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Translational Relevance

In this study, we showed that the cell adhesion molecule 1 (CAMD1) versus CD7 plot reflects the progression of disease in patients infected with human T-cell lymphotrophic virus type I (HTLV-I), in that the proportion of $CADM1^{+}$ subpopulations (D, $CADM1^{pos}$ $CD7^{dim}$ and N, $CADM1^{pos}CD7^{neg}$) increased with the progression from HTLV-I asymptomatic carrier (AC) to indolent adult T-cell leukemia–lymphoma (ATL) to aggressive ATL. We confirmed the purity of the clonal HTLV-I–infected cells in these subpopulations of various clinical subtypes, including asymptomatic carriers. The results from the flow-cytometric analysis will help physicians assess disease status. The analysis is also practical in screening for putative high-risk HTLV-I asymptomatic carriers, which show nearly identical flow-cytometric and gene expression profiles with those of smoldering-type ATL patients. Furthermore, cell sorting by flow cytometry enables purification of clonally expanding cells in various stages of oncogenesis in the course of progression to aggressive ATL. Detailed molecular analysis of these cells will provide valuable information about the molecular events involved in multistep oncogenesis of ATL.

(indolent ATLs and HTLV-I asymptomatic carriers; AC) revealed that HTLV-I–infected and clonally expanded cells were purified similarly and that the subpopulations with downregulated CD7 grew concomitantly with the progression of HTLV-I infection (15). Although this type of flow-cytometric analysis was shown to be a useful tool, a substantial subpopulation of T cells shows downregulated expression of CD7 under physiologic (16, 17) and certain pathologic conditions, including autoimmune disorders, viral infection, and hematopoietic stem cell transplantation (18–23).

Recently, Sasaki and colleagues reported ectopic overexpression of the cell adhesion molecule 1/tumor suppressor in lung cancer 1 (CADM1/TSLC1) gene in primary acute-type ATL cells based on expression profile analysis (24, 25). CADM1 (/TSLC1) is a cell-adhesion molecule that was originally identified as a tumor suppressor in lung cancers (25, 26). In addition, numbers of $CD4^{+}$ $CADM1^{+}$ cells have been found to be significantly correlated with the proviral load (PVL) in both ATLs and HTLV-I asymptomatic carriers (25, 27). Thus, CADM1 is a good candidate marker of HTLV-I–infected cells. In the present study, we incorporated CADM1 into our flow-cytometric analysis. In the CADM1 versus CD7 plot of $CD4^{+}$ cells, HTLV-I–infected and clonally expanded cells were efficiently enriched in the $CADM1^{+}$ subpopulations regardless of disease status. In these cells, stepwise CD7 downregulation (from dimly positive to negative) occurred with disease progression. The proportion of the three subpopulations observed in this plot [P, $CADM1^{negative(neg)}CD7^{positive(pos)}$; D, $CADM1^{pos}CD7^{dim}$; and N, $CADM1^{pos}$ $CD7^{neg}$] accurately reflected the disease status in HTLV-I infection. The analysis of comprehensive gene expression in each subpopulation revealed that the expression profile of $CADM1^{+}$ subpopulations in indolent ATLs showed similarities with that in asymptomatic carriers with high PVL; yet, it was distinct from that in aggressive ATLs. These D and N subpopulations were indicative of HTLV-I–infected cells in the intermediate stage of ATL development.

Materials and Methods

Cell lines and patient samples

TL-Om1, an HTLV-I–infected cell line (28), was provided by Dr. Sugamura (Tohoku University, Sendai, Japan). The MT-2 cell line was a gift from Dr. Miyoshi (Kochi University, Kochi, Japan) and ST-1 was from Dr. Nagai (Nagasaki University, Nagasaki, Japan). Peripheral blood samples were collected from in-patients and out-patients at our hospital, as described in our previous reports (14, 15). As shown in Supplementary Table S1, 26 cases were analyzed (10 cases of asymptomatic carrier; 5 cases of smoldering-type; 6 cases of chronic-type; and 5 cases of acute-type). All patients with ATL were categorized into clinical subtypes according to Shimoyama's criteria (12, 29). Patients with various complications, such as autoimmune disorders and systemic infections, were excluded. Lymphoma-type patients were also excluded because ATL cells are not considered to exist in the peripheral blood of this clinical subtype. Samples collected from six healthy volunteers (mean age 48.8 years; range 34–66 years) were used as normal controls. The present study was approved by the Institutional Review Board of our institute (the University of Tokyo, Tokyo, Japan). Written informed consent was obtained from all patients and healthy volunteers.

Flow cytometry and cell sorting

Peripheral blood mononuclear cells (PBMC) were isolated from whole blood by density gradient centrifugation, as described previously (14). An unlabeled CADM1 antibody (clone 3E1) and an isotype control chicken immunoglobulin Y (IgY) antibody were purchased from MBL. These were biotinylated (primary amine biotinylation) using biotin N-hydroxysuccinimide ester (Sigma-Aldrich). Pacific Orange-conjugated anti-CD14 antibody was purchased from Caltag-Invitrogen. All other antibodies were obtained from BioLegend. Cells were stained using a combination of biotin-CADM1, allophycocyanin (APC)-CD7, APC-Cy7-CD3, Pacific Blue-CD4, and Pacific Orange-CD14. After washing, phycoerythrin-conjugated streptavidin was applied. Propidium iodide (Sigma-Aldrich) was added to the samples to stain dead cells immediately before flow cytometry. A FACSAria instrument (BD Immunocytometry Systems) was used for all multicolor flow cytometry and fluorescence-activated cell sorting (FACS). Data were analyzed using FlowJo software (TreeStar). The gating

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procedure for a representative case is shown in Supplementary Fig. S1.

Quantification of HTLV-I proviral load by real-time quantitative PCR

PVL in FACS-sorted PBMCs was quantified by real-time quantitative PCR (TaqMan method) using the ABI Prism 7000 sequence detection system (Applied Biosystems), as described previously (14, 30).

