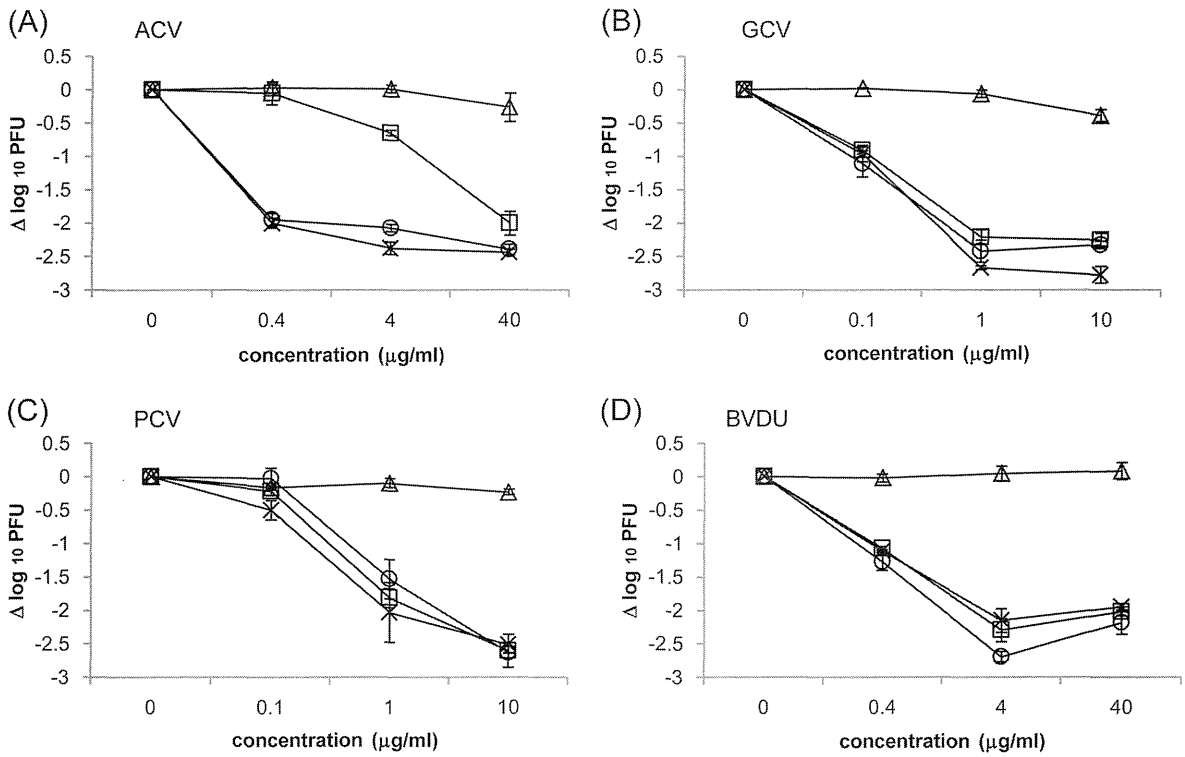

FIG 2 The inhibitory effects of antiviral compounds on replication of TAR in 293T cells transfected with each of the plasmids *vTK*-375G (○), *vTK*-375T (□), *vTK*-TAS (×), and empty pTARGET (Δ). Each experiment was performed in triplicate, and the error bars indicate standard deviations.

used in the present study may be suitable for such a condition. However, it should be kept in mind that the method can be applied only for *vTK*-related ACV resistance and not for DNA polymerase-related resistance.

ACV-resistant HSV is usually seen in patients with a history of ACV treatment (16). In the present case, administration of ACV may possibly have induced the appearance of the ACV-resistant HSV-1 strain. Although a recent study showed a benefit of oral ACV suppressive therapy for survivors of NHE (17), emergence of ACV-resistant HSV during the suppressive therapy was also reported (18). Thus, sensitivity of the causative HSV to antiviral drugs should be carefully monitored. This patient did not receive the suppressive therapy because he suffered from NHE before the beneficial effect of the therapy was reported.

