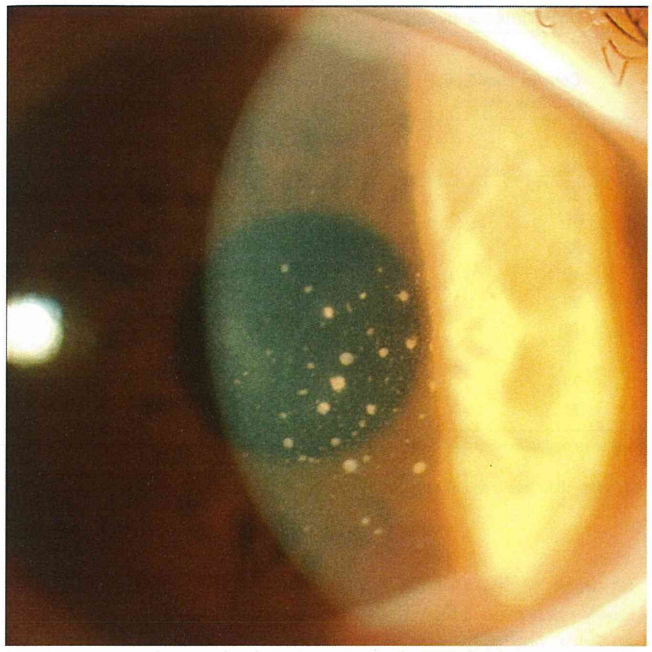

Figure 1 Case 4: Slit-lamp microscopy photo with cytomegalovirus-associated iridocyclitis. Mutton fat keratic precipitates with some pigmentation were scattered within the central area of the cornea. There was mild inflammation found within the anterior chamber.

anti-glaucoma agents effectively controlled the inflammation in the anterior segment of the eye as well as the high IOP.

Corneal endothelial cell loss

Specular microscopic examination revealed significant corneal endothelial cell loss (≥35%) in all 11 patients (table 2). Severe corneal endothelial cell loss larger than 70% was recorded in more than one-half of the endotheliitis group eyes. In contrast, this severe cell loss was observed in one of the seven patients with iridocyclitis.

There were several patients (cases 1, 8, 10 and 11; see table 2) with corneal endothelial cell counts <700 cells/mm^2. Among the patients, three cases had a low visual acuity between 0.3 and 0.6. However, one patient had a good visual acuity of 1.5.

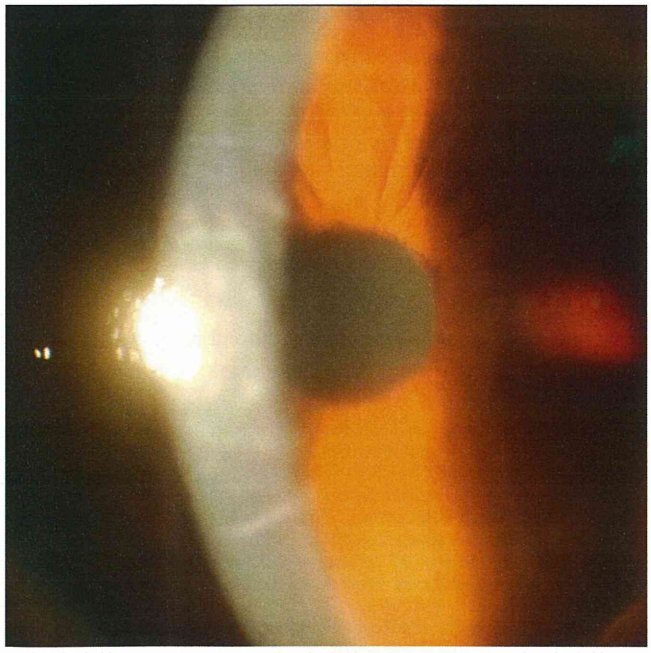

Figure 2 Case 8: Slit-lamp microscopy photo with cytomegalovirus-associated corneal endotheliitis. Diffuse corneal stromal oedema with folds in Descemet's membrane was observed.

PCR analysis of the aqueous humour samples

Multiplex PCR analyses confirmed the presence of CMV genomic DNA, but none of the other human herpes viruses (HSV-1, HSV-2, VZV, EBV, HHV-6, HHV-7 or HHV-8) in all 11 of the patients (table 2).

Quantitative real-time PCR detected significant viral loads of CMV genomic DNA in the aqueous humour of all 11 patients, with values ranging from 5.4×10^3 to 5.9×10^6 copies/ml (table 2). The mean values for the CMV viral load in the iridocyclitis and corneal endotheliitis groups were 9.4×10^5 and 1.2×10^6 copies/ml, respectively. The differences in CMV viral load between the two groups were not significant (p=0.571).

The corneal endothelial cell damage intensity was correlated to the CMV viral load in the aqueous humour. Results of the linear regression analysis demonstrated a positive correlation between the CMV viral load and the corneal endothelial cell loss (Spearman's correlation coefficient by rank test, r=0.664; p=0.036; figure 3).

However, there was no correlation between the interval from the disease onset to the aqueous sampling and the viral load in the aqueous humour (Spearman's correlation coefficient by rank test, r=0.445; p=0.159). Furthermore, the interval from the disease onset to the sampling was not correlated with the corneal endothelial cell damage intensity (Spearman's correlation coefficient by rank test, r=0.373; p=0.239). In addition, there was also no correlation between the viral load and many other ocular findings, such as cells and flare in the anterior chamber, types of KPs, gonioscopic findings, IOP and post-treatment BCVA.

DISCUSSION

The present study analysed ocular manifestations and CMV viral loads in the aqueous humour of patients with CMV-associated iridocyclitis and corneal endotheliitis. Our major findings included: (1) presence of significant corneal endothelial cell loss in both corneal endotheliitis and iridocyclitis tested eyes; and (2) a significant correlation between corneal endothelial cell loss and CMV viral load in the aqueous humour.

Even though it has been demonstrated that viral infections play a significant role in many inflammatory diseases, a qualitative PCR method that is capable of determining the pathological role of these viral infections has yet to be elucidated. If the presence of viral DNA in an affected disease site could be proven, the quantitative determination and correlation with the clinical manifestations of the viral infection could lead to a much deeper understanding of the role of the virus as a pathogenic disease candidate. For example, we have previously reported on two intraocular inflammatory disorders: one involving uveitis associated with human T-cell leukaemia virus type 1 (HTLV-1)[13 14] and the other involving anterior uveitis associated with VZV.[14] In HTLV-1 uveitis, a significantly higher HTLV-1 viral load was detected in the peripheral blood mononuclear cells of the patients compared with asymptomatic HTLV-1 carriers.[13] This viral load was significantly correlated with the vitreous inflammation of the disease.[14] In our report on anterior uveitis associated with VZV, we demonstrated there was a high VZV viral load within the patient's aqueous humour. Furthermore, there was a significant correlation between the viral load and the intensity of the iris atrophy in these patients.[15]