Evaluation of HTLV-I *HBZ* gene amplification by semiquantitative PCR

HTLV-I *HBZ* gene amplification was performed as described previously (25). Briefly, the 25-μL PCR mixture consisted of 20 pmol of each primer, 2.0 μL of mixed deoxynucleotide triphosphates (2.5 mmol/L each), 2.5 μL of 10× PCR buffer, 1.5 μL of $MgCl_2$ (25 mmol/L), 0.1 μL of AmpliTaq Gold DNA Polymerase (Applied Biosystems), and 20 ng of DNA extracted from cell lines and clinical samples. The PCR consisted of initial denaturation at 94°C for 9 minutes, 30 cycles of 94°C for 30 seconds, 57°C for 30 seconds, and 72°C for 45 seconds, followed by 72°C for 5 minutes. The β-actin gene (*ACTB*) was used as an internal reference control. The primer sequences used were as follows: HBZ forward, 5′-CGCTGCCGATCACGATG-3′; HBZ reverse, 5′-GGAGGAATTGGTGGACG-3′; ACTB forward, 5′-CGTGCTCAGGGCTTCTT-3′; and ACTB reverse, 5′-TGAAGGTCTCAAACATGATCTG-3′. Amplification with these pairs of oligonucleotides yielded 177-bp HBZ and 731-bp β-actin fragments.

FISH for quantification of HTLV-I–infected cells

FISH analysis was performed to detect HTLV-I proviral DNA in mononuclear cells that had been FACS-sorted on the basis of the CADM1 versus CD7 plot. These samples were sent to a commercial laboratory (Chromosome Science Labo Inc.), where FISH analysis was performed. Briefly, pUC/HTLV-I plasmid containing the whole-HTLV-I genome was labeled with digoxigenin by the nick translation method, and was then used as a FISH probe. Pretreatment, hybridization, and washing were performed according to standard laboratory protocols. To remove fluorochrome-labeled antibodies attached to the cell surface, pretreatment consisted of treatment with 0.005% pepsin and 0.1 N HCl. The FISH probe was detected with Cy3-labeled anti-digoxigenin antibody. Cells were counterstained with 4′, 6 diamidino-2-phenylindole. The results were visualized using a DMRA2 conventional fluorescence microscope (Leica) and photographed using a Leica CW4000 cytogenetics workstation. Hybridization signals were evaluated in approximately 100 nuclei.

Inverse long PCR to assess the clonality of HTLV-I–infected cells

For clonality analysis, inverse long PCR was performed as described previously (14). First, 1 μg genomic DNA extracted from the FACS-sorted cells was digested with *Pst*I or *Eco*RI at 37°C overnight. RNase A (Qiagen) was added to remove residual RNA completely. DNA fragments were purified using a QIAEX2 Gel Extraction Kit (Qiagen). The purified DNA was self-ligated with T4 DNA ligase (Takara Bio) at 16°C overnight. After ligation of the *Eco*RI-digested samples, the ligated DNA was further digested with *Mlu*I, which cuts the pX region of the HTLV-I genome and prevents amplification of the viral genome. Inverse long PCR was performed using Tks Gflex DNA Polymerase (Takara Bio). For the *Pst*I-treated group, the forward primer was 5′-CAGCCCATTCTATAGCACTCTCCAGGAGAG-3′ and the reverse primer was 5′-CAGTCTCCAAACACGTAGACTGGGTATCCG-3′. For the *Eco*RI-treated template, the forward primer was 5′-TGCCTGACCCTGCTTGCTCAACTCTACGTCTTTG-3′ and the reverse primer was 5′-AGTCTGGGCCCTGACCTTTTCAGACTTCTGTTTC-3′. Processed genomic DNA (50 ng) was used as a template. The reaction mixture was subjected to 35 cycles of denaturation (94°C, 30 seconds) and annealing plus extension (68°C, 8 minutes). Following PCR, the products were subjected to electrophoresis on 0.8% agarose gels. Fourteen patient samples were analyzed. For samples from which a sufficient amount of DNA was extracted, PCR was generally performed in duplicate.

Gene expression microarray analysis of each subpopulation in the CADM1 versus CD7 plot

Total RNA was extracted from each subpopulation in the CADM1 versus CD7 plot using TRIzol (Invitrogen) according to the manufacturer's protocol. Details of the clinical samples used for microarray analyses are shown in Supplementary Table S1. Treatment with DNase I (Takara Bio) was conducted to eliminate genomic DNA contamination. The quality of the extracted RNA was assessed using a BioAnalyzer 2000 system (Agilent Technologies). The RNA was then Cy3-labeled using a Low Input Quick Amp Labeling Kit (Agilent Technologies). Labeled cRNA samples were hybridized to 44K Whole Human Genome Oligonucleotide Microarrays (Agilent Technologies) at 65°C for 17 hours. After hybridization, the microarrays were washed and scanned with a Scanner C (Agilent Technologies). Signal intensities were evaluated by Feature Extraction 10.7 software and then analyzed using Gene Spring 12.0 software (Agilent Technologies). Unsupervised two-dimensional hierarchical clustering analysis (Pearson correlation) was performed on 10,278 genes selected by one-way ANOVA ($P < 0.05$). The dataset for these DNA microarrays has been deposited in Gene Expression Omnibus (accession number GSE55851).

Expression analysis of miR-31 and *Helios* transcript variants of each subpopulation in the CADM1 versus CD7 plot

The expression levels of the microRNA miR-31 were quantified using a TaqMan-based MicroRNA Assay (Applied Biosystems), as described previously (31), and normalized to RNU48 expression level. Helios mRNA transcript variants were examined using reverse transcription

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PCR (RT-PCR) with Platinum Taq DNA Polymerase High Fidelity (Invitrogen), as described previously (32). To detect and distinguish alternative splicing variants, PCR analyses were performed with sense and antisense primer sets specific for the first and final exons of the Helios gene. The PCR products were then sequenced to determine the exact type of transcript variant. A mixture of Hel-1, Hel-2, Hel-5, and Hel-6 cDNA fragments was used as a "Helios standard" in the electrophoresis of RT-PCR samples.