It is recommended to repeat the lumbar puncture after 21 days from the initiation of ACV administration in the treatment of NHE (19). On the other hand, persistence of CSF HSV DNA is reported to be associated with poor neurodevelopmental outcomes of NHE patients (20). CSF HSV DNA quantification was conducted weekly in this patient to monitor the HSV-1 genome level in a real-time manner. We considered that the practice was beneficial, although further discussion is needed. In fact, the frequent monitoring enabled us to treat NHE with an appropriate choice of antiviral drugs.

In conclusion, ACV-resistant HSV-1 was virologically confirmed for the first time in a NHE patient. A nucleotide mutation, G375T in the HSV-1 TK gene, leading to a Q125H amino acid substitution, conferred ACV resistance.

Nucleotide sequence accession numbers. The vTK DNA sequence data have been deposited in the DNA Data Bank of Japan (DDBJ) under accession no. AB713519 (CSF sample 1) and AB713520 (CSF sample 2).

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We have no conflicts of interest.

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Factors Associated with Steroid Phobia in Caregivers of Children with Atopic Dermatitis

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Abstract: **Topical corticosteroids (TCS) are first-line therapeutic agents for atopic dermatitis (AD). Some patients express irrational fear and anxiety about using TCS, which leads to poor outcomes for AD. Although it is important to understand the factors underlying steroid phobia so that its effects can be minimized, few studies have addressed this subject. Here, we used a questionnaire to investigate predictive factors for steroid phobia in the caregivers (usually mothers) of children with AD. We studied 436 children with AD (mean age 47.6 mos, range 2–236 mos) who newly visited our AD outpatient unit. The caregivers were asked to complete a medical history questionnaire regarding AD. Steroid phobia was analyzed for correlations with other patient and caregiver variables. Overall, 38.3% of the caregivers were reluctant to use TCS on their children's skin. Patient characteristics female sex (odds ratio [OR] = 1.85 vs male; p = 0.005), child's paternal history of AD (OR = 1.94; p = 0.03), and frequent changing of clinics (OR = 1.25; p = 0.03) were predictive factors for steroid phobia. AD severity did not correlate with steroid phobia. Our findings suggest that greater attention to the patient's sex and clinical background of patients with AD is important to the success of AD therapy, regardless of AD severity.**

Topical corticosteroids (TCS) are first-line therapeutic agents for atopic dermatitis (AD) (1). In daily clinical practice, it is common for patients to express irrational fear and anxiety about using TCS (steroid phobia) (2–6). Steroid phobia may lead to poor patient adherence to TCS therapy, resulting in poor control of AD (3,5), and poor control of AD may lead to physical, psychological, and social isolation, including sleep disturbance, teasing, and school refusal, which are thought to be more serious problems than the adverse effects of TCS (3,7).

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Furthermore, those problems result in lower quality of life, not only for children with AD, but also for their families (8), sometimes even leading to family disruption (3). Some patients and caregivers with steroid phobia choose alternative and unproven therapies, which may cause exacerbation of AD (9). Others choose strict dietary therapy that results in malnutrition or failure to thrive (10).

In Japan, steroid phobia is widespread because of confusion and misinformation regarding AD therapies, including strict dietary restriction therapy, in the 1980s and negative media publicity that has exaggerated the adverse effects of TCS since the 1990s (11). Moreover, alternative and unproven therapies have gained popularity as an "atopy industry" (12). Incorrect information has often been disseminated over the Internet (13). In addition to these problems, Japan has experienced scams and lawsuits regarding alternative therapies, deaths due to malnutrition caused by extreme dietary restriction therapy, teasing-linked suicides, and at worst, family suicides (13).

Although steroid phobia is a serious problem, few studies have investigated the factors associated with it (14). The present study was designed to shed light on this, with the ultimate objective of improving the treatment of AD.