Table 2 Virological analysis and corneal endothelial cell findings in patients with CMV anterior uveitis

Case	Herpes virus DNA		Endothelial cell count (cells/mm^2)		Corneal endothelial cell loss (%)†	Post-treatment BCVA	Interval from onset to sampling (months)
	CMV (copies/ml)	Others*	Affected eye	Fellow eye			
1	2.3×10^5	-	642	2738	77	0.4	96
2	5.5×10^3	-	1633	2869	43	0.8	8
3	1.3×10^4	-	1695	2789	39	1.5	48
4	6.5×10^4	-	1618	3576	55	1.5	24
5	3.5×10^5	-	1445	2608	38	1.2	14
6	5.9×10^6	-	919	2288	45	1.2	16
7	5.4×10^3	-	2512	3917	60	1.2	6
8	1.0×10^6	-	573	2427	76	0.6	12
9	2.8×10^4	-	1427	2262	35	0.7	5
10	1.2×10^4	-	593	2092	72	0.3	4
11	3.6×10^6	-	620	2674	77	1.5	20

Using aqueous humour samples, genomic DNA of the human herpes viruses was measured by qualitative multiplex PCR and quantitative real-time PCR. Corneal endothelial cell count was examined by specular microscopy.
*Herpes viruses excluding CMV, ie herpes simplex virus type 1 and type 2, varicella zoster virus, Epstein–Barr virus, and human herpes virus types 6, 7 and 8.
†Corneal endothelial cell loss was calculated as described in the methods section.
BCVA, best-corrected visual acuity (decimal fraction); CMV, cytomegalovirus.

Although we found that there was a positive correlation between the corneal endothelial cell loss and the CMV viral load in the aqueous humour, there was no correlation between the viral load and many other ocular signs such as cells and flare in the anterior chamber, types of KPs, gonioscopic findings, IOP, post-treatment visual acuity and the interval from the disease onset to the aqueous sampling. These patients had been treated with topical corticosteroids (eg betamethasone) and anti-glaucoma agents (eg timolol and latanoprost) before they were referred to us by local ophthalmologists. These treatments are known to reduce the intensity of anterior uveitis, IOP and other ocular manifestations, but have no effect on recovering the corneal endothelial cell damage, because the corneal endothelial cell damage is barely reversible.

The cells and flare in the anterior chamber were mild in all 11 patients. A possible explanation why the intensity of the inflammatory reaction in the anterior chamber was so mild in this disease might be related to the involvement of the anterior chamber-associated immune deviation (ACAID).[16 17] In an experimental rabbit corneal endotheliitis model, eyes inoculated with inactivated HSV-1 prior to an active HSV-1 infection exhibited less severe inflammatory reactions and corneal endotheliitis. In addition, they also developed an immune deviation to HSV-1.[18] Although CMV-related ACAID has not been previously reported, real-time PCR in the present study demonstrated that CMV genomic DNA was present at high levels within the anterior chamber of the patients. Therefore, it may be that ACAID in response to CMV occurs in the eye, resulting in a relatively mild inflammatory reaction.

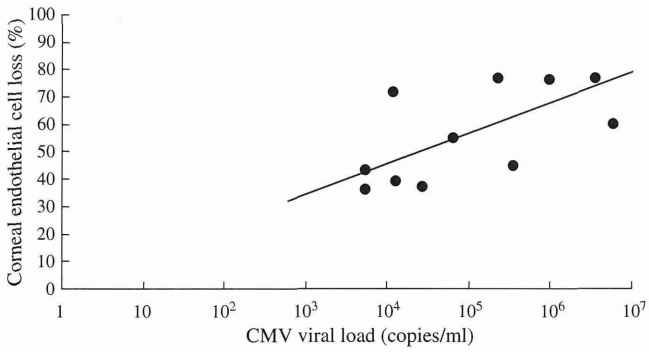

Figure 3 Correlation between cytomegalovirus (CMV) viral load and corneal endothelial cell damage. The CMV viral load was plotted on a logarithmic graph versus the corneal endothelial cell loss (%). The scatter plot shows significant correlation between the CMV viral load and the corneal endothelial cell loss (Spearman's correlation coefficient by rank test, r=0.664; p=0.036).

While our results showed CMV infection in the anterior segment of the eye caused inflammation and corneal endothelial cells loss in immunocompetent hosts, our study cannot answer many other questions. For example, why does CMV cause intraocular inflammation in immunocompetent hosts? Where does the CMV that is detected in the aqueous humour come from? And how is CMV able to cause inflammatory disorder only within the anterior segment of the eye? One possible explanation why our patients developed CMV anterior uveitis is that all our patients had been given topical corticosteroids for a long period of time. This may have contributed to induce local immunosuppressive condition in the anterior segment of the eye and resulted in reactivation of CMV.[8] Further clinical and experimental investigations are necessary to clarify these important questions.

In conclusion, significant corneal endothelial cell damage was detected in all CMV-associated iridocyclitis- and corneal endotheliitis-tested eyes. In addition, a significant correlation was found between corneal endothelial cell loss and the CMV viral load in the aqueous humour.

Competing interests None.

Ethics approval This study was conducted with the approval of the Institutional Ethics Committee of Tokyo Medical and Dental University.

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

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A significant association of viral loads with corneal endothelial cell damage in cytomegalovirus anterior uveitis

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Acute Cerebellitis and Concurrent Encephalitis Associated with Parvovirus B19 Infection

To the Editors:

Central nervous system (CNS) infections caused by parvovirus B19 (PVB19) have been rarely documented, especially the involvement of the cerebellum.[1] We describe an immunocompetent girl with acute cerebellitis associated with PVB19 infection.

A 5-year-old girl was hospitalized because of seizures of the upper extremities after a 3-day history of fever. On arrival, consciousness was disturbed (Glasgow coma scale: E2V1M4). Laboratory findings on admission included white blood cells 10.6 $\times 10^9$/L, blood glucose 74 mg/dL and sodium 130 mmol/L. Cerebrospinal fluid (CSF) examination showed 229 cells/μL with predominance of polymorphonuclear cells, 144 mg/dL protein and 56 mg/dL glucose. A brain computed tomography image was normal.

The patient began treatment with intravenous ceftriaxone, panipenem/betamipron, dexamethasone and acyclovir. Owing to persistent consciousness disturbance, methylprednisolone therapy was added on the fourth day in a dose of 30 mg/kg for 3 days. Electroencephalography on the sixth day showed high-voltage delta activity in the bilateral occipital regions, leading to the diagnosis of encephalopathy. On the sixth day, brain diffusion-weighted magnetic resonance images (see Fig. A, Supplemental Digital Content 1, http://links.lww.com/INF/B90) showed marked hyperintensity in the bilateral dentate nuclei in the cerebellum, suggesting a diagnosis of acute cerebellitis. These dentate nuclear lesions disappeared on the 10th day, and instead hyperintensity in the cerebellar hemisphere became prominent (see Fig. B, Supplemental Digital Content 1, http://links.lww.com/INF/B90). Thereafter, the patient could gradually follow simple verbal instructions, could sit alone on the 16th day, and could walk with a wide stance on the 26th day. Mutism had remained until the 20th day. Three months later, slurred speech and intention tremor persisted. A follow-up magnetic resonance study 6 months later showed cerebellar atrophy (see Fig. C, Supplemental Digital Content 1, http://links.lww.com/INF/B90).

