Leukemia
Original article

Title: Clonal origin of Epstein-Barr virus-infected T/NK-cell subpopulations in chronic active Epstein-Barr virus infection

Authors: Shouichi Ohga, MD, PhD,[1] Masataka Ishimura, MD,[1] Goichi Yoshimoto, MD, PhD,[2] Toshihiro Miyamoto, MD, PhD,[2] Hidetoshi Takada, MD, PhD,[1] Tamami Tanaka, PhD,[1] Koichi Ohshima, MD, PhD,[5] Ken-Ichi Imadome, PhD,[4] Yasunobu Abe, MD, PhD,[3] Koichi Akashi, MD, PhD,[2] Toshiro Hara, MD, PhD[1]

Affiliations: 1. Department of Pediatrics, 2. Department of Medicine and Biosystemic Science, 3. Department of Medicine, and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, 4. Department of Infectious Diseases, National Research Institute for Child Health and Development, Tokyo, 5. Department of 2nd Pathology, Faculty of Medicine, Kurume University School of Medicine, Kurume, Japan

Correspondence: Shouichi Ohga, MD, PhD. Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. TEL: +81-92-642-5421, FAX: +81-92-642-5435, E-mail: ohgas@pediatr.med.kyushu-u.ac.jp

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Running title: Clonal EBV-infected T/NK-cell subsets in CAEBV

Abbreviations: BM: bone marrow, CAEBV: chronic active Epstein-Barr virus infection, EBV: Epstein-Barr virus, EBER: Epstein-Barr virus-encoded mRNA, EBNA: Epstein-Barr virus-nuclear antigen, HLH: hemophagocytic lymphohistiocytosis, HIV: human immunodeficiency virus, HMB: hypersensitivity to mosquito bite, Ig: immunoglobulin, IM: infectious mononucleosis, LMP: latent membrane protein, MNC: mononuclear cell, Lin: lineage, LPD: lymphoproliferative disease, NK natural killer, PB: peripheral blood, PCR: polymerase chain reaction, TCR: T-cell receptor

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ABSRTACT

Clonal expansion of Epstein-Barr virus (EBV) infected B-cells occasionally occurs in immunocompromized subjects. EBV-infected T/natural killer (NK)-cells proliferate in patients with chronic active EBV infection (CAEBV) that is a rare mononucleosis syndrome. It is classified into either T-cell type or NK-cell type according to the primary target of infection, while the pathogenesis remains unclear. To search the clonal origin of EBV-infected T/NK-cells, virus distribution and clonotype were assessed by using highly purified cell fractions obtained from 6 patients. Patient 1 had a monoclonal proliferation of EBV-infected T-cell receptor Vδ2/Vγ9-expressing cells, and carried lower copy number of EBV in αβT-cells. Patients 2 and 3 had a clonal expansion of EBV-infected $CD4^+$T-cells, and lower EBV load in $CD56^+$cells. Patients 4, 5 and 6 had an expansion of $CD56^+$cells with higher EBV load than $CD3^+$cells. EBV-terminal repeats were determined as clonal bands in the minor targeted populations of 5 patients. The size of terminal repeats indicated the same clonotype in minor subsets as in major subsets of 4 patients. However, EBV was not detected in bone marrow-derived lineage negative $CD34^+$cells of patients. These results suggested that EBV could infect T/NK-cells at differentiation stage, but spared bone marrow $CD34^+$hematopoietic stem cells in CAEBV patients.

INTRODUCTION

More than 90% adults are infected with Epstein-Barr virus (EBV), and the infection persists for life. The primary infection often causes acute infectious mononucleosis (IM) in adolescents or young adults. The γ herpes virus enters $CD21^{+}$B cells, and establishes latent infection in the memory B-cell pools evading immune elimination by EBV-specific cytotoxic T-cells.[1] EBV-infected B-cell lymphoproliferative disease (LPD) occurs in immunocompromized patients. Recently, novel EBV-driven T/natural killer (NK)-cell LPD has been identified in non-immunocompromised hosts such as chronic active EBV infection (CAEBV) and EBV-associated hemophagocytic lymphohistiocytosis (HLH).[2,3] CAEBV is a rare mononucleosis syndrome typified by high circulating levels of EBV DNA and recurrent IM-like symptoms along with coronary artery lesions, chorioretinitis, and brain involvement.[4] It was first described as a progressive EBV infection targeting B-cells, but most cases are reported from Asia as EBV-infected T/NK-cell LPD. The Asian type CAEBV is classified into T-cell type or NK-cell type according to the target of infection.[5-7] The NK-cell type might be linked with hypersensitivity to mosquito bite (HMB) and slow progression. However, affected patients with either type result in fetal demise without allogeneic stem cell transplantation (SCT).[8,9] $CD8^{+}$T cells are exclusively targeted in EBV-HLH patients, while the virus spreads over T/NK-cell subsets in CAEBV patients. The cell-type specific infection does not stipulate the transformation or tissue infiltration. Despite the increasing number of reports,[10] the cause of T/NK-cell infection remains unclear.

The episome of EBV DNA has a highly variable reiteration of terminal repeat (TR) sequences that are joined by random recombination upon circularization of the linear genome at entry into cells. As the TR number is inherent in a round of EBV

reactivation and reinfection, the diversification denotes the latently infected cell clones. Nonetheless, cell purity and blotting sensitivity have hampered the precise analysis of CAEBV T/NK-cells. EBV-producing T/NK-cell lines established from patients do not represent the *in vivo* clonal nature.

In the present study, EBV load and clonality of T/NK-cell subsets were analyzed in CAEBV patients by using the high-accuracy cell sorting. EBV DNA was detected in the major targeted subsets as well as in the other subsets with the evidence of clonotypic infection. These results first implied the infection of T/NK-cell precursors. Whereas, bone marrow (BM)-derived $CD34^+$ cells, supposed to include common lymphoid progenitors, were not involved in the infection. The pathogenesis of CAEBV was discussed with special reference to the cellular origin of EBV infection.