MATERIALS AND METHODS

Patients

The present study was conducted on new outpatients (and their caregivers) with AD aged 1 month to 20 years of age who visited the Outpatient Unit of the Division of Allergy, National Center for Child Health and Development, Tokyo, from April 2003 to June 2006. Patients were excluded if they had other forms of inflammatory dermatitis. The hospital ethics committee of the National Center for Child Health and Development approved this study, and it was conducted in accordance with the principles outlined in the Declaration of Helsinki.

Clinical Background and Data

Allergists diagnosed AD. The severity of AD in each patient was assessed using the scoring of atopic dermatitis (SCORAD) index (15) and then classified into one of four categories according to that index (remission 0, mild 1–25, moderate 25–49, and severe ≥50) (16). Age, sex, age at onset of AD, duration of eczema, parental history of AD, current usage of TCS, and results of blood tests (eosinophil and total immunoglobulin E [IgE] levels) were confirmed from the medical records for each patient.

Questionnaire

The caregiver attending a child patient—in most cases (approximately 95%) the mother—was asked to complete a questionnaire before the doctor examined the child. The questionnaire consisted of items concerning perceptions regarding TCS and the history of AD therapy. The items regarding perceptions of TCS included caregiver's steroid phobia, adverse effects, and perceived image of TCS therapy. Steroid phobia was assessed by asking "Would you agree to use TCS on your child's skin?" Those who answered "never" or "no, I'd rather not use TCS if I can avoid it" were defined as having steroid phobia. Preconceptions regarding adverse effects and the image of TCS therapy were ascertained by asking open-ended questions. The history of AD therapy was also ascertained using open-ended questions, and frequency of changing clinics, use of alternative therapies, and negative experiences with doctors were determined. Caregivers were also asked about the history of nonadherence to TCS therapy, how they applied TCS to the child, and the use of soap to bathe the child (Table 2).

Statistical Analyses

To check the validity of the single-item question about steroid phobia, the chi-square test was used to analyze for associations between the question "Would you agree to use TCS on your child's skin?" and the caregiver's image of TCS, history of nonadherence to TCS therapy, the caregiver's preconceptions regarding adverse effects, application of TCS, and use of alternative care. Then we performed bivariate logistic analysis of steroid phobia against patient sex, age, onset age, duration of eczema, severity of AD, parental history of AD, eosinophil count, total IgE level, frequency of changing clinics, and negative experience with doctors. Finally, we performed multiple logistic analysis to identify factors associated with steroid phobia, using as explanatory variables the factors that showed a marginal association with steroid phobia with $p < 0.4$ in the bivariate analysis. Data were analyzed using STATA software (Windows version 8.0; StataCorp, College Station, TX). $p < 0.05$ was considered to indicate statistical significance in all comparisons. The postestimated goodness-of-fit (Hosmer-Lemeshow) was confirmed for logistic regression analysis.

RESULTS

Patients

Four hundred forty-eight caregivers completed the questionnaire. We excluded 12 patients who had other

forms of inflammatory dermatitis, leaving 436 patients for the analyses: 286 male (65.6%) and 150 female (34.4%). Their mean age was 47.6 ± 48.9 months (range 2–236 mos). The characteristics of the patients are summarized in Table 1.

Confirmation of the Single-Item Question on Steroid Phobia

The incidence of steroid phobia measured using a single-item question was 38.3% of all caregivers (Table 1) and 58.7% of those with a history of nonadherence to TCS therapy (Table 2). We used the chi-square test to confirm the validity of the single-item question about steroid phobia, "Would you agree to use TCS on your child's skin?" There were strong correlations between negative perceptions regarding TCS (negative image of TCS, history of nonadherence to TCS therapy, strong apprehension regarding adverse effects of TCS, not a current user of TCS, and preference for alternative care) and the question (Table 2). Because these negative perceptions of TCS are a crucial component of steroid phobia, we considered this single-item question about steroid phobia to be useful.

Bivariate Analyses

In the bivariate analyses, female sex (odds ratio [OR] = 1.59, 95% confidence interval [CI] = 1.06–2.39), duration of eczema (OR = 0.93, 95% CI = 0.88–0.99), and paternal history of AD (OR = 1.91, 95% CI = 1.06–3.44) were significantly associated with steroid phobia; severity of AD and blood sample data were not (Table 3).