Results

CADM1 expression based on the CD3 versus CD7 plot in CD4$^+$ cells in primary HTLV-I–infected blood samples

The clinical profiles of the 32 cases analyzed are shown in Supplementary Table S1. We first examined CADM1 expression in each subpopulation (H, I, and L) of the CD3 versus CD7 plot. Representative data (for a case of smoldering ATL) are shown in Fig. 1A. The results demonstrate that

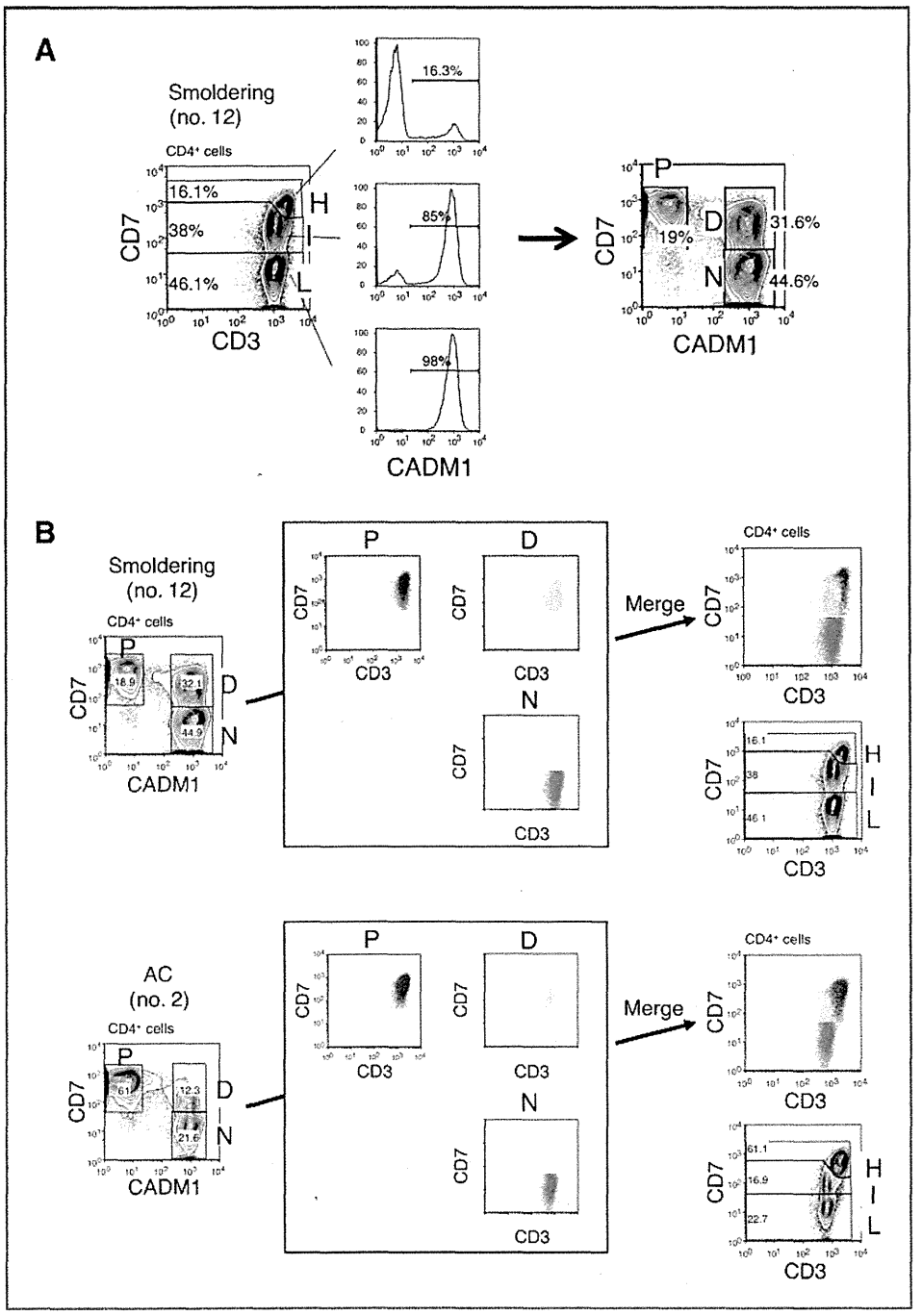

Figure 1. CADM1 versus CD7 plot for CD4$^+$ cells from HTLV-I–infected blood samples analyzed by flow cytometry. A, representative flow-cytometric analysis of a patient with smoldering-type ATL. Three subpopulations (H, I, and L) were observed in the CD3 versus CD7 plot for CD4$^+$ cells (left). Expression of CADM1 in each subpopulation is shown (middle). The right-hand panel shows how the CADM1 versus CD7 plot for CD4$^+$ cells was constructed. B, the P, D, and N subpopulations in the CADM1 versus CD7 plot correspond to the H, I, and L subpopulations in the CD3 versus CD7 plot. Blue, yellow, and red dots, respectively, indicate the P, D, and N subpopulations in the CADM1 versus CD7 plot, and are redrawn in the CD3 versus CD7 plot. Two representative cases are shown. In the upper case, the P and D subpopulations in the CADM1 versus CD7 plot are partly intermingled in the CD3 versus CD7 plot. Unlike the CD3 versus CD7 plot, the CADM1 versus CD7 plot clearly distinguishes three subpopulations.

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CADM1 was expressed almost exclusively in the I and L subpopulations. Drawing a CADM1 versus CD7 plot for $CD4^+$ cells revealed three distinct subpopulations (P, $CADM1^{neg}CD7^{pos}$; D, $CADM1^{pos}CD7^{dim}$; and N, $CADM1^{pos}$ $CD7^{neg}$). As shown in Fig. 1B, the P, D, and N subpopulations corresponded to the H, I, and L subpopulations in the CD3 versus CD7 plot. In the previous CD3 versus CD7 plot, the lower case (AC no. 2) showed three distinct subpopulations. However, in the upper case (smoldering no. 12), the H and I subpopulations substantially intermingled with each other and were not clearly separated. In contrast, the CADM1 versus CD7 plot clearly revealed three distinct subpopulations in both cases.

HTLV-I–infected cells are highly enriched in $CADM1^+$ subpopulations

On the basis of previous reports (25, 27), we expected HTLV-I–infected cells to be enriched in the $CADM1^+$ subpopulations in our analysis. Figure 2A shows the PVL measurements of the three subpopulations in the CADM1 versus CD7 plot for three representative cases. HTLV-I–infected cells were highly enriched in the $CADM1^+$ subpopulations (D and N). The PVL data indicate that most of the cells in the D and N subpopulations were HTLV-I infected. Figure 2B shows the results of semiquantitative PCR of the *HBZ* gene in representative cases. In the D and N subpopulations, the *HBZ* gene was amplified to the same degree as in the HTLV-I–positive cell line. To confirm these results, FISH was performed in one asymptomatic carrier. As shown in Supplementary Fig. S2, HTLV-I–infected cells were highly enriched in the D and N subpopulations, which supports the results of the PVL analysis and semiquantitative PCR of the *HBZ* gene. In the FISH analysis, percentages of HTLV-I–infected cells in D and N did not reach 100%. This may have been due to a technical issue. Because the cells subjected to FISH analysis were sorted by FACS, several fluorochrome-conjugated antibodies may have remained on their surfaces, even after treatment with protease.