MATERIALS and METHODS

Patients

Six Japanese patients treated in Kyushu University Hospital between 2002 and 2008, were recruited. All fulfilled the diagnostic criteia.[11] Clinical profiles are shown in **Table 1**. Median age at the onset of illness was 3.5 years (range: 1~8). The unique symptoms included HMB (n=4), hydroa vacciniforme (n=4), and coronary artery lesion (n=1). Three patients had abnormal patterns of anti EBV-antibodies (Abs). Five had increased number of activated T-cells and/or high CD4/CD8 ratio. Primary target was T-cell in 3 and NK-cell in 3 patients. Clonality was screened for peripheral blood (PB) mononuclear cells (MNCs) by EBV-TR and immunoglobulin (Ig)/T-cell receptor (TCR) genes. No patients received cytotoxic agents at the time of study. Controls included EBV-seropositive subjects or patients with immune thrombocytopenia.

Antibodies, cell staining, and sorting

Mononuclear cells were separated by centrifugation using separation medium (Cappel, Aurora, OH). Each cell fraction was positively selected by immunomagnetic beads with Vario-MACS columns (Miltenyi Biotec, Bergisch Gladbach, Germany) after the staining with monoclonal Abs (mAbs) against CD3, CD4, CD8 and CD56. TCR $V\delta2^+$cells and $TCR\alpha\beta^+$cells were procured by using MACS anti-FITC or anti-PE microbeads (Miltenyi Biotec) after the staining with FITC-conjugated anti-Vδ2 mAb (Immunotech/Beckman Coulter, Marseille, France), or PE-conjugated TCRαβ mAb. $CD56^+$cells were positively collected using Lymphocyte Separation column (Miltenyi Biotec, Bergisch Gladbach, Germany), after the depletion of $CD3^+$cells by Lymphocyte Depleting column. The procedures yielded cell samples with more than 97% of purity, which was determined by the flow cytometry using EPICS XL (Immunotech Coulter [IC], Miami, FL). To raise the maximum purity (>99.9%) of $CD3^+$cells, $V\delta2^+$cells, $TCR\alpha\beta^+$cells, and $CD34^+$cells, all sorting and analyses were performed on FACS Aria (BD Biosciences, Oxnald, PA, USA). $CD3^+$cells, $V\delta2^+$cells, and $TCR\alpha\beta^+$cells were positively sorted after staining with each FITC-conjugated mAbs. BM $CD34^+$cells were sorted as follows; $CD34^+$cells were enriched from BM cells by using the Indirect CD34 MicroBead Kit (Miltenyi Biotec) as described previously.[12] For analyses and sorting, cell samples were stained with Cy5-PE- or PC5-conjugated lineage mixture, including Cy5-PE- or PC5-conjugated anti-CD3, CD4, CD8, CD10, CD20, CD11b, CD14, GPA, FITC-conjugated anti-CD3, CD4 or Vδ2, PE-conjugated anti-Vαβ, CD16, or CD56, allophycocyanin (APC)-conjugated anti-CD34, and nonviable cells were excluded by propidium iodide (PI) staining. $CD34^+$cells were isolated from BM cells by FACS Aria. Appropriate isotype-matched control mAbs were used to determine the

background staining level in each channel. To avoid any contamination, the second round of sorting was performed routinely with the same sorting gates as the first round. FACS data were analyzed with FlowJo software (Tree Star, Inc.). The re-analyses warranted the consistent purity of > 99.9% procured cells **(Supplementary figure 1)**.

DNA extraction and real-time PCR for EBV-DNA

High molecular DNA was extracted from cell samples. DNA was used for PCR and Southern blotting. The real-time PCR for EBV DNA was performed according to the established method.[4,6] Briefly, TaqMan probe was labeled with the reporter dye molecule at the 5' end, and with the quencher flour at the 3' end via a linker arm nucleotide. Gene dosages were analyzed by ABI PRISM 7700 (PE Biosystems, Foster City, CA). DNA was mixed with TaqMan Universal PCR Master Mix (PE Biosystems), primers, and TaqMan probe. PCR conditions were 50 ^{o}C for 2 min and 95 ^{o}C for 10 min, followed by 50 cycles at 95 ^{o}C for 15 sec, and 60 ^{o}C for 1 min. Healthy persons with past EBV infection show <200 copies/ml and <40 copies/μgDNA in PB and MNCs, respectively.

Southern blotting probed with EBV-tandem repeats (TR) or TCR/Ig genes

Southern blotting was performed as the established method.[2,13] Briefly, 5μg of high molecular weight DNA were digested with *Eco*RI and/or *Bam*HI. The digested DNA was electrophoresed on 0.9% agarose gel and transferred to Byodine-B, which was hybridized with a ^{32}P-labeled probe. Clonality of lymphocytes was assessed probed with a 5.2-kb *Bam*HI-*Eco*RI fragment containing the TR sequence of the EBV genome, and/or TCR/immunoglobulin (Ig) gene probes of Cβ1, Jβ1, Jβ2, Jγ, and JH. Normal control DNA was extracted from MNC of healthy EBV-seropositive adults.

Gene analysis of γδT-cells

Genomic DNA was extracted from highly purified PB panTCRγδ positive PB MNCs by the conventional methods. Direct sequencing of PCR product of N-regions was completed for TCRδ-gene and TCRγ-gene. The exon and exon–intron boundary regions of each gene were amplified by PCR, and the products were then subjected to direct sequencing using 3130*xl* Genetic Analyzer (Life Technologies Corp., CA, USA).