Multivariate Analyses

To evaluate the effects of confounding factors, a logistic regression model was adjusted for patient sex, age, duration of eczema, parental history of AD, frequency of changing clinics, and negative experience with doctors. In the multivariate analyses, female sex (adjusted OR [aOR] = 1.85, 95% CI = 1.20–2.85), child's paternal history of AD (aOR = 1.94, 95% CI = 1.03–3.58), and frequent changing of clinics (aOR = 1.25, 95% CI = 1.03–1.53) were significantly associated with steroid phobia (Table 4).

DISCUSSION

We found that the predictive factors for steroid phobia in caregivers of children with AD are patient female sex,

TABLE 1. *Characteristics of Patients*

	Mean (range) or	SD or %
Demographic		
Patient's sex M/F	286/150	65.6/34.4
Patient's age (months)	47.6 (2–236)	48.9
< 12	120	27.5
12–72	217	49.8
> 72	99	22.7
Clinical characteristic		
Onset age	10.4 (1–191)	21.3
< 12	338	77.5
12 < < 36	74	17.0
36 <	16	3.7
Duration of eczema	36.8 (0–224)	43.3
Severity of (SCORAD) index		
Remission (0)	12	2.8
Mild (1–25)	159	36.5
Moderate (> 25)	175	40.1
Severe (> 50)	90	20.6
Parental history of	102	23.4
Mother	60	13.8
Father	51	11.7
Both	9	2.1
Eosinophil count (/μL)	662.3 (0–5,592.4)	689.5
< 294	93	
294–467	93	
467–793	93	
> 793	93	
Eosinophil count (%)	6.74 (0–34.8)	4.86
< 3.55	93	
3.55 < < 5.7	95	
5.7 < < 8.7	92	
8.7 <	92	
Total IgE (IU/mL)	2259.2 (2–97,600)	8692
< 53.9	93	
53.9 < < 310	93	
310 < < 1,141	93	
1,141 <	94	
Steroid phobia		
Caregivers who were reluctant to use	167	38.3
Past consultation for AD		
Frequency of changing	2.1 (0–6)	1.2
Caregivers who had negative experiences with	80	18.4
Application of TCS		
Use of TCS		
Non-current	24	5.5
Past user	64	14.7
Current user	334	76.6
Caregivers who apply TCS sparingly at doctor's	171	39.2
Caregivers who apply TCS sparingly at own	81	18.8
Caregivers who apply TCS liberally at doctor's	58	13.3
Caregivers who apply TCS liberally at own	35	8
Alternative		
Caregivers who preferred alternative	71	16.3
Caregivers who don't use soap to bathe the child	25	5.7

AD, atopic dermatitis; SCORAD, Scoring Atopic Dermatitis; TCS, topical corticosteroids.