On the 10th day, maculopapular rash appeared, extending to the face and extremities, suggesting erythema infectiosum. This was confirmed by elevation of serum PVB19 IgM and IgG antibodies (11.63 and 7.79 titers, respectively) using enzyme immunosorbent assay. Polymerase chain reaction analyses detected PVB19 DNA in the CSF (4.5×10^4 copies/mL) and in the plasma (2.8×10^5 copies/mL) samples stored from the time of admission. Polymerase chain reaction was also applied for herpes simplex virus 1 and 2, human herpes virus (HHV) 6, 7 and 8, varicella-zoster virus, cytomegalovirus, Epstein-Barr virus, JC virus and BK virus, showing negative results for all viruses examined except for HHV6 (5.5×10^2 and 3.5×10^3 copies/mL in the CSF and plasma, respectively). Serologically, HHV6-IgG antibody was positive and HHV6-IgM antibody was negative, findings consistent with history of exanthema subitum. Collectively, cerebellitis and concurrent encephalitis were likely caused by CNS PVB19 infection with reactivation of latent HHV6.

It is important to note that clinical and radiologic features in our patient were consistent with those of rotavirus-associated cerebellitis.[2] Takanashi et al[2] documented 11 such cases and proposed it as a novel clinicoradiologic entity because of the homogeneous characteristics. Although rotavirus predominated as causative pathogens,[2] adenovirus type 3,[3] HHV-6[4] and influenza virus[5] have also been reported. Among 31 childhood PVB19 CNS infections,[1] only 2 developed ataxia, but their cerebellar involvement was not radiologically demonstrated.

Thus, this is the first describing PVB19 as a cause for this novel type of cerebellitis and suggests a wider entity and a common mechanism.

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Primary Cutaneous Aspergillosis in Two Pediatric Trauma Patients

To the Editors:

Aspergillosis is the most common mold infection in immune compromised patients,[1] and can be nosocomially acquired. We report 2 cases of primary cutaneous aspergillosis (PCA) in previously healthy pediatric patients with multiple traumatic injuries after motor vehicle accidents.

CASE 1

A previously healthy 6-year-old boy with a history of prematurity and mild intermittent asthma was admitted to the pediatric intensive care unit after he suffered pulmonary contusions and a mediastinal hematoma in a motor vehicle accident. He developed acute respiratory distress syndrome requiring venoarterial extracorporeal membranous oxygenation. He received systemic corticosteroids for his lung injury and vancomycin for *Staphylococcus aureus* bacteremia. He developed erythematous bullous lesions on his left proximal forearm, which underwent necrosis with central ulceration (see Fig. 1). Tissue culture grew *Aspergillus fumigatus* and he was treated with intravenous voriconazole for 21 days, followed by 10 days of oral therapy.

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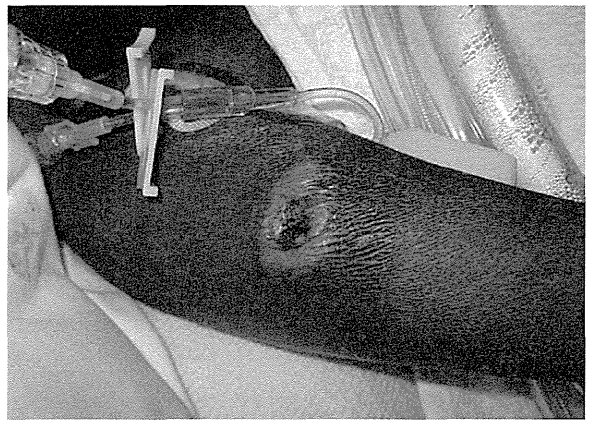

FIGURE 1. Bullous lesion of the left forearm after undergoing necrosis and ulceration.

CASE 2

A previously healthy 2-year-old girl suffered pulmonary contusions and thoracic spondylolisthesis in a motor vehicle accident. She developed acute respiratory distress syndrome requiring high-frequency oscillatory ventilation and completed a 2-week course of methylprednisolone. Sputum cultures grew *Acinetobacter baumanii* and *Moraxella catarrhalis* for which she was treated with vancomycin and cefotaxime. She developed multiple indurated erythematous papules with central eschar on her extremities. Wound culture grew *A. fumigatus*. She completed a 6-week course of voriconazole, with resolution of the lesions.

DISCUSSION

PCA is typically associated with alterations in skin integrity and immune compromise. The morphology of lesions varies. It may first manifest as erythema and induration, but can progress to papules, nodules, macules, plaques, pustules, vesicles, bullae or ulcers.[1] Diagnosis is typically made by skin biopsy for histopathology and culture. Amphotericin B has historically been first-line therapy; however, voriconazole has been shown to be superior in adults, has a good safety profile in children and offers oral administration.[2]

Our patients were previously healthy, without underlying immune suppression, but other factors likely caused transient alteration in their defenses. They suffered multiple traumatic injuries, and may have had unrecognized alterations in skin integrity. Trauma is known to alter cellular and humoral immunity, with an initial proinflammatory response to the injury characterized by activation of nuclear factor-κB with resultant increases in acute phase reactants such as cytokines, specifically interleukin (IL)-1b, IL-6, IL-8 and tumor necrosis factor-α. After this proinflammatory state, a compensatory antiinflammatory response syndrome or immune paralysis can occur. Patients develop impaired monocyte function with decreased expression of human leukocyte antigen DR and decreased production of tumor necrosis factor-α, resulting in predominance of the Th2 lymphocyte pattern.[3] Posttraumatic impairment of monocyte activity has been shown to correlate with severity of injury, and is predictive of the development of systemic inflammatory response syndrome.[4] Corticosteroids have been associated with PCA, with impairment of glycemic control, phagocytic function and strength of the skin barrier.[5] Broad spectrum antibiotics can also increase susceptibility to fungal infections in immune compromised patients.

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Letter to the Editor

Common variable immunodeficiency classification by quantifying T-cell receptor and immunoglobulin κ-deleting recombination excision circles

To the Editor:

Common variable immunodeficiency (CVID) is the most frequent primary immunodeficiency associated with hypogammaglobulinemia and other various clinical manifestations. CVID was originally reported to be a disease primarily caused by defective B-cell function, with defective terminal B-cell differentiation rendering B cells unable to produce immunoglobulin. However, combined immunodeficiency (CID) involving both defective B and T cells is often misdiagnosed as CVID.[1] Indeed, one study reported that $CD4^+$ T-cell numbers were decreased in 29% of 473 patients with CVID[2]; similarly, another study found that naive T-cell numbers were markedly reduced in 44% (11/25) of patients with CVID.[3] These observations indicated that a subgroup of patients with clinically diagnosed CVID is T-cell deficient. Consistently, some patients with CVID have complications that might be related to T-cell deficiency, including opportunistic infections, autoimmune diseases, and malignancies, which is similar to that observed in patients with CID.[1,4] Therefore identifying novel markers to better classify CVID and distinguish CID from CVID will be required to best manage medical treatment for CVID.