Immunohistology

BM clot samples fixed in buffered formalin and embedded in paraffin wax, were immunostained with Abs against CD3, CD4, CD8, CD56, latent membrane protein (LMP1) and EBNA-2 (Dako, Copenhagen, Denmark). For *in situ* hybridization (ISH) to detect EBV-RNA, the samples was hybridized with an oligo-probe (EBER-1 region) and a sense probe (negative control), and then was labeled with digoxigenin as our methods.[14] Briefly, after de-waxing, dehydration and proteinase-K digestion, the sections were hybridized overnight in a solution of 50% formamide containing 5 ng DIG-labeled probes. After washing, detection was accomplished using an avidin-alkaline-phosphatase conjugate.

RESULTS

Target cells and clinical expression

T-cell type Patient (Pt) 1 presented fever and skin eruption. $CD4^-CD8^-$γδ T cells increased to 30 % of PB MNC, which expressed Vδ2/Vγ9 **(Figure 1)**. PCR products amplified by Vδ2 or Vγ9 primer indicated clonal proliferation of Vδ2/Vγ9 T-cells. Sequencing of Vγ9-JγP1 junctional transcripts revealed an N-region sequence **(Table 2)**. $Vδ2^+$cells and $TCRαβ^+$cells had $4×10^5$ and $4×10^3$ copies EBV/μgDNA, respectively. Screening blotting of MNC showed a single EBV-TR and TCRγ gene rearrangement.

The clonal expansion of EBV-infected Vδ2/Vγ9 T-cells has continued for 6 years. Pt 2 suffered hydroa vacciniforme. $CD3^+$cells, $CD8^+$cells and $CD56^+$cells carried 2×10^5, $5x10^3$ and 9×10^3 copies/μgDNA, respectively. Screening blotting indicated diclonal expansion of EBV^+CD4^+T-cells. At 25 years of age, she underwent a sibling donor SCT because of cutaneous lymphoma with $EBER1^+$ $CD4^+$T-cells or $CD56^+$cells. She attained EBV-free remission, but died of graft-versus-host disease. Pt 3 had fever and hepatitis. $CD4^+$cells, $CD8^+$cells and $CD56^+$cells had 3×10^4, 90 and <40 copies/μgDNA, respectively. He attained remission after an HLA-matched sibling donor BMT.

NK-cell type Pt 4 showed aggressive course with cardiac and enteral involvement. EBV-infected $CD56^+$cells infiltrated vesicular HMB lesions. $CD4^+$cells, $CD8^+$cells and $CD56^+$cells carried 2×10^3, 1×10^3, and 4×10^5 copies/μgDNA, respectively. Screening of MNC showed diclonal TR bands. She attained EBV-free remission after cord blood transplantation. Pt 5 had fever and HMB. $CD3^+$cells and $CD56^+$cells carried 3×10^4 and 8×10^5 copies/μgDNA, respectively. Skin infiltrations with $EBER1^+$ $CD4^+$cells subsided by low dose prednisolone. Pt 6 showed fever and HMB. $CD4^+$cells, $CD8^+$cells and $CD56^+$cells had 8×10^3, 4×10^4, and 4×10^5 copies/μgDNA, respectively.

Clonality of EBV-infected subsets

The clonotype of EBV-infected subsets were assessed by Southern blotting with TR probe for highly purified cell fractions. The blotting sensitivity was estimated to detect more than 0.5% contamination of EBV-infected cells by the add-back experiments **(Supplementary figure 2)**. As shown in Figure 2, TCRαβ cell fraction (>99.9%) showed the same sized single band as $V\delta2^+$ cells in Pt 1 after either *Eco*RI or *Bam*HI digestion **(Figure 2)**. Pt 2 and Pt 3 having high EBV load in $CD4^+$ T-cells showed a single TR band in $CD56^+$cells and no detectable band in $CD8^+$ T-cells, respectively.

The TR size in $CD56^+$cells of Pt 2 was same as one of diclonal bands in $CD3^+$ T-cells.

The clonotype in highly purified T-cells was assessed in NK-cell type CAEBV. Clonal TR bands were detected in $CD3^+$cells (>99.5%) in all 3 patients. The diclonal bands and monoclonal band in $CD3^+$cells of Pt 4 and Pt 5 were in the same size as those in the $CD56^+$cells, respectively. On the other hand, in Pt 6, $CD3^+$cells showed a distinct monoclonal band in larger size than $CD56^+$cells **(Figure 2)**. The screening of MNC from Pt 6 showed the same sized TR band as $CD56^+$ cells.

EBV load in BM $CD34^+$ cells

To further assess the cellular origin of EBV-infection, the virus load was re-quantified using highly purified cells. EBV DNA was undetectable in BM derived lin^-CD34^+ cells of 5 patients studied, and seropositive controls **(Figure 3)**. Immunohistochemical staining and/or *in situ* hybridization of BM clot samples revealed no detectable $CD34^+$ cells positive for LMP1, EBNA or EBER **(Figure 4)**.

DISCUSSION

Clonal expansion of EBV-infected T/NK-cells was determined not only in the major targeted subsets but also in the less infected subsets of CAEBV patients. The clonotype of EBV in the minor targeted cells notably coincided with that in the major EBV-infected T/NK-cell fractions. On the other hand, BM-derived $CD34^+$ cells did not carry EBV DNA. These suggested that EBV infection could occur at differentiation stages of lymphocytes and then clonally expand to diverse subsets of T/NK-cells, but not involve $CD34^+$ hematopoietic stem cells in CAEBV patients.