The CADM1 versus CD7 plot accurately reflects disease progression in HTLV-I infection

Compared with the CD3 versus CD7 plot, the CADM1 versus CD7 plot was revealed to be clear in its distinction of the three subpopulations and efficient in enrichment of HTLV-I–infected cells. On the basis of these findings, we analyzed clinical samples of asymptomatic carriers and three clinical subtypes of ATL: the smoldering, chronic, and acute subtypes. Data for representative cases, presented in Fig. 3A, suggest that the continual changes in the proportions of the three subpopulations are associated with disease progression. In the CADM1 versus CD7 plot, normal control samples showed a P-dominant pattern. With progression of the disease from the asymptomatic carrier state with a low PVL to that with a high PVL, and to indolent-type ATL, the D and N subpopulations increased gradually. As the disease further progressed to acute-type ATL, the N subpopulation showed remarkable expansion. Data for all analyzed samples are presented in Fig. 3B. The results suggest that the CADM1 versus CD7 plot of peripheral blood samples represents progression of the disease in HTLV-I carriers. Data for the normal control cases analyzed are shown in Supplementary Fig. S3. In all normal controls, the percentages of the D and N subpopulations were low. Supplementary Fig. S4 shows temporal data for a patient with chronic-type ATL who progressed from stable disease to a relatively progressive state and the concomitant change in the flow cytometry profile.

Clonality analysis of the three subpopulations in the CADM1 versus CD7 plot

To characterize the three subpopulations further, the clonal composition of each subpopulation was analyzed by inverse long PCR, which amplifies part of the provirus

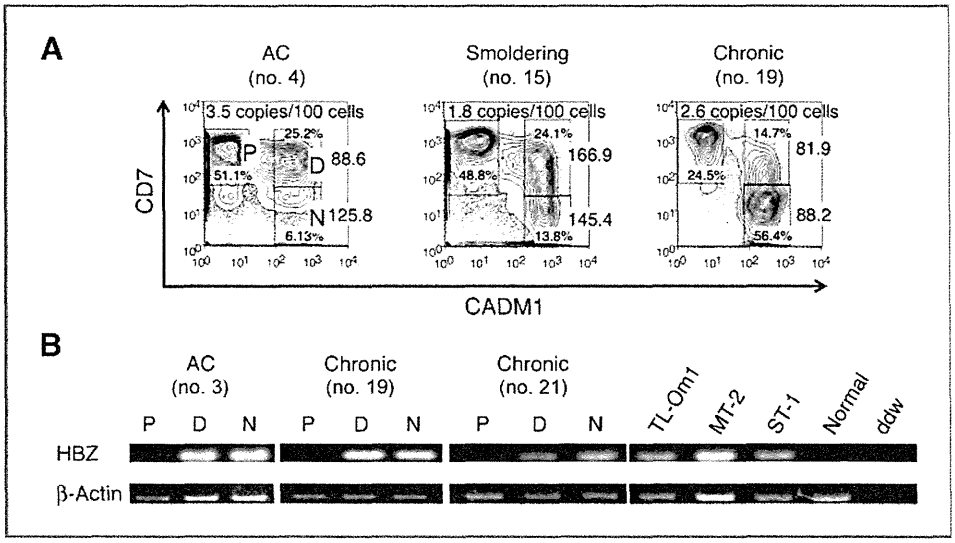

Figure 2. HTLV-I–infected cells are highly enriched in the $CADM1^+$ subpopulations. A, analysis of PVL in the three subpopulations. Three representative cases are shown. PVL data (copies/100 cells) are shown in red. Percentages of each subpopulation are shown in black. B, semiquantitative PCR of the *HBZ* gene in the three subpopulations in three representative cases. Normal, DNA from PBMCs from a normal control; ddw, deionized distilled water.

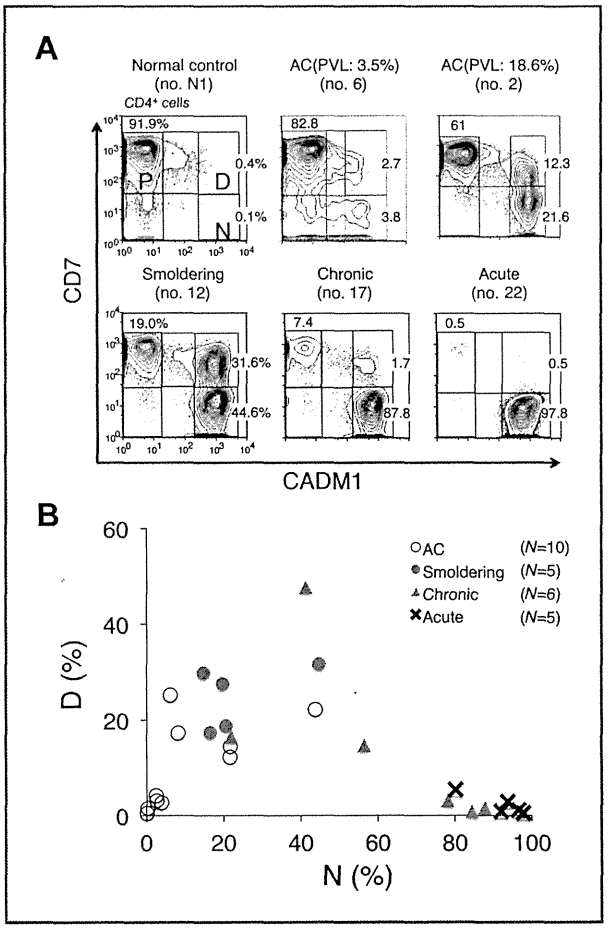

Figure 3. Proportion of each subpopulation in the CADM1 versus CD7 plots for asymptomatic HTLV-I carriers (asymptomatic carriers) and ATLs of various clinical subtypes. A, data of representative cases are shown. B, a two-dimensional plot of all analyzed samples showing the percentages of the D and N subpopulations.

long terminal repeat and the flanking genomic sequence of the integration sites. Cells in each subpopulation were sorted by FACS, and subjected to inverse long PCR analysis. Representative results for smoldering-, chronic-, and acute-type ATL samples are presented in Fig. 4A. Major clones, indicated by intense bands, were detected in the D and N subpopulations. The major clones in the D and N subpopulations in each case were considered to be the same based on the sizes of the amplified bands, suggesting that clonal evolution is accompanied by downregulation of CD7 expression. Fig. 4B shows representative results for three cases of asymptomatic carrier. In all cases, weak bands in the P subpopulation were visible, indicating that this population contains only minor clones. In these asymptomatic carriers, the proportion of abnormal lymphocytes and PVL increases from left to right. The consistent increase in the D and N subpopulations, together with growth of major clones as shown in the inverse PCR analysis, were considered to reflect these clinical data.