TABLE 2. *Confirmation of Single-item Question About Steroid Phobia*

Category	Steroid phobia (+) (N = 167)	Steroid phobia (−) (N = 262)	p-Value†
Image of TCS held by caregiver			
Negative	96 (62.3)	88 (37.1)	<0.001*
Positive	7 (4.5)	65 (27.4)	
Both negative and positive	50 (32.5)	75 (31.6)	
Other	1 (0.6)	9 (3.8)	
History of non-adherence with TCS (by caregiver)			
Yes	90 (57.7)	63 (25.0)	<0.001*
No	66 (42.3)	189 (75.0)	
Preconceptions regarding adverse effects of TCS (by caregiver)			
Adverse effects on the skin (including skin thinning and darkening)			
Yes	112 (67.1)	128 (48.9)	<0.001*
No	55 (32.9)	134 (51.1)	
Skin thinning			
Yes	54 (32.3)	59 (22.5)	0.024*
No	113 (67.7)	203 (77.5)	
Skin darkening			
Yes	35 (21.0)	28 (10.7)	0.003*
No	132 (79.0)	234 (89.3)	
Systemic adverse effects of TCS			
Yes	57 (34.1)	53 (20.2)	0.001*
No	110 (65.9)	209 (79.8)	
Application of TCS			
Use of TCS			
Non-current user	11 (6.7)	10 (4.0)	<0.001*
Past user	41 (25.2)	23 (9.1)	
Current user	111 (68.1)	219 (86.9)	
Caregivers who apply TCS sparingly at doctor's instruction	66 (50.0)	105 (49.3)	0.16
Caregivers who apply TCS sparingly at own judgment	38 (28.8)	43 (20.2)	
Caregivers who apply TCS liberally at doctor's instruction	17 (12.9)	41 (19.2)	
Caregivers who apply TCS liberally at own judgment	11 (8.3)	24 (11.3)	
Alternative care			
Caregivers who preferred alternative care			
Yes	37 (22.2)	34 (13.0)	0.013*
No	130 (77.8)	228 (87.0)	
Caregivers who don't use soap to bathe the child			
Yes	8 (4.9)	17 (6.6)	0.46
No	156 (95.1)	240 (93.4)	

*$p < 0.05$.
†Chi-square test.
Data given in parentheses are expressed as percentage.

child's paternal history of AD, and frequent changing of clinics for the patient but not severity of AD. These results suggest that greater attention to the clinical background of patients with AD is important in addressing steroid phobia, regardless of the severity of AD.

The incidence of steroid phobia among caregivers was 38.3% (Table 1), which is consistent with previous studies. A report from Hong Kong showed that 40% of patients with moderate and 60% of patients with severe AD expressed concern about using TCS, but there was no association between steroid phobia and severity of AD (6). In the United Kingdom, 72.5% of patients with AD worried about using TCS, and 36.5% of those had been nonadherent to TCS therapy (4). Of caregivers of children with AD in Australia, 40% answered that TCS was dangerous, and 20% said it was too dangerous to use on their child's skin (17). In France, 80.7% of the parents of children with AD and people with AD reported having fears about TCS, and 36% admitted nonadherence to treatment (18). Although methodologic differences make it difficult to compare these percentages directly, approximately one-third of parents are reluctant to use TCS on their children. Medical care providers need to be sensitive to this anxiety about using TCS in their daily clinical practice.

We also confirmed the validity of the single question about steroid phobia, "Would you agree to use TCS on your child's skin?" by finding strong correlations with negative perceptions regarding TCS (negative image of TCS, history of nonadherence to TCS therapy, strong apprehension regarding adverse effects of TCS, not a current user of TCS and preference for alternative care) (Table 2). This result was similar to a recent study that reported a correlation between steroid phobia and the need for reassurance, the belief that topical corticosteroids

TABLE 3. *Correlation with Steroid Phobia (Bivariate)*

	Bivariate OR	95% CI	p-Value
Patient's sex			
Male	Reference		0.025*
Female	1.59	1.06–2.39	
Patient's age (mos)			
< 12	Reference		0.027*
12–72	1.06	0.67–1.67	
> 72	0.54	0.30–0.96	
Onset age (mos)			
< 12	Reference		0.87
12–36	1.14	0.69–1.90	
> 36	1.05	0.38–2.88	
Duration of eczema (yrs)	0.93	0.88–0.99	0.018
Severity of AD (SCORAD index)			
Remission (0)	Reference		0.8
Mild (1–25)	0.59	0.18–1.90	
Moderate (25–50)	0.63	0.20–2.04	
Severe (> 50)	0.7	0.21–2.33	
Parental history of AD			
Mother			
No	Reference		0.39
Yes	1.28	0.73–2.22	
Father			
No	Reference		0.031*
Yes	1.91	1.06–3.44	
Eosinophil count (/μL)			
< 294	Reference		0.82
294–467	0.89	0.49–1.63	
467–793	0.86	0.47–1.58	
793 <	1.12	0.62–2.03	
Eosinophil count (%)			
< 3.55	Reference		0.51
3.55–5.7	0.75	0.40–1.38	
5.7–8.7	1.06	0.58–1.92	
> 8.7	1.16	0.64–2.10	
Total IgE (IU/ml)			
< 53.9	Reference		0.81
53.9–310	0.75	0.41–1.37	
310–1,141	0.93	0.51–1.68	
> 1,141	0.91	0.50–1.65	
Past consultation for AD			
Frequency of changing clinics	1.14	0.96–1.34	0.13
Caregivers with negative experience with doctors			
No	Reference		0.14
Yes	1.45	0.89–2.37	

*p < 0.05.