It has been controversial whether CAEBV is defined as chronic infection or clonal disorder. Diverse subsets of T-cells and/or NK-cells of CAEBV patients carried EBV

DNA as expected. T-cell and NK-cell lines shedding EBV have been established from CAEBV patients.[15,16] EBER1$^+$ T-cells and NK-cells are found in the tonsils of patients with acute IM.[17] EBV could infect T/NK-cells during the primary infection. If the excessive viral load raises the chance of ectopic infection, the diverse infection in T/NK-cell subsets might be explained by the frequent reactivation and reinfection of lytic virus during the prolonged course of CAEBV. Reactive T-cells oligoclonally expand in CD8$^+$ but not CD4$^+$ subsets of patients with acute IM.[18] In the current study, EBV-infected T/NK-cells clonally proliferated in the major targeted populations (non-CD8$^+$ T-cells) as well as other subsets of CAEBV patients. The primary targeted cells (Vδ2 in Pt 1, NK-cells in Pt 2 and Pt 3) and other minor subsets (αβT cells in Pt 1, T-cells in Pt 4 and Pt 6) shared the same sized TR. PB γδT-cells of healthy human consist entirely of polyclonal Vδ2/Vγ9 T-cells, while those of Pt 1 originate from a single EBV-carrying Vδ2/Vγ9 clone. Toyobe *et al.*[19] reported a CAEBV patient who had biclonal expansion of Vβ7$^+$ and Vβ9$^+$ cells in PB CD4$^+$ T-cells. These T-cell clones were primarily targeted by EBV, and the strain was indistinguishable from each other by genotype analyses. Endo *et al.*[20] analyzed the clonotype of EBV in 2 CAEBV patients who had biphenotypic expansions of CD4$^+$ and CD8$^+$ T cells. Both T-cell subsets carried the same TR sized clone of EBV with hypermethylated status in the Cp region. Germinal center B-cells retaining latent EBV could be the source of B-cell LPD.[21] In this line, the diverse T/NK-cell infection in CAEBV patients might not stem from the spread of lytic infection but the differentiation and expansion of EBV-infected T/NK-cells retaining the latent episome. Alternative explanation of the clonotypic virus is that EBV gene was integrated into the host genome.[22] Proliferating EBV-infected T/NK-cells of CAEBV patients lack malignant morphology. EBV latent genes are

expressed in heterogeneous and restricted patterns in the EBV-infected T/NK-cells of CAEBV patients.[23] The integration of a specific site of EBV genome is unlikely in CAEBV T/NK-cells. Taken together, it may raise the possibility that EBV infection occurs at an early stage of differentiation, and the clonotypic EBV-infected T/NK-cells enter the circulating lymphocytes. Multiple EBV strains are differently associated with EBV-associated tumors in a single host.[24] Further study on the site specific clonality might provide a clue to the pathophysiology of CAEBV.

The major concern is how clonal EBV-infected T/NK-cells are allowed in patients. The etiological analogy was inferred between CAEBV and X-linked LPD, while EBV^+ B-cell LPD arises in the latter. Only a Caucasian patient with the B-cell type CAEBV had defective cytotoxicity due to *PRF1* mutations.[25] However, no genetic defects have been identified in T/NK-cell type CAEBV or EBV-HLH.[26,27] Cytotoxic defect has been implicated in CAEBV patients,[28] though EBV-infected T/NK-cells may have altered cytotoxicity. No familial occurrence was determined in the cases with T/NK-cell type CAEBV reported in Asians and Latin Americans. To the best of our knowledge, in two Japanese pairs of identical twins, one suffered from CAEBV but each counterpart was healthy carrier. The end-to-end length of fused TRs may itself drive clonal selection consequent to the opposing growth advantages and disadvantages of LMP1.[29] The dynamics of primary infection may provide a clue to the evolution of CAEBV.

CAEBV patients die without successful allogeneic SCT, which eradicates EBV-infected hematopoietic cells and reconstitutes the normal EBV-specific immunity. Based on the clonotypic EBV in CAEBV patients, we hypothesized that $CD34^+$ hematopoietic stem cells might be targeted by the infection. HIV-1-infected $CD34^+$ hematopoietic stem cells have been detected in a subpopulation of HIV-positive

patients, ranging from 14 to 36%.[30] HTLV-1 or HHV8 infects $CD34^+$ hematopoietic stem cells,[31] although those cells appeared not to be the major reservoir in carriers. The current results first indicated that BM $CD34^+$ cells were not a niche of EBV infection in CAEBV patients. The primitive hematopoietic stem cells are resistant to HIV-1 infection, although once these cells begin to differentiate and become committed hematopoietic stem cells they become increasingly susceptible to HIV-1 infection and permissive to viral gene expression and infectious virus production.[32] EBV might target CD34 negative T-cell progenitors prior to the differentiation into αβ T cells and γδ T cells. Quiescent hematopoietic stem cells are resistant to *in vitro* infection of pathogens. Dormant $CD34^+$ cells in BM might be protected from infection, or promptly eliminated after infection. CAEBV may be a clonal disease of EBV-infected lymphoid progenitor cells, arising from cytotoxic defect of unknown cause. It explains the rationale of the allogeneic SCT that is necessary for complete recovery of CAEBV patients. Further study on the origin of EBV-infected T/NK-cells may shed some lights not only on the relationship between infection and clonal disease, but also on the therapeutic target of EBV-associated T/NK-cell LPD/lymphoma.

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FIGURE LEGENDS

Figure 1. Representative flow cytometry for lymphocytes of Patient 1. TCRγδ cells increased to ~30% of peripheral blood lymphocytes, exclusively expressing Vγ9/Vδ2 but negligible expression of HLA-DR. Vγ4- and Vδ3-expressing cells were <1% of lymphocytes. More than 99% of $V\delta2^+$ lymphocytes were $CD4^-CD8^-$ T cells.

Figure 2. Southern blotting analysis of high molecular DNA obtained from highly purified cell fractions (>99.5%) probed with the EBV-terminal repeated (TR) sequence. *Eco*RI (E) or *Bam*HI (B)-digested DNA was electrophoresed (2~5μg/lane) and hybridized with a ^{32}P-labeled 5.2-kb *Bam*HI-*EcoR*I fragment containing the TR repeat sequences. The blotting sensitivity was confirmed to detect >0.5% contamination of EBV-infected cells. Arrow, a clonal band. * non-specific band in each digestion. NC: normal control of healthy human lymphocytes, PC: positive control of Raji cell lines.