We recently performed real-time PCR-based quantification of T-cell receptor excision circles (TREC) and signal joint immunoglobulin κ-deleting recombination excision circles (KREC) for mass screening of severe combined immunodeficiency (SCID)[5] and B-lymphocyte deficiency[6] in neonates. TREC and KREC are associated with T-cell and B-cell neogenesis, respectively.[7] Here we retrospectively report that TREC and KREC are useful for classifying patients with clinically diagnosed CVID.

Hypogammaglobulinemic patients (n = 113) were referred to our hospital for immunodeficiency from 2005-2011, and the following patients were excluded from the CVID pool by estimating their SCID genes based on clinical manifestations and lymphocyte subset analysis: 18 patients with SCID diagnoses; 14 patients less than 2 years of age (transient infantile hypogammaglobulinemia); 10 patients with IgM levels of greater than 100 mg/dL (hyper-IgM syndrome); 26 patients with diseases other than CVID caused by known gene alterations (10 with X-linked agammaglobulinemia and 11 with hyper-IgM syndrome [*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.

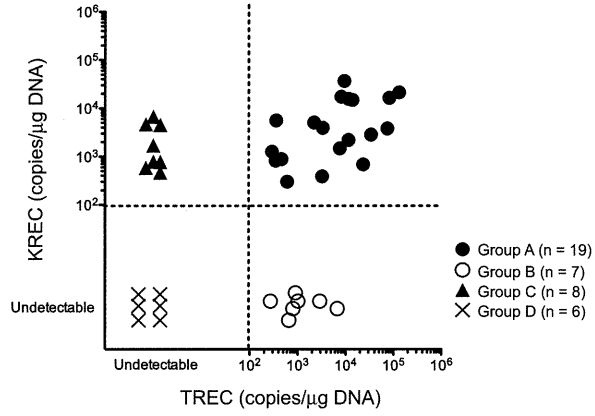

FIG 1. Quantifying TREC and KREC classifies patients with CVID into 4 groups. Patients with CVID were classified as follows: TREC(+)/KREC(+), group A (19 patients); TREC(+)/KREC(−), group B (7 patients); TREC(−)/KREC(+), group C (8 patients); and TREC(−)/KREC(−), group D (6 patients). *Undetectable,*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

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

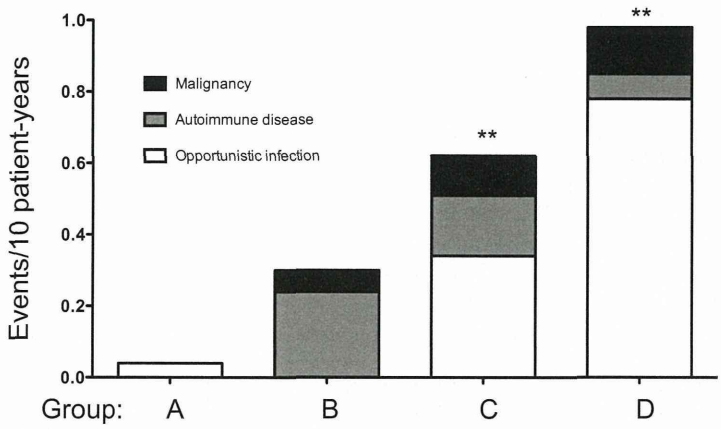

FIG 2. Cumulative incidence of complication events per 10 patient-years differs among groups. Opportunistic infections, autoimmune diseases, and malignancies were evaluated for each patient group. Complication incidences in group D (0.98 events/10 patient-years), group C (0.63 events/10 patient-years), 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: $P = .0692$.

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 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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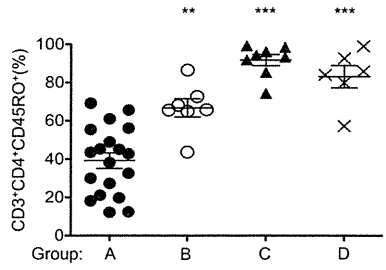
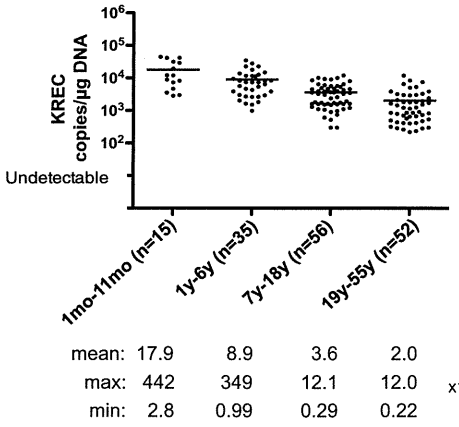


FIG E1. $CD45RO^+CD3^+CD4^+$ T-cell frequency within $CD4^+CD3^+$ lymphocytes was analyzed among groups. $CD45RO^+CD3^+CD4^+$ lymphocyte counts were significantly higher in groups B, C, and D compared with those in group A ($P < .0001$). Group A: 37% ± 16%; group B: 67% ± 13% ($^{**}P < .01$); group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



	1mo-11mo (n=15)	1y-6y (n=35)	7y-18y (n=56)	19y-55y (n=52)	
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; $P < .0001$).