Gene expression profiling of the three subpopulations in the CADM1 versus CD7 plot

To determine the molecular basis for the biologic differences among the three subpopulations in the CADM1 versus CD7 plot, we next characterized the gene-expression profiles of the subpopulations of the following clinical subgroups: asymptomatic carriers ($n = 2$), smoldering-type ATLs ($n = 2$), chronic-type ATL ($n = 1$), acute-type ATLs ($n = 3$), and normal controls ($n = 3$). The two asymptomatic carriers (nos. 5 and 9) had high PVLs (11.6 and 26.2%, respectively) and relatively high proportions of D and N subpopulations (Supplementary Table S1). Unsupervised hierarchical clustering analysis of the results revealed three clusters (A, B1, and B2) or two major clusters A and B, where A is composed solely of the samples of the acute-type N subpopulation and B is subdivided into two clusters (B1 and B2; Fig. 5A). The B2 cluster is composed of the P subpopulation of all clinical subtypes and of normal controls, whereas the B1 cluster is composed of the D and N subpopulations of

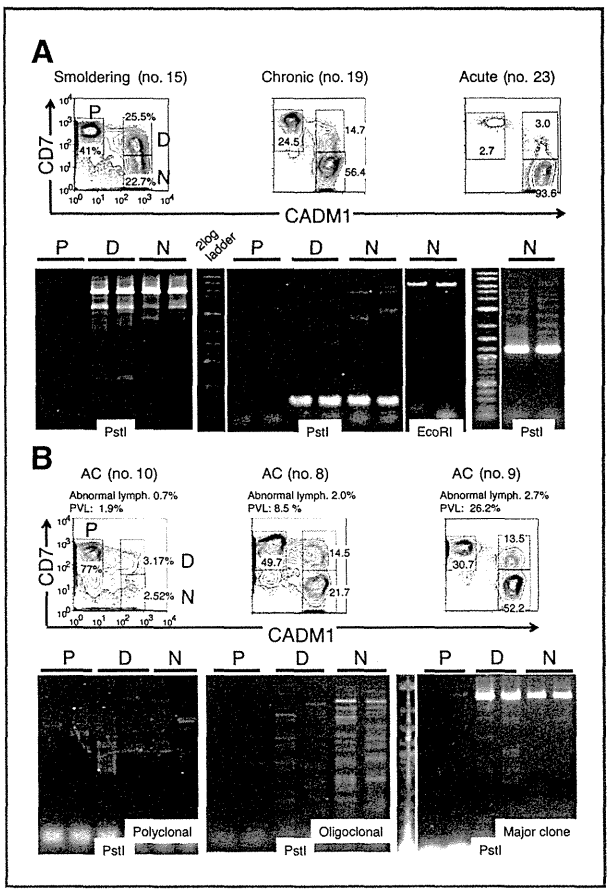

Figure 4. Clonality of subpopulations in the CADM1 versus CD7 plot analyzed by inverse long PCR. FACS-sorted cells (P, D, and N) were subjected to inverse long PCR. The black bar indicates duplicate data. Flow-cytometric profiles and clinical data are also presented. A, representative cases of smoldering-, chronic-, and acute-type ATL are shown. B, representative cases of asymptomatic carriers are shown.