Figure 3. Quantification of EBV DNA in highly purified cell fractions (>99.9%) obtained from CAEBV patients assessed by real-time PCR. High molecular DNA was extracted from Vδ2, αβT, CD4, CD3, and CD56-expressing cells fractionated from peripheral blood, and $CD34^+$ cells (*hatched bar*) obtained from bone marrow cells. Solid bars represent the major infected subsets with high EBV load (*black bar*), and the minor infected subsets with low EBV load (*gray bar*).

Figure 4. Immunohistochemical staining of bone marrow (BM) cells obtained from Patient 5 and Patient 6. BM clot samples were stained with anti-CD34 antibody using fuchsin as a chromogen for visualization of alkaline phosphatase activity (positive: red cytosol, *open arrow*), and were then employed for *in situ* hybridization of EBER1 using diaminobenzidine as a chromogen of peroxidase (positive: brown nucleus, *closed arrow*). There were no double positive cells for CD34 and EBER1 in BM cells.

Table 1. Clinical profiles of patients with chronic active EBV infection

Patient	1	2	3	4	5	6
Sex	f	f	m	f	m	m
Age (yrs) at onset, at the study	6, 7	8, 25	2, 5	4, 4	1, 10	3, 8
Involvement						
CAL	no	no	no	yes	no	no
Enteral	no	no	no	yes	no	no
HMB	no	yes	no	yes	yes	yes
HV	yes	yes	no	no	yes	yes
Anti EBV-Abs						
VCA-IgG	160	640	1280	640	160	160
-IgM	<10	<10	<10	<10	<10	<10
-IgA	<10	40	20	<10	<10	<10
EADR-IgG	<10	160	640	40	10	10
-IgA	na	na	<10	160	<10	<10
EBNA	40	10	40	40	80	80
EBV-DNA[1)] PB/MNC	100/106	300/104	600/na	3000/na	300/105	2000/na
Major targeted subsets	γδT	CD4	CD4	CD56	CD56	CD56
CD3/CD19/CD56 (%)	70/28/2	75/18/7	69/24/7	82/5/13	47/17/36	58/15/27
CD3+HLA-DR+ (%)	14.4	21.5	15.8	14.2	0.6	5.9
CD4/CD8	1.6	0.8	5.3	2.0	3.3	2.9
Clonality[2)] EBV-TR	M	D	M	D	M	M
TCR	R	R	na	G	G	G
Outcome	AOD	Death post-SCT	ADF post-SCT	ADF post-SCT	AOD	AOD

1) Each value means the copy number of EBV DNA (PB: /ml, MNC: /μgDNA).
2) Clonality was screened by Southern blotting for PB-MNC derived DNA probed with EBV-TR, TCR and Ig genes. There was no evidence of clonally proliferating B cells. CAEBV: chronic active Epstein-Barr virus infection, EBV: Epstein-Barr virus, CAL: coronary artery lesion, CNS: central nervous system, HMB: hypersensitivity to mosquito bite, HV: hydroa vacciniforme, VCA: viral capsid antigen, EBNA: EBV nuclear antigen, PB: peripheral blood, MNC: mononuclear cells, TR: terminal repeat, TCR: T-cell receptor, M: monoclonal, D: diclonal, R: rearrangement, G: germ line, AOD: alive on disease, ADF: alive on disease free state, SCT stem cell transplantation

Table 2

Table 2. Nucleotide Sequence of Vγ9-JγP1Junctional Transcripts Expressed by γδT-Cells

	Vγ9	N region	JγP1
Germline	ATT CCG TCA GCC ****TAC TGT GCC TTG TGG GAG GTG		ACC ACT GGT TGG ATC ← *JγT3 primer*
	Vγ9 primer →		
Pt	TGT GCC TTG TGG GAG GTG	CAG	ACC ACT GGT TGG TTC AAG ATA TTT GCT GAA GGG ACT AAG
	C A L W E V	Q	T T G W

The germline sequences of the 3' end of Vγ9, N region, and the 5' end of JγP1 gene segments are at the top.