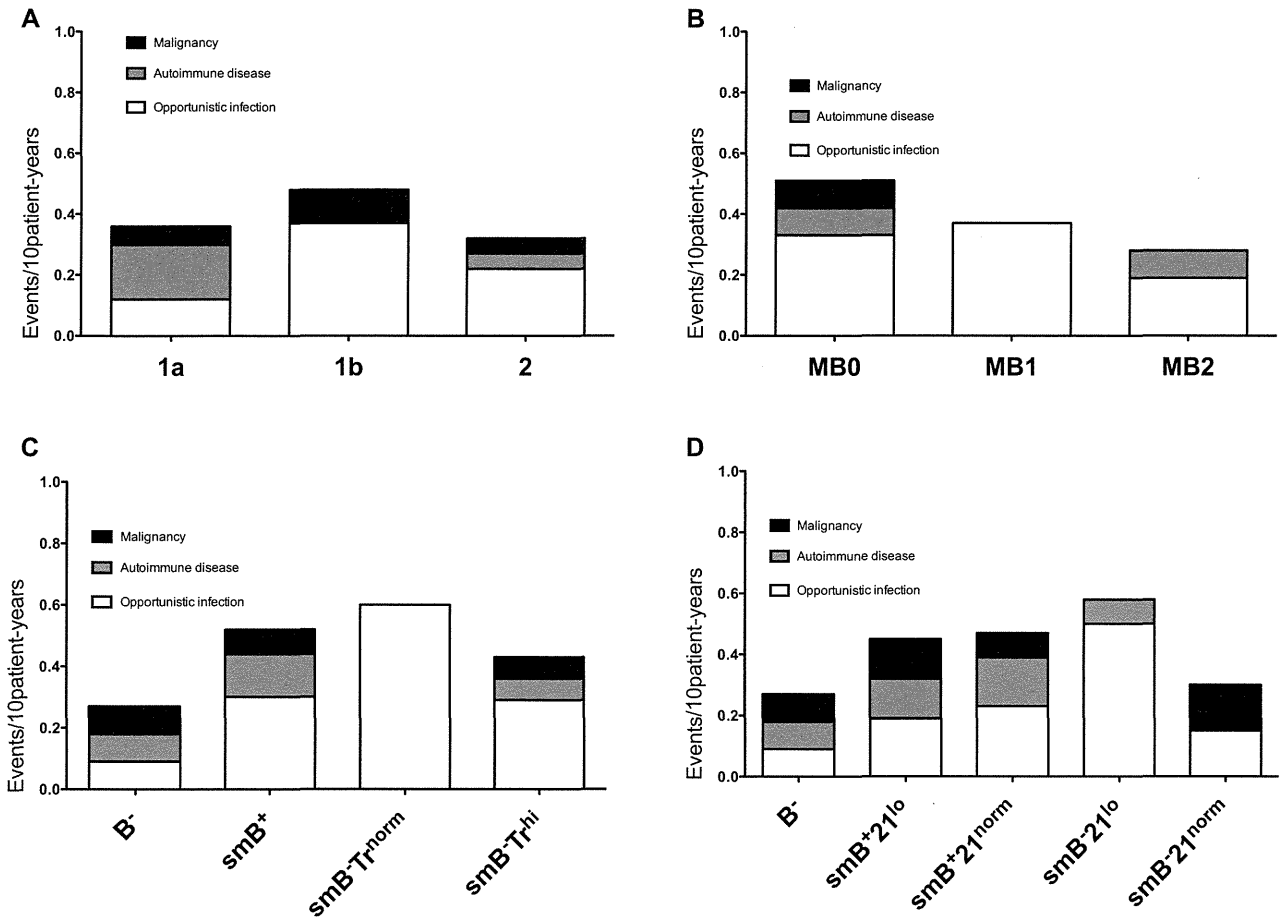


FIG E3. Patients were classified in the following way and analyzed for cumulative incidence of complications: **A,** Freiburg; **B,** Paris; and **C,** EUROclass classifications, according to $CD38^{hi}IgM^{hi}$ transitional B cells (Fig E3, *A-C*) or $CD21^{low}$ B cells **(D)**. Five patients were 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.

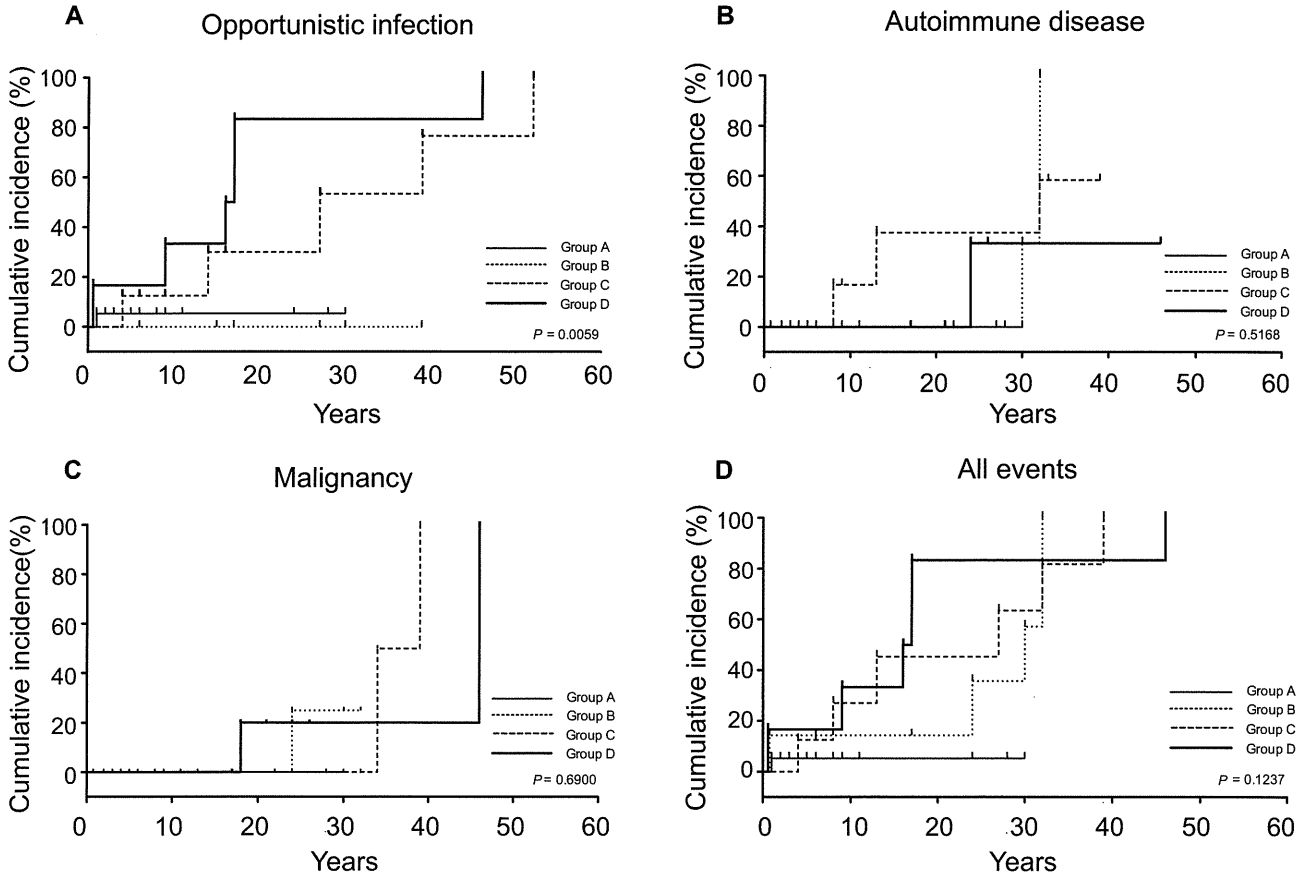


FIG E4. Comparing longitudinal cumulative incidence of complication events among groups. Cumulative incidence was estimated separately and longitudinally by using the Kaplan-Meier method and statistically compared between groups by using the log-rank test. The cumulative incidence of opportunistic infections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