TABLE 4. *Correlation with Steroid Phobia (Multivariate)*

	†Multivariate OR	95% CI	p-Value
Patient's sex			
Male	Reference		0.005*
Female	1.85	1.20–2.85	
Patient's age (mos)			
< 12	Reference		
12–72	1.03	0.62–0.73	0.9
> 72	0.55	0.20–1.53	0.25
Duration of eczema (years)	0.96	0.86–1.07	0.45
Parental history of AD			
Mother			
No	Reference		0.39
Yes	1.15	0.64–2.05	
Father			
No	Reference		0.034*
Yes	1.94	1.03–3.58	
Past consultation for AD			
Frequency of changing clinics	1.25	1.03–1.53	0.026*
Caregivers with negative experience with doctors			
No	Reference		0.78
Yes	1.08	0.63–1.85	

*p < 0.05.
†Adjusted for all values listed above.

pass through the skin into the bloodstream, a prior adverse event, inconsistent information about the quantity of cream to apply, a desire to self-treat for the shortest possible time, or poor treatment adherence (18). Our observation suggests that appropriate education of patients to remedy negative perceptions of TCS would be one good strategy for addressing steroid phobia, as others have noted (2,4,19).

In this study, most patients and caregivers (91.3%) had used TCS, at least in the past (Table 1), which suggests that only a few caregivers had totally rejected TCS therapy for their child at the onset of AD. This implies that, whether the caregivers of children with AD become steroid phobic or not depends on the experience during AD therapy that they have received. An earlier study on factors related to steroid phobia suggested that a preconception of ineffectiveness or adverse effects of TCS was associated with steroid phobia (14).

Our results indicate that the patient's sex, a paternal history of AD, and frequent changing of clinics for the patient were associated with steroid phobia (Table 4).

There have been no studies focusing on patient sex as an association factor for steroid phobia. In our study, we found that misinterpretation of skin darkening as a TCS side effect was significantly associated with steroid phobia (Table 3). Light skin, bright eyes, and black hair have long been considered essential factors for beauty in Japanese girls (20). Thus, we assume that our finding may be due to a cultural factor in Japan that—together with the misinterpretation—leads the parents of girls to be reluctant to use TCS for their children.

We found that the child's paternal—but not maternal—history of AD was associated with steroid phobia (Tables 3 and 4). If parents had experienced treatment failure in the past, they tended to feel steroid phobia for their children. On the other hand, parental history of AD could be a remedy for steroid phobia because they may have been advised about or read correct knowledge regarding TCS during their previous experience with TCS therapy. We should have asked whether the parents had experienced treatment failure in the past, but in Japan, steroid phobia was widespread in 1980s (12). Therefore some parents with AD had not received

appropriate or successful TCS therapy and might have negative perceptions of TCS. Although our results showed that one of the predictive factors for steroid phobia was child's paternal history of AD, this might be because the caregivers of most Japanese children with AD are their mothers. Mothers would have more chances to acquire the correct knowledge regarding TCS from doctors, and any steroid phobia they might have might be relieved. On the other hand, fathers would have less opportunity to be in contact with their children's doctor, and their steroid phobia might remain. Family or relatives have also been reported to be major sources of steroid phobia (4,6,9). Thus, a father with a history of AD might cause steroid phobia in the mother–caregiver. We believe that these results indicate that correct information regarding TCS is needed not only for mother–caregivers, but also for father–partners.