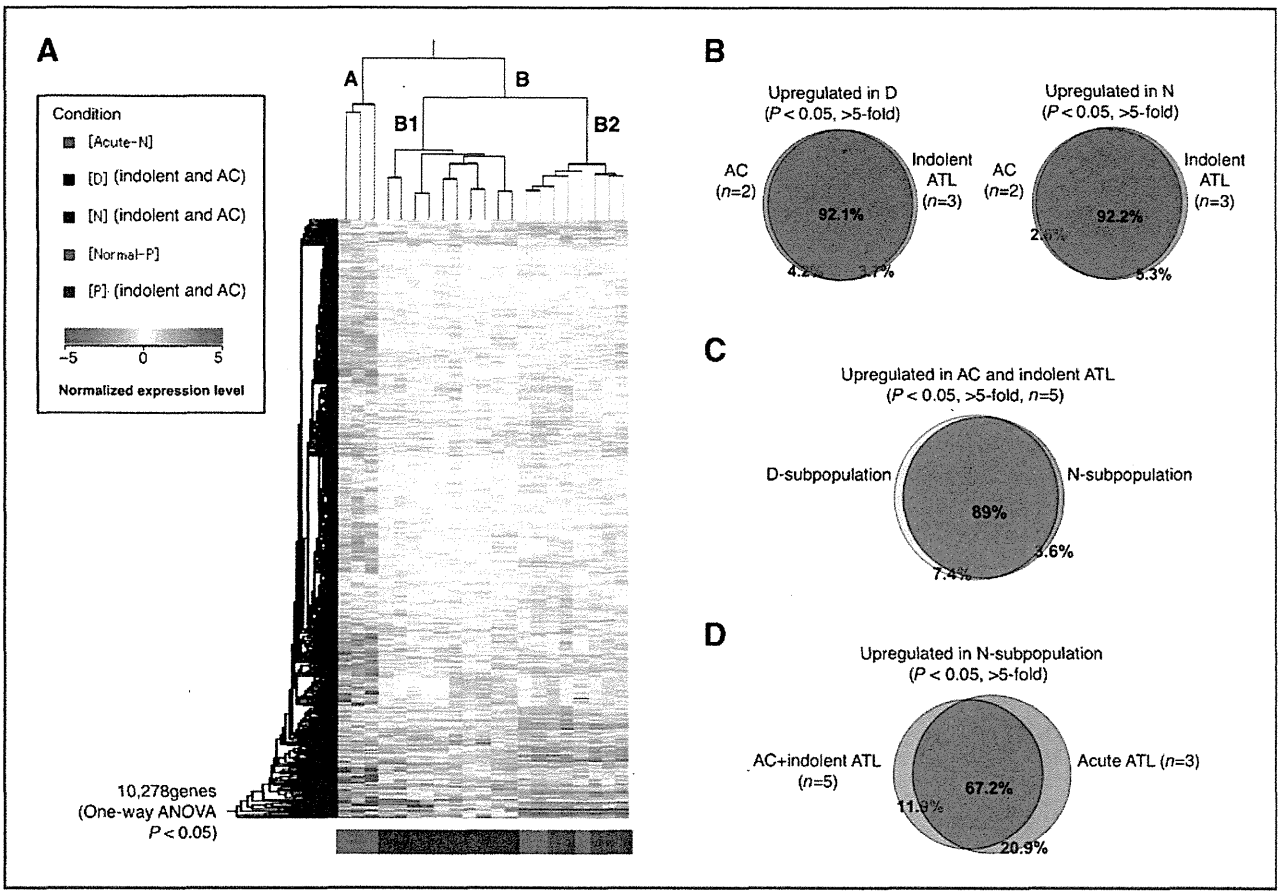

Figure 5. Comprehensive gene expression analysis of the three subpopulations in the CADM1 versus CD7 plot. A, we conducted an unsupervised hierarchical clustering analysis of 10,278 genes whose expression levels were significantly changed in the P subpopulation of normal controls ($n = 3$); P, D, and N subpopulations of asymptomatic carriers and indolent ATLs ($n = 5$); and N subpopulation of acute-ATLs ($n = 3$; one-way ANOVA, $P < 0.05$). The P and D subpopulations of acute ATLs and D and N subpopulations of normal controls could not be analyzed because of insufficient numbers of cells. Clustering resulted in three major clusters: (i) P subpopulations of normal controls (gray) and asymptomatic carriers/indolent ATLs (green); (ii) D and N subpopulations of asymptomatic carriers/indolent ATLs (blue and brown, respectively); and (iii) N subpopulations of acute ATLs (red). These results indicate that the P subpopulations of asymptomatic carriers/indolent ATLs have characteristics similar to those of normal uninfected cells, whereas the D and N subpopulations of asymptomatic carriers/indolent ATLs have genetic lesions in common. The N subpopulations of acute ATLs are grouped in an independent cluster, meaning that these malignant cell populations have a significantly different gene expression profile, even compared with the N subpopulations of indolent ATLs. B, similarity between asymptomatic carriers and indolent ATLs. The Venn diagrams show that 92.1% and 92.2% of genes upregulated in the D and N subpopulations, respectively, compared with "Normal-P" ($P < 0.05$), were common to asymptomatic carriers ($n = 2$) and indolent ATLs ($n = 3$). C, similarity between the D and N subpopulations. The Venn diagram shows that 89% of genes upregulated in the D and N subpopulation, compared with Normal-P ($P < 0.05$), overlapped. D, comparison of the N subgroups between acute-ATLs ($n = 3$) and asymptomatic carriers/indolent ATLs ($n = 5$). As shown in the Venn diagram, 67.2% of genes were upregulated ($P < 0.05$) in the N subpopulations of both acute ATLs and asymptomatic carrier/indolent ATLs. However, a significant number of genes (20.9%) were upregulated only in the N subpopulation of acute ATLs.

asymptomatic carriers and indolent ATLs (smoldering- and chronic-type).

Figure 5B shows a Venn diagram of the upregulated genes in the D subpopulation (left) or the N subpopulation (right) common to asymptomatic carriers ($n = 2$) and indolent ATLs ($n = 3$). These diagrams demonstrate that the changes in the gene expression profiles of the D and N subpopulations of asymptomatic carriers were similar to those of indolent ATLs. Furthermore, the gene expression profiles of the D and N subpopulations of asymptomatic carriers and indolent ATLs were similar (Fig. 5C). In contrast, the upregulated genes showed distinct differences between the N subpopulation of acute-type ATL and that of indolent ATLs and asymptomatic carriers, although approximately 70% were common to both (Fig. 5D).

Expression of a tumor suppressor microRNA and splicing abnormalities of Ikaros family genes in the three subpopulations

To determine whether the novel subpopulations identified had other properties in common with ATL cells, we examined miR-31 levels and *Helios* mRNA patterns in sorted subpopulations (31, 32). Expression of miR-31 decreased drastically in the D subpopulation derived from indolent ATLs and asymptomatic carriers, and was

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even lower in the N subpopulation derived from asymptomatic carriers and indolent/acute ATLs (Fig. 6A). In addition, examination of *Helios* mRNA transcript variants revealed that expression levels of *Hel-2*, which lacks part of exon 3, were upregulated in the D and N subpopulations of asymptomatic carriers and indolent ATLs, and it was dominantly expressed in the N subpopulation of acute ATLs (Fig. 6B).

Supplementary Fig. S5 presents a summary of this study. The representative flow-cytometric profile shows how the CADM1 versus CD7 plot reflects disease progression in HTLV-I infection. The plot together with the gene expression profiles clearly distinguished the subpopulations of distinct oncogenic stages. The groups classified according to gene expression profile are shown as blue, yellow, and red and are superimposed on the CADM1 versus CD7 plot. Collectively, our data suggest that CADM1 expression and stepwise downregulation of CD7 were closely associated with clonal expansion of HTLV-I–infected cells in ATL progression.

Discussion

We showed that the CADM1 versus CD7 plot is capable of discriminating clonally expanding HTLV-I–infected cells in indolent ATLs and even in asymptomatic carriers, as well as in acute-type ATLs. Our analysis demonstrated efficient enrichment of HTLV-I–infected cells in the $CADM^+$ subpopulations (D and N in the CADM1 vs. CD7 plot), based on the results of real-time PCR (PVL analysis), semiquantitative PCR analysis of the *HBZ* gene, and FISH analysis (Fig. 2 and Supplementary Fig. S2). Furthermore, the CADM1 versus CD7 plot was shown to discriminate the three subpopulations more clearly than the CD3 versus CD7 plot (Fig. 1). Clonality analysis of ATLs and asymptomatic carriers (Fig. 4A and B) revealed that $CADM1^+$ subpopulations (D and N) contained