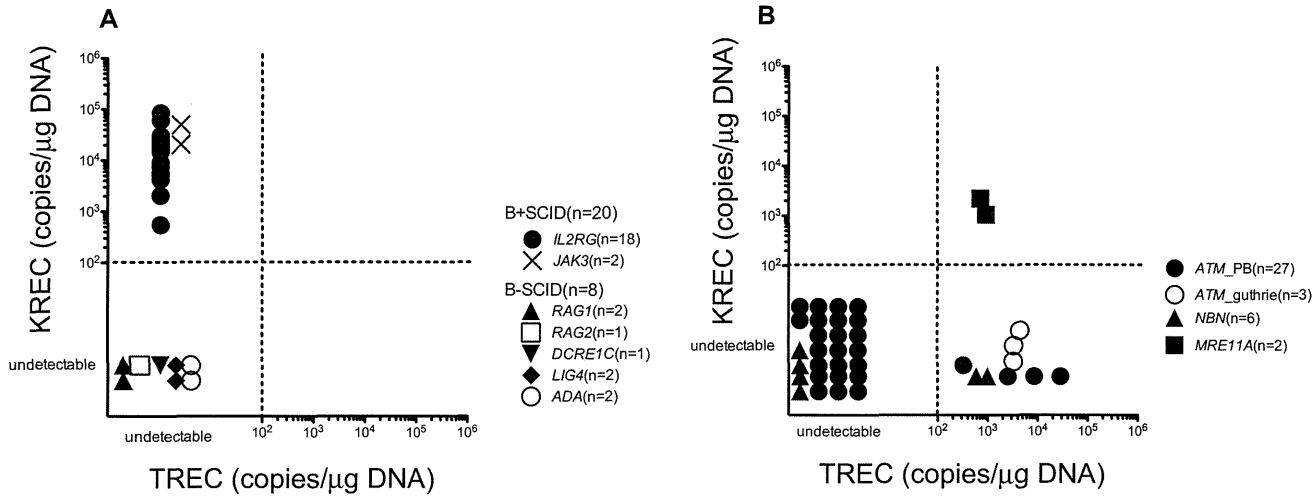


FIG E5. TREC and KREC quantification classifies patients with SCID, AT, NBS, or ataxia-telangiectasia–like disease *(ATLD)* into 4 groups. **A,** Patients with B^+SCID (n = 20) were classified as group C, and patients with B^-SCID (n = 8) were classified as group D; these patients were included in the 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.

4．出血性膀胱炎

5）多項目ウイルスモニタリングからの情報

診療のコツ

出血性膀胱炎（hemorrhagic cystitis：HC）の診断と治療に当たり，その迅速性・感度・定量性から，ウイルスPCRは重要な役割を果たす。特に体系的かつ定量的なアプローチが肝要である[1]。

ウイルスとしては，健常人においても尿細管上皮に潜伏感染しているBKウイルス（BKV）が検出されることが最も多いが，非HCでもBKVが検出されることが多い。したがって，HCがBKVによるか否かの判断には，他の感染症の否定や，量的な評価などが求められる。一方，アデノウイルス（AdV：adenovirus）による膀胱炎は，ウイルスが陽性であることが重要であるが，量的なデータは経過のフォローアップに有用である。

HCの尿ウイルス定量測定についてのまとまったデータは，Leungらによる報告（2001年）が最も早いものであり[2]，その中で既に，BKVは移植後に高頻度に尿中に検出されること，BKVによるHCではコピー数が，非HCにおけるBKVコピー数や，AdV HCにおけるウイルスコピー数に比べて4～5オーダー高いことを示している。

BKV HCについては，前処置（骨髄破壊的前処置），GVHD，移植前の尿ウイルス検出，血中BKVの検出などと関係する，との報告もある。

BKV，AdV以外では，HHV-6(human herpesvirus-6：ヒトヘルペスウイルス6型)やJCV（JCウイルス）も関与しているのではと推測されているが，因果関係の証明は困難である。さらに，CMV（cytomegalovirus：サイトメガロウイルス）などのヘルペス属も検出されることがあるため，可能性のあるウイルスにつき多項目測定を行わない限り，どのウイルスがどれくらいの量で存在するとHCに至るのか，あるいは腎障害に至るのか，などの問いかけには答えられない。

筆者らは，以下の11種類のDNAウイルス（HSV-1，HSV-2，VZV，EBV，CMV，HHV-6，HHV-7，HHV-8，BKV，JCV，ParvovirusB19）を高感度・迅速・安価に測定するMultiplex PCR系を立ち上げ，陽性検体では定量測定も実施し，AdVは別途定量検査を行った[3]。その

結果，造血細胞移植後膀胱炎症状を呈した患者77人125検体において，73人96検体で何らかのウイルスが陽性になることが明らかになった。最も多く検出されたウイルスはBKV（65検体），次いでJCV(32検体)であり，次いでAdVが23検体であった。その他，EBV(7)，CMV(15)，HHV-6(15)も検出されるが，単独陽性群はそれぞれ3，3，6であり，EBV(Epstein-Barr virus：エプスタイン・バーウイルス）に関してはコピー数も低く，病態とは無関係と考えられる。JCVが陽性となった検体では25/32でBKVが陽性，1例でAdVが陽性であり，それ以外の6症例でのコピー数は105以下であった。ウイルスが単独で陽性になるものはBKVが31/54検体と最も多く，次いでAdV(7検体)であった。また，1/3の検体では複数以上のウイルスが検出された（図1，2）。

ウイルス量からもいくつかの情報が得られた(図2)。まずBKVのウイルスコピー数は，AdVに比して2オーダーは高く，また10^7～10^8以上のものでは明らかに出血性膀胱炎を呈している