Frequent changing of clinics suggests a history of treatment failure or distrust of medical care services, because in Japan patients can go to clinics without any referral. This finding of frequent changing of clinics might indicate that negative experiences with doctors, including ineffectiveness of TCS, are associated with steroid phobia.

On the other hand, AD severity was not associated with steroid phobia, which is consistent with previous studies (6,18). Furthermore, as others have noted (14,21), in our study, personal negative experiences and attitudes such as a preconception of ineffectiveness might have been more important as factors underlying steroid phobia than objective factors such as AD severity evaluated by a doctor. This finding suggests that AD severity should not be a factor in evaluating whether a patient has steroid phobia.

This study has a number of limitations. First, it was conducted at a national medical center where most patients would know that doctors would use TCS for AD therapy. Therefore, strongly phobic patients might have been underrepresented. Further studies of such patients should be undertaken to compare with our results. Second, the questionnaire about steroid phobia was not validated in other studies (4,6,17,18), although we confirmed that a single-item question, "Would you agree to use TCS on your child's skin?" was associated with negative perceptions of TCS (Table 2). Although we tried to identify negative experiences with doctors using open-ended questions regarding history of AD therapy, a future study should have multiple-choice questions and focus on a history of treatment failure and the patient–doctor relationship. Third, because of the cross-sectional study design, the presumed cause-and-effect relationship between predictive factors and steroid phobia may be the reverse; for example, steroid phobia may have been the reason for frequent changing of clinics. Fourth, other unmeasured factors, such as parental level of education, social status, and personality, might have confounded the results. Further studies should be done regarding these factors in steroid phobia, although medical expense would not be burden for Japanese caregivers because there are medical care subsidies for children in Japan.

In conclusion, our study suggests that greater attention to patient sex, paternal history of AD, and frequency of changing clinics for the patient will aid physicians in addressing steroid phobia, whereas AD severity does not play any role. It is necessary to devote sufficient time to careful elucidation of the clinical background of patients with AD from their caregivers. This would help physicians understand any steroid phobia of caregivers and contribute to overcoming steroid phobia in patients with AD and their caregivers.

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Salivary Cortisol Response to Stress in Young Children with Atopic Dermatitis

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Abstract: **Poor responsiveness of the hypothalamic–pituitary–adrenal (HPA) axis under stress may be one explanation for stress-induced exacerbation of atopic dermatitis (AD) symptoms. In previous studies, children and adults with AD showed attenuated salivary cortisol responses to psychosocial stress, suggesting hyporesponsiveness of the HPA axis, but few studies have been conducted in young children, who are vulnerable to systemic side effects of topical corticosteroid (TCS) therapy. We evaluated whether salivary cortisol responses to the stress of venipuncture in young children with AD were related to the severity of AD or performance of TCS therapy. We studied 38 young children with AD (median age 16.5 mos, range 3–66 mos) being treated at our outpatient unit. Patients were divided into three groups according to the scoring of atopic dermatitis index: mild (n = 12), moderate (n = 14), and severe (n = 12). To evaluate the responsiveness of the HPA axis to stress, salivary cortisol was determined before and after venipuncture. Salivary cortisol responsiveness to stress correlated negatively with severity of AD (p = 0.048) but not with previous use of TCS (p = 0.43) in young children with AD. Our findings suggest that the disease activity of AD, rather than TCS use, is responsible for HPA axis dysfunction in children with AD.**

Clinical observations and experimental findings have emphasized that exacerbation of atopic dermatitis (AD) symptoms is closely related to psychosocial stress (1,2). Stress itself can reportedly cause epidermal barrier dysfunction and mast cell activation through release of neuropeptides, which in turn facilitates exacerbation of allergic inflammation (reviewed in reference 1). Stress also activates the hypothalamic–pituitary–adrenal (HPA) axis to release cortisol, a potent attenuator of inflammatory reactions in general, although previous studies indicated that children and adults with AD showed attenuated salivary cortisol responses to psychosocial stress, suggesting hyporesponsiveness of the HPA axis (3,4). Therefore, poor responsiveness of

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