Figure 1

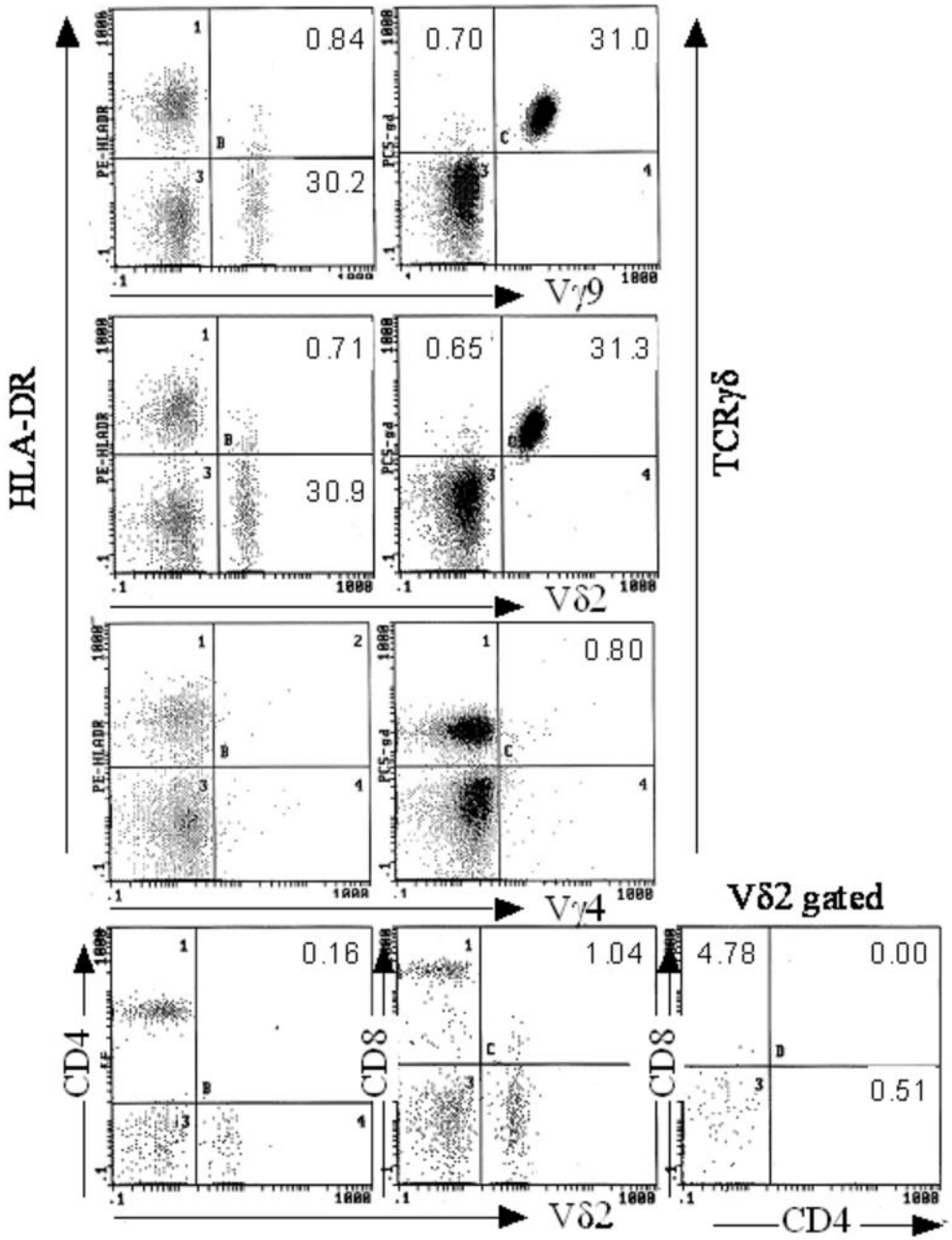

HLA-DR
TCRγδ
0.84
30.2
0.70
31.0
Vγ9
0.71
30.9
0.65
31.3
Vδ2
0.80
Vγ4
Vδ2 gated
0.16
1.04
4.78
0.00
0.51
CD4
CD8
Vδ2
CD4