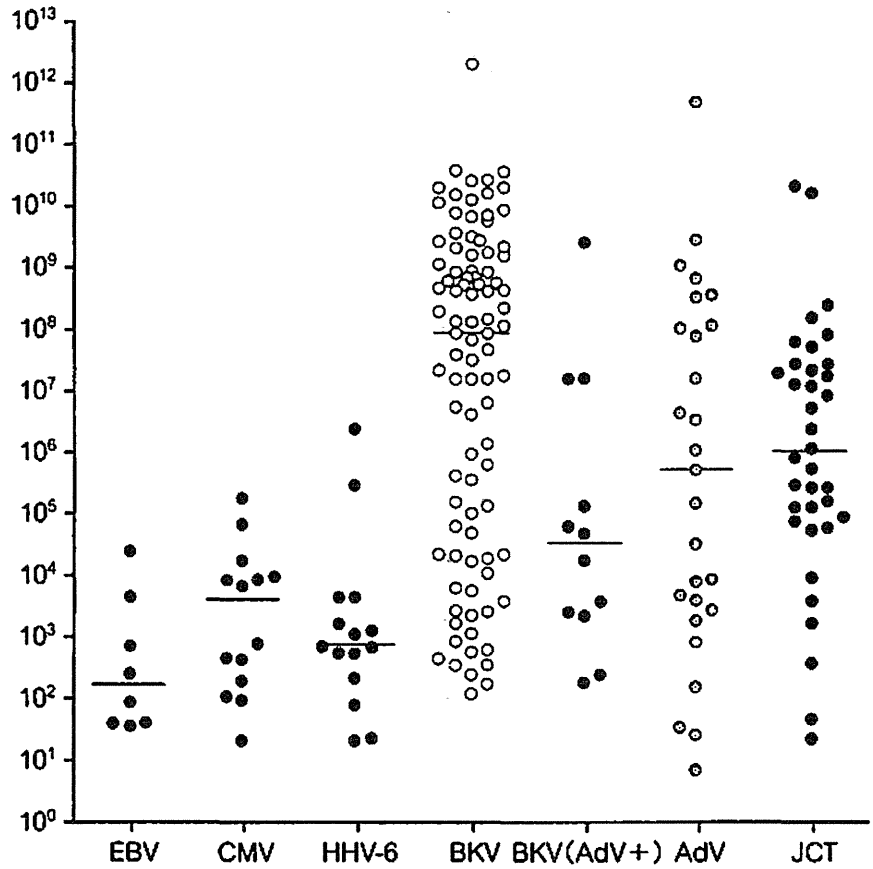


図1　造血細胞移植後出血性膀胱炎において検出されたウイルスとそのコピー数（77人125検体での検討）

尿検体mL当たりの各ウイルスのコピー数を，リアルタイムPCRにて算定してプロットした。横線は中央値を表している。AdV陽性検体でBKVが検出される場合の多くではBKVのコピー数が10^7以下（中央値：10^6～10^5）であり，主体はAdVであろうと推測される。

（自験例）

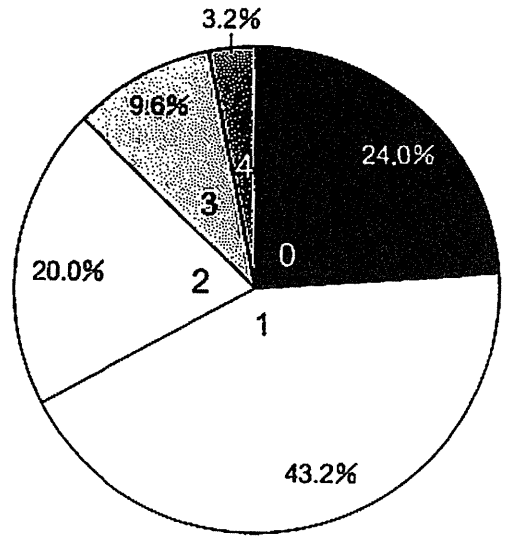


図2　造血細胞移植後出血性膀胱炎にて検出されたウイルス数（125検体の集計）

造血細胞移植後の出血性膀胱炎では3/4以上でウイルスが検出され，また2種類以上のウイルスが検出される例が30%以上であった。3～4種類が同定される症例も散見される。

（自験例）

という点である。血液検体にてBKVが検出されるものでは，尿中のBKV量の中央値が10^9であり，BKV膀胱炎の診断の一助になる可能性がある。AdV出血性膀胱炎においてもBKVが検出される場合があるが，図1で明らかなように，コピー数の中央値は10^4～10^5であり，AdV膀胱炎に随伴しての上皮障害で検出されたものと考えられる。一方，コピー数が10^8以上のものでは，どちらが主体か慎重に判断する必要がある。

（森尾 友宏）

▷ 文　献

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A novel BMT technique for treatment of various currently intractable diseases

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A recently-developed BMT method combines a "Perfusion Method" (PM) for collecting bone marrow cells (BMCs) with the Intra-Bone Marrow (IBM) injection of BMCs (IBM-BMT). As distinct from the conventional aspiration method (AM), the PM allows rapid (within 1 h) collection of BMCs without T cell contamination (T cells < 10%). Therefore, no GvHD occurs. Moreover, the burden on donors, such as back pain, bleeding and infection, can be reduced.

Full chimerism can be achieved even with only mild conditioning regimens if IBM-BMT is carried out, since IBM-BMT replaces not only the recipient's hemopoietic stem cells (HSCs) but also mesenchymal stem cells (MSCs) with donor-derived HSCs and MSCs.

Using this method, we show that most currently intractable diseases are HSC or MSC disorders, and that this novel strategy (PM + IBM–BMT) can be used to treat various otherwise intractable diseases (including autoimmune diseases and age-associated diseases).

We believe that the development of this technique will herald a revolution in the field of BMT, regeneration medicine and also organ transplantation.

Introduction

In 1985, we found that allogeneic bone marrow transplantation (BMT) (but not autologous BMT) could be used to prevent and treat autoimmune diseases in autoimmune-prone mice [1,2]. In addition, we succeeded in inducing autoimmune diseases in normal mice by the transplantation of T cell-

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depleted bone marrow cells (BMCs) or partially purified hemopoietic stem cells (HSCs) from autoimmune-prone mice to normal mice [3,4].

Based on these findings, we have proposed that autoimmune diseases originate from defects in HSCs [3–7], and have also found that abnormal HSCs of autoimmune-prone mice are more resilient than normal HSCs [4,8,9]; abnormal HSCs can proliferate even in the allogeneic microenvironments, whilst normal HSCs can proliferate in collaboration with major histocompatibility complex (MHC)-compatible stromal cells (mesenchymal stem cells: MSCs), but not MHC-incompatible MSCs [4,8,9].

From these findings, we realized that, in the case of BMT across MHC barriers, we would have to transplant both donor-derived HSCs and MSCs to ensure that the donor-derived normal HSCs grow and survive in the allogeneic environments.

Recently, we have discovered that the injection of whole BMCs directly into the bone cavity (intra-bone marrow-BMT: IBM-BMT) provides distinct advantages, since IBM-BMT can efficiently recruit not only donor-derived HSCs but also MSCs. We here review our data regarding IBM-BMT plus the perfusion method (PM) (capable of efficiently collecting MSCs).

Advantages of novel BMT

As shown in Fig. 1, conventional BMT is carried out as follows: Bone marrow needles are inserted into the iliac bones more than 100 times, and the BMCs are collected by the aspiration method (AM). Therefore, contamination with peripheral blood (particularly T cells) is inevitable. When thus-collected cells are intravenously injected (IV-BMT), most cells become trapped in the lung and only a few cells migrate into the bone marrow (Fig. 1).

To apply our new BMT methods to humans, we established, using cynomolgus monkeys, a "PM", which minimizes the contamination of BMCs with T cells. As shown in Fig. 2, two needles are inserted into a long bone such as the humerus, femur, or tibia. The end of the extension tube is connected to a needle. The other end is placed in a syringe containing 0.5 ml heparin. The other needle is connected to a syringe containing 30 ml of saline, and the saline is then pushed gently from the syringe into the medullary cavity to flush out the bone marrow (BM). The saline containing the BM fluid is then collected.