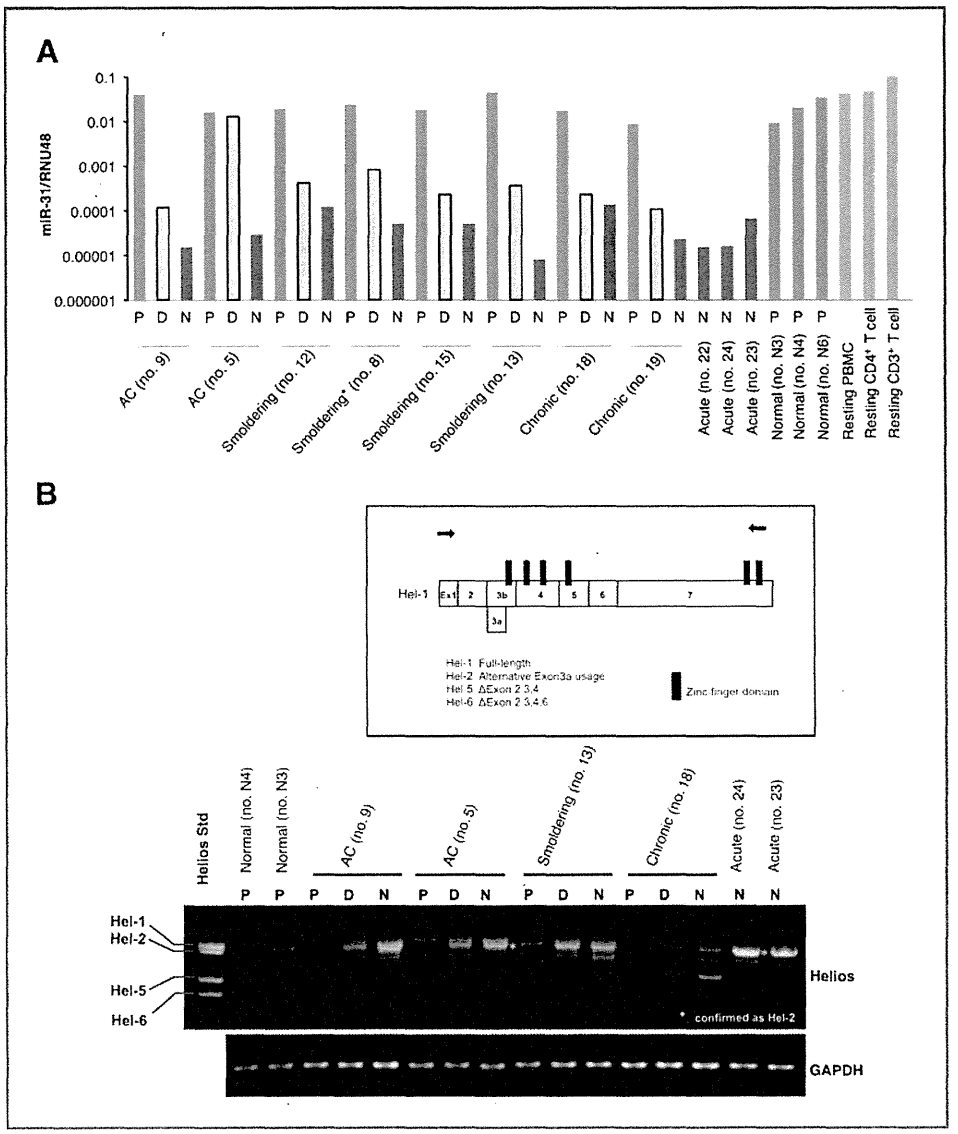

Figure 6. Gene expression pattern in the CADM1/CD7 subpopulation. A, miR-31 expression levels quantified by TaqMan-based real-time PCR. Total RNAs derived from each subpopulation were isolated and analyzed by RT-real-time PCR. RNU48 levels were also measured as an internal normalizer. *Smoldering (no. 8), this patient was considered to be at the asymptomatic carrier/smoldering borderline, because the proportion of abnormal lymphocytes fluctuated around 5%. On the day of sampling, the patient's hemogram showed 6.5% abnormal lymphocytes. B, expression analysis of *Helios* transcript variants in the subpopulations of normal controls ($n = 2$), asymptomatic carriers ($n = 2$), and ATLs (smoldering-type ATL, $n = 1$; chronic-type ATL, $n = 1$; acute-type ATL, $n = 2$). Comparisons of transcript variants among the P, D, and N subpopulations were performed by RT-PCR using primer sets specific for full-length *Helios* cDNA (top). The primer locations for *Helios* PCR are indicated by arrows in the schematic representation of *Hel-1*. The amplified cDNA (asterisk) was confirmed to be the *Hel-2* variant. The *Helios* standard (left lane), a mixture of cDNA fragments of *Hel-1*, *Hel-2*, *Hel-5*, and *Hel-6*, was used as a size indicator for each transcript variant. The glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) mRNA was analyzed as an internal control (bottom).

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clonally expanded HTLV-I–infected cells, whereas cells in the P subpopulation ($CADM1^-$) did not show clonal expansion in this analysis. Current molecular analyses of ATL cells have been limited to HTLV-I–infected cell lines and primary cells from acute/lymphoma type ATL, because in these cases, the predominant expanding clones are readily available with relatively high purity. However, the separation of clonally expanding ATL cells from indolent ATLs and asymptomatic carriers has not yet been achieved. The CADM1 versus CD7 plot from FACS allows efficient purification of such clones *in vitro*.

In an unsupervised clustering analysis of the gene expression data, the D and N subpopulations of asymptomatic carriers/indolent ATLs were grouped together, suggesting that the biologic characteristics of these subpopulations are similar (Fig. 5A and B) but distinct from the N subpopulation of acute-type ATLs (Fig. 5D). These results support the notion that in indolent ATLs and even in asymptomatic carriers, the D and N subpopulations are clonally expanding cells representing the intermediate oncogenic stage. Although the D and N subpopulations have similar gene expression profiles (Fig. 5C), there are potentially important differences distinguishing these subpopulations, according to the apparent decrease in the D subpopulation and increase in the N subpopulation that were observed as the disease progressed from indolent to acute-type ATL (Fig. 3). Detailed analysis of the genomic and epigenomic differences between these two subpopulations will provide us with information about the genomic and epigenomic lesions that are involved in disease progression. Another important finding is that the expression profiles of cells in the N subpopulation of indolent and acute-type ATLs showed significant differences, even though the majority of the genes were common to both groups (Fig. 5D). Characterization of the genes that show distinct expression patterns will reveal the molecular events that contribute to the progression from indolent to aggressive ATLs.