Figure 2

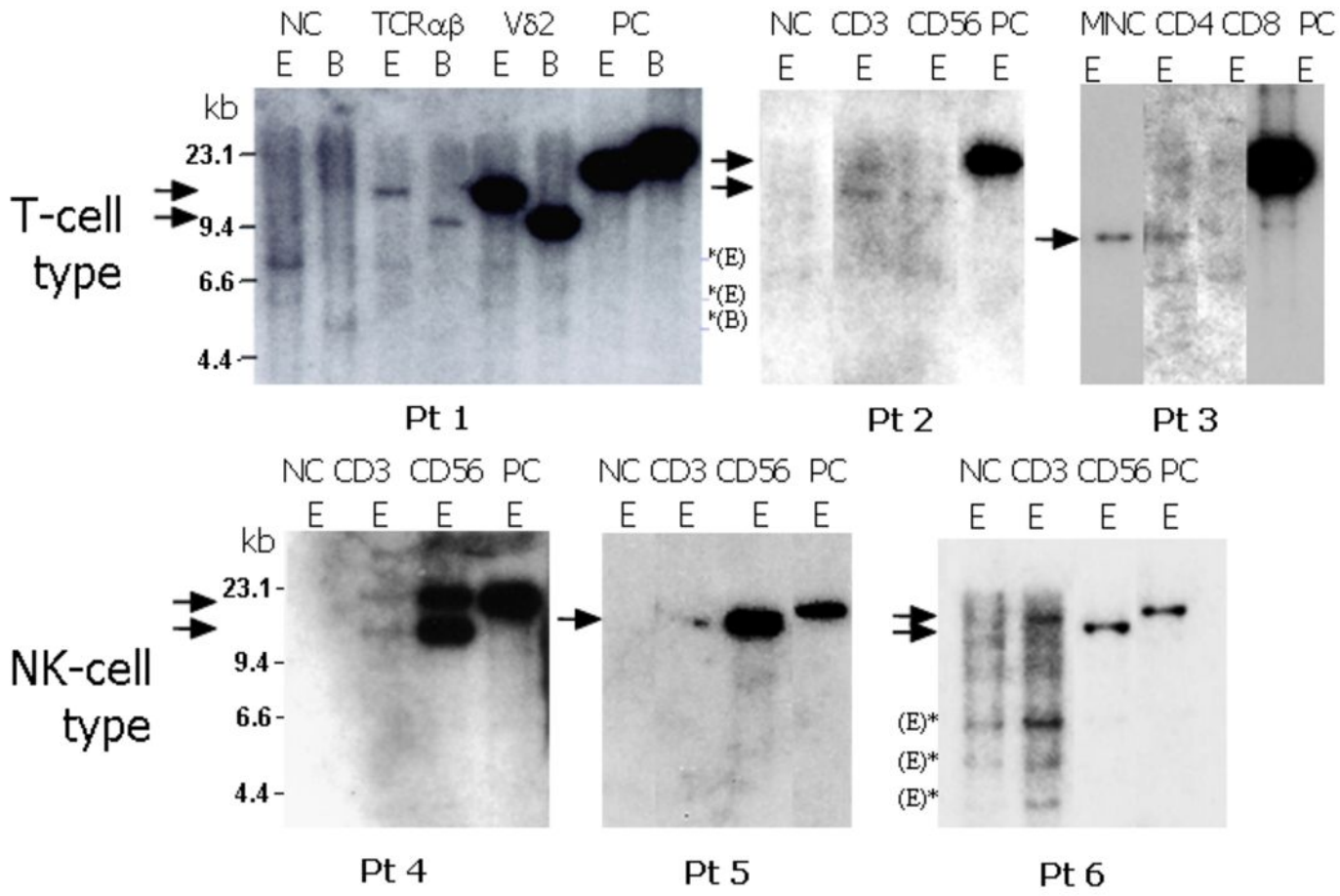

NC TCRαβ Vδ2 PC
E B E B E B E B
kb
23.1
9.4
6.6
4.4
T-cell type
K(E)
K(E)
K(B)
NC CD3 CD56 PC
E E E E
MNC CD4 CD8 PC
E E E E
Pt 1
Pt 2
Pt 3
NC CD3 CD56 PC
E E E E
kb
23.1
9.4
6.6
4.4
NK-cell type
NC CD3 CD56 PC
E E E E
NC CD3 CD56 PC
E E E E
(E)*
(E)*
(E)*
Pt 4
Pt 5
Pt 6