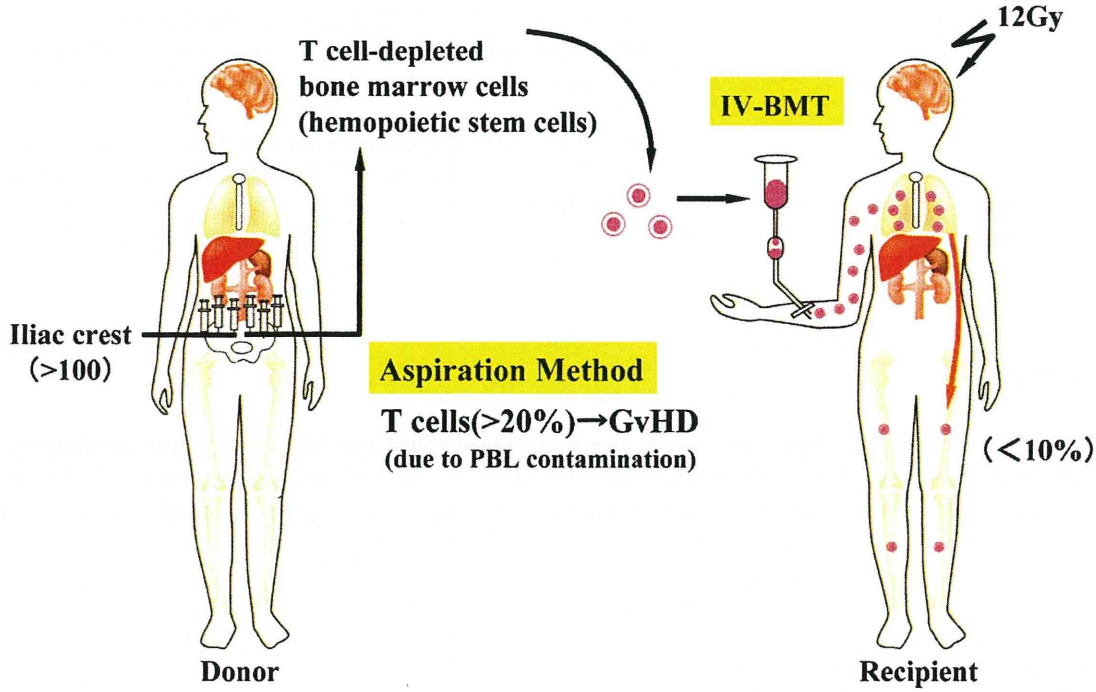

Fig. 1. Conventional BMT for allogeneic BMT. Conventional BMT is carried out using an aspiration method (AM), followed by the intravenous injection of BMCs (IV-BMT).

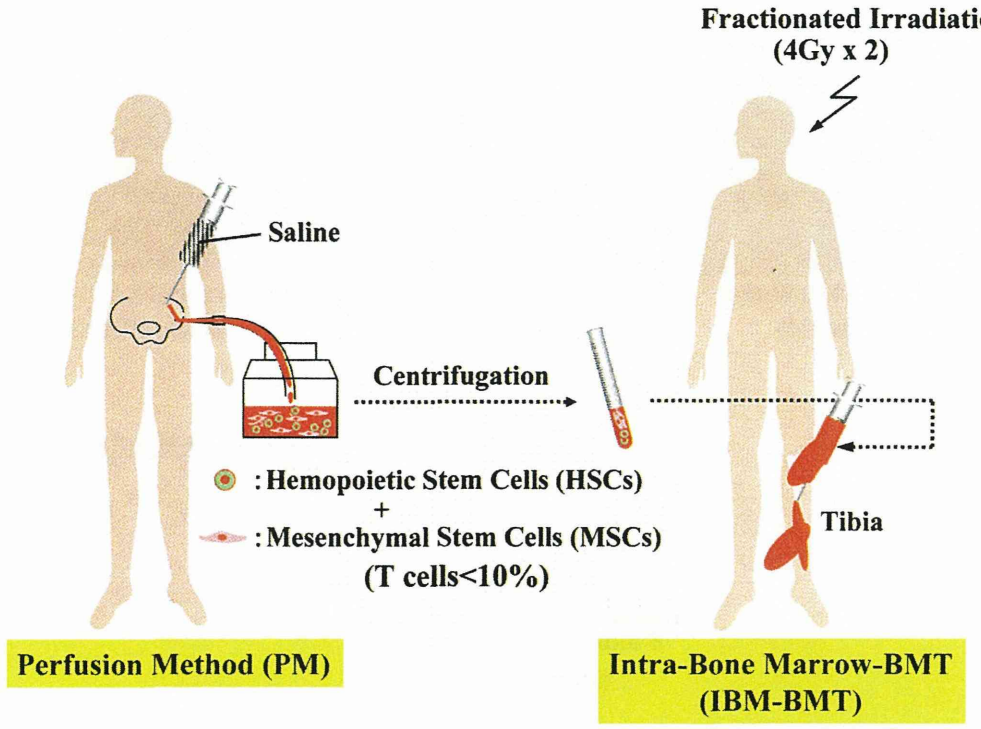

Fig. 2. New BMT method for allogeneic BMT. The new BMT method is carried out using a perfusion method (PM), followed by IBM–BMT.

There is significantly less contamination with T cells when using the PM (<10%) than with the conventional AM (>20%) [10,11]. Therefore, T cell-depletion is unnecessary with the PM, and whole BMCs can be used. However, in the case of the conventional AM, T cell-depletion is necessary, and the loss of some important cells such as MSCs during the process of T cell-depletion is inevitable. Furthermore, the number and progenitor activities of the cells harvested using the PM are greater than when using the conventional AM [10,11].

We have also found that the PM is applicable to the iliac bones as well as the long bones not only in monkeys but also in humans.

We are now starting a Phase I Study for the clinical application of PM + IBM–BMT.

IBM–BMT for organ transplantation

Since we have previously found that the combination of organ allografts and conventional IV-BMT from the same donors prevents the rejection of organ allografts [12], we attempted to apply IBM–BMT to organ allografts. IBM–BMT was the most effective strategy, since the radiation dose could be reduced to 4.0Gy × 2 in skin allografts [12,13]. In addition, we found that IBM–BMT is applicable to allografts of other organs and tissues in rats, such as pancreas islets [14] legs [15], lungs [16], and heart [17].

IBM–BMT for regeneration therapy

As it was apparent that donor stromal cells could be effectively recruited by "IBM–BMT", we next attempted to treat osteoporosis in SAMP6 mice; the SAMP6 mouse (a substrain of senescence-accelerated mice) spontaneously develops osteoporosis early in life and is therefore a useful model for examining the mechanisms underlying osteoporosis. After IBM–BMT, the hematolymphoid system was completely reconstituted with donor-type cells. Thus-treated SAMP6 mice (8 months after IBM-BMT) showed marked increases in trabecular bone even at 20 months of age (Fig. 3), and the bone mineral density (BMD) remained similar to that of normal B6 mice. Bone marrow stromal cells in "IBM-