Figure 3

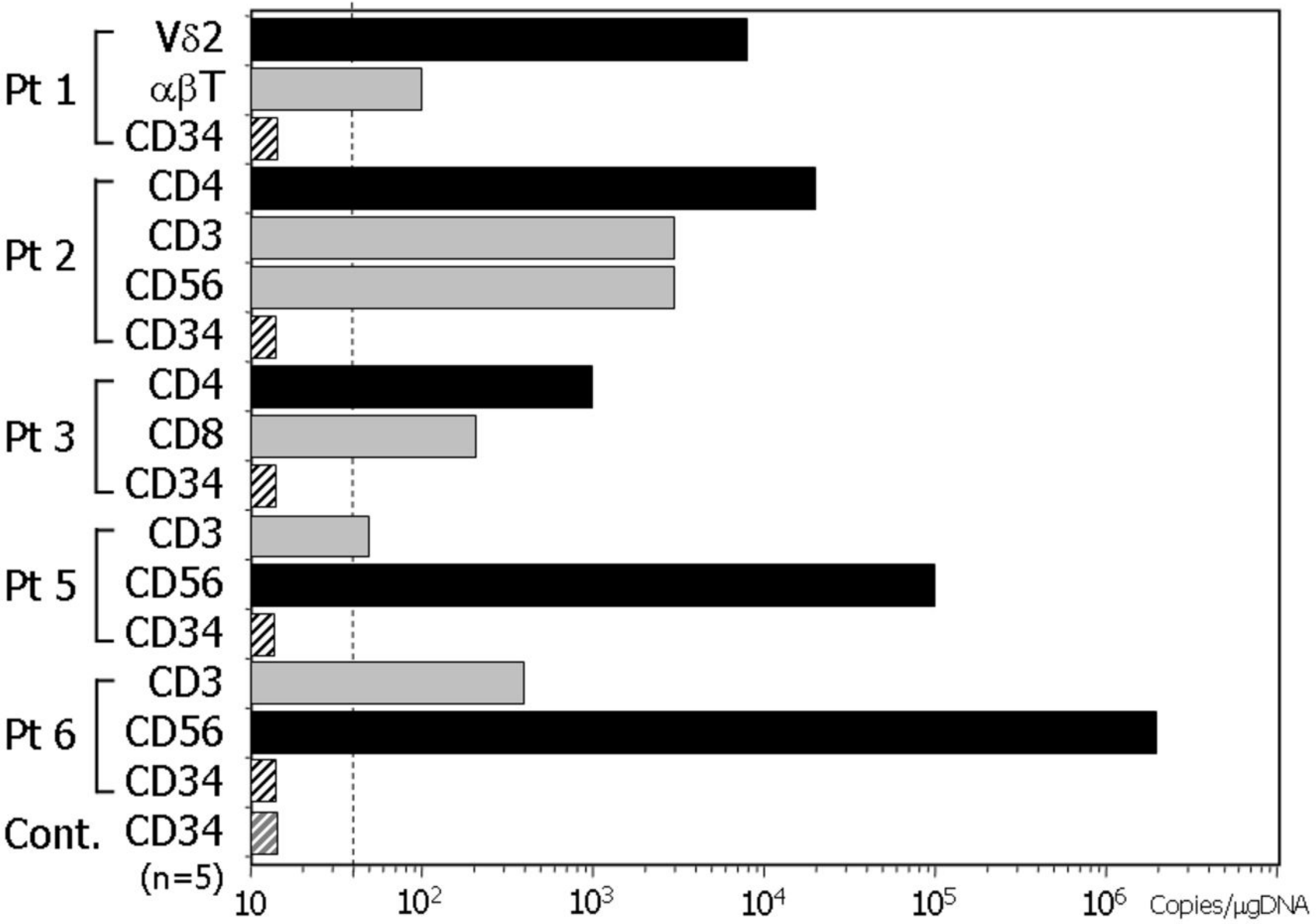
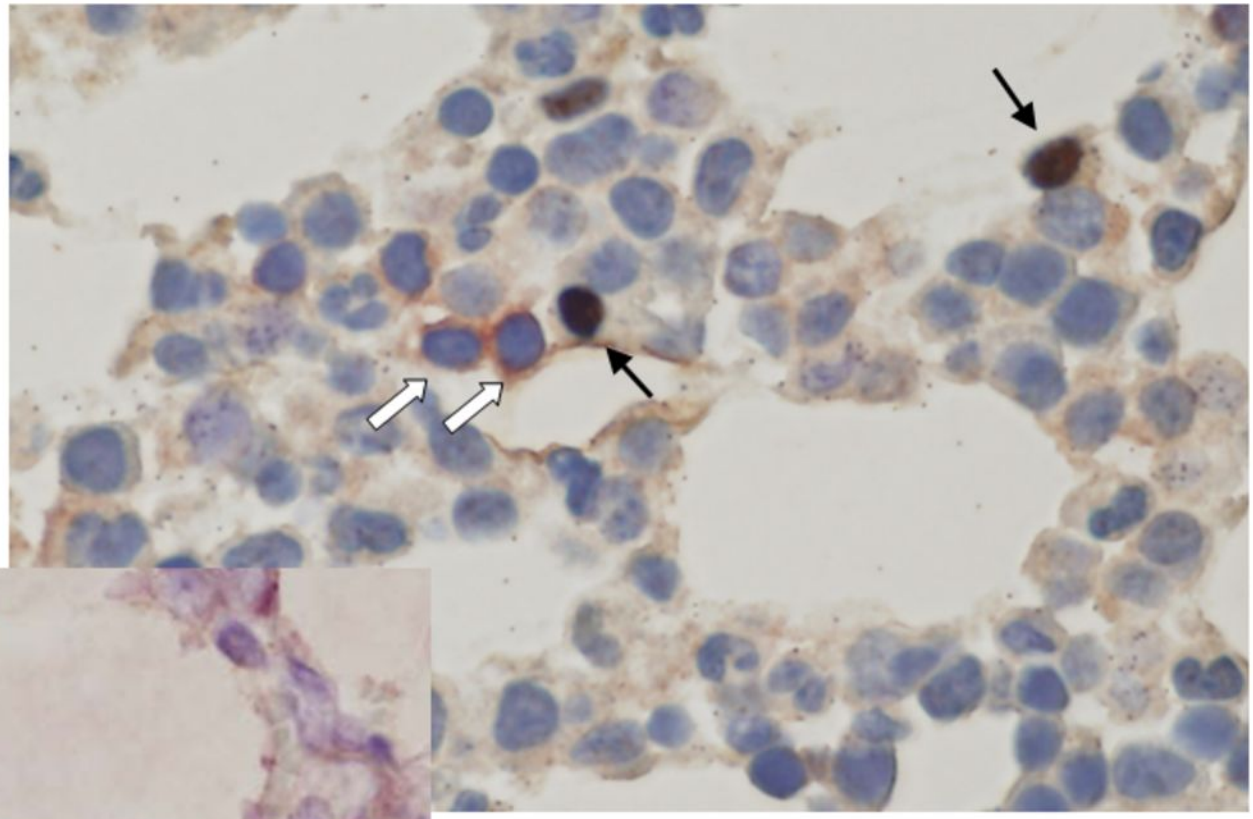
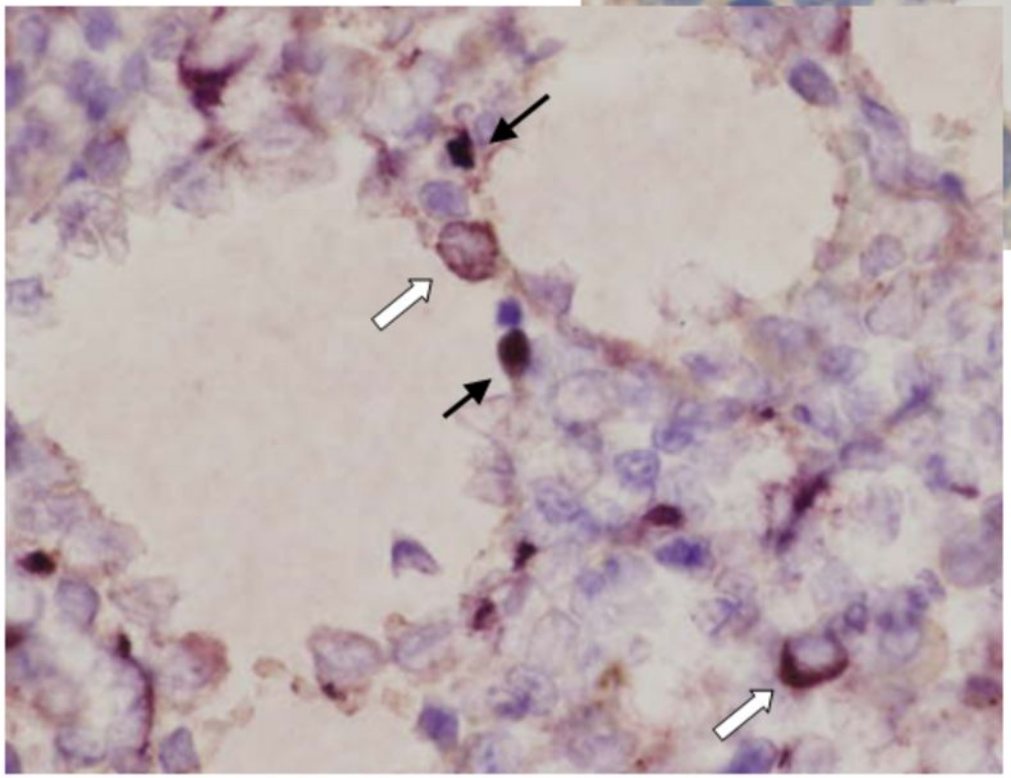

Pt 1
Vδ2
αβT
CD34
Pt 2
CD4
CD3
CD56
CD34
Pt 3
CD4
CD8
CD34
Pt 5
CD3
CD56
CD34
Pt 6
CD3
CD56
CD34
Cont.
CD34
(n=5)
10
10²
10³
10⁴
10⁵
10⁶
Copies/μgDNA

Figure 4



Pt 5: BM
CD4 & EBER



Pt 6: BM
CD34 & EBER