To address whether the emerging molecular hallmark of ATL was conserved in the novel subpopulations identified, we examined the miR-31 level and *Helios* mRNA pattern in sorted subpopulations (Fig. 6). Through integrative analyses of ATL cells, we recently showed that the expression of miR-31, which negatively regulates noncanonical NF-κB signaling by targeting NIK, is genetically and epigenetically suppressed in ATL cells, leading to persistent NF-κB activation, and is thus inversely correlated with the malignancy of the cells (31). The miR-31 levels in the P subpopulations in asymptomatic carriers and indolent ATLs were as high as those in normal P subpopulations, PBMCs, and resting T cells, whereas those in the D subpopulations decreased significantly and those in the N subpopulations were as low as in acute-type N subpopulations (Fig. 6A). Previously, we also identified ATL-specific aberrant splicing of *Helios* mRNA and demonstrated its functional involvement in ATL (32). As shown in Fig. 6B, the *Hel-2* type variant, which lacks part of exon 3 and thus lacks one of the four DNA-binding zinc-finger domains, accumulated in the D and N subpopulations of asymptomatic carriers and indolent ATLs, and was dominantly expressed in the N subpopulation of acute-type ATLs. Collectively, the molecular abnormality of ATL cells became evident in the gradual progression from P to D to N, even in asymptomatic carriers, strongly supporting the notion that the CADM1/CD7 expression pattern correlates with the multistep oncogenesis of ATL.

One of the more remarkable findings in the expression profile analysis was that the D and N subpopulations of asymptomatic carriers clustered within the same group as those of the indolent ATL cases (Fig. 5A and B). The asymptomatic carriers used in this analysis had high PVLs and relatively high proportions of the D and N subpopulations (Supplementary Table S1). In addition, mono- or oligoclonal expansion of the HTLV-I–infected cells was demonstrated in these cases. HTLV-I–infected cells in the D and N subpopulations of these asymptomatic carriers comprise clonally expanding cells that are potentially at the premalignant and intermediate stages according to their clonality, comprehensive gene expression profile, miR31 expression, and aberrant RNA splicing, all indicating that they can be categorized as asymptomatic carriers with high risk of developing into ATL, requiring careful follow-up (15, 30, 33, 34). Our flow-cytometric analysis of PBMCs from asymptomatic carriers using the CADM1 versus CD7 plot may provide a powerful tool for identifying high-risk asymptomatic carriers. Certain indolent ATL cases are difficult to distinguish from asymptomatic carriers, according to Shimoyama's criteria based on the morphologic characteristics determined by microscopic examination. Characterization of peripheral blood T cells by the CADM1 versus CD7 plot may provide useful information for clinical diagnosis.

According to Masuda and colleagues, manipulation of *CADM1* gene expression in leukemic cell lines suggested that CADM1 expression confers upon ATL cells tissue invasiveness and a growth advantage (35). The mechanism by which HTLV-I infection regulates CADM1 expression and the significance of CADM1 expression in ATL oncogenesis will require clarification by future studies.

Finally, as summarized in Supplementary Fig. S5, we demonstrated that (1) HTLV-I–infected and clonally expanded cells are efficiently enriched in $CADM1^+$ subpopulations; (2) the proportions of the three subpopulations in the CADM1 versus CD7 plot, discriminated by CADM1 expression and stepwise downregulation of CD7, accurately reflect the disease stage in HTLV-I infection; and (3) the $CADM1^+CD7^{dim/neg}$ subpopulations are at the intermediate stage of ATL progression and can be identified even in asymptomatic carriers. These findings will help to elucidate the molecular events involved in multistep oncogenesis of ATL.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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Development of methodology: T. Ishigaki, T. Yamochi, N. Watanabe

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特集 ATL/HTLV-1研究の最近の進展

わが国におけるHTLV-1キャリアとATL患者に対する相談機能と知識の普及*

内丸　薫**

Key Words：HTLV-1 asymptomatic carrier, prenatal check up, blood donation, public health center

はじめに

2007年の全国調査によればわが国のHTLV-1感染者数は推定108万人とされており[1]，1988年の調査における120万人から若干減少しているものの，現在でもわが国には多数のHTLV-1感染者が存在している．また年間1,100人前後がATLを新規に発症していると推定されている[2]．これらのHTLV-1キャリアおよびATL患者はさまざまな不安や悩みを抱えることになり，これに対する相談機能の整備が進められつつある．本稿ではHTLV-1キャリア・ATL患者に対する相談機能の整備に関するこれまでの背景と現状，今後の課題などについて概説する．

HTLV-1キャリアの分布の変化

日本におけるHTLV-1感染者の分布には地域的な偏りがあり，九州・沖縄地方を中心とした西日本地区に多いことはよく知られている．しかし，近年の人口の大都市圏への移動，集中によりHTLV-1キャリアの分布に変化が生じることは容易に想像される．図1は東京大学医科学研究所附属病院血液内科に設置されているHTLV-1キャリア専門外来の受診者のうち首都圏在住者のみを抽出し，その背景因子を解析した結果であるが，全体の約40％は九州・沖縄をはじめとするendemic area(高浸淫地域)出身者であり，さらに本人はnon-endemic areaの出身(主に首都圏)で母親がendemic area出身であるのが約15％，本人，母親ともnon-endemic area出身で，配偶者が

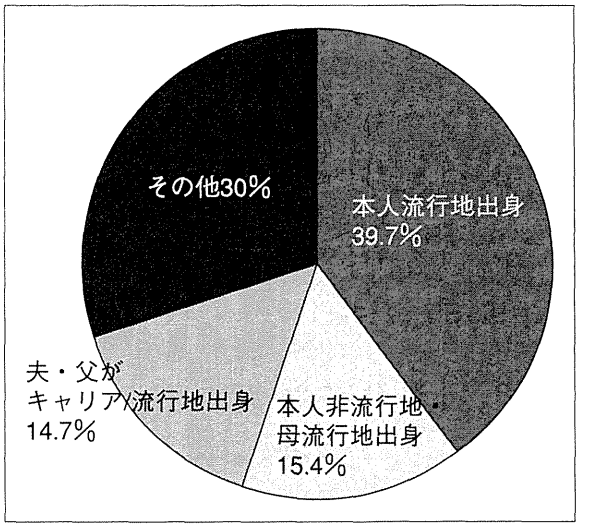

図1　東大医科研病院HTLV-1キャリア専門外来を受診した首都圏在住者

2006年3月から2007年12月の間の東京大学医科学研究所附属病院血液内科HTLV-1キャリア専門外来受診者のうち首都圏(関東地方)在住者88名のendemic areaとの関連の解析結果を示す．全体の少なくとも70％はendemic areaからの移住に関連したキャリアと考えられた．

* Counseling system for HTLV-1 asymptomatic carriers and ATL patients in Japan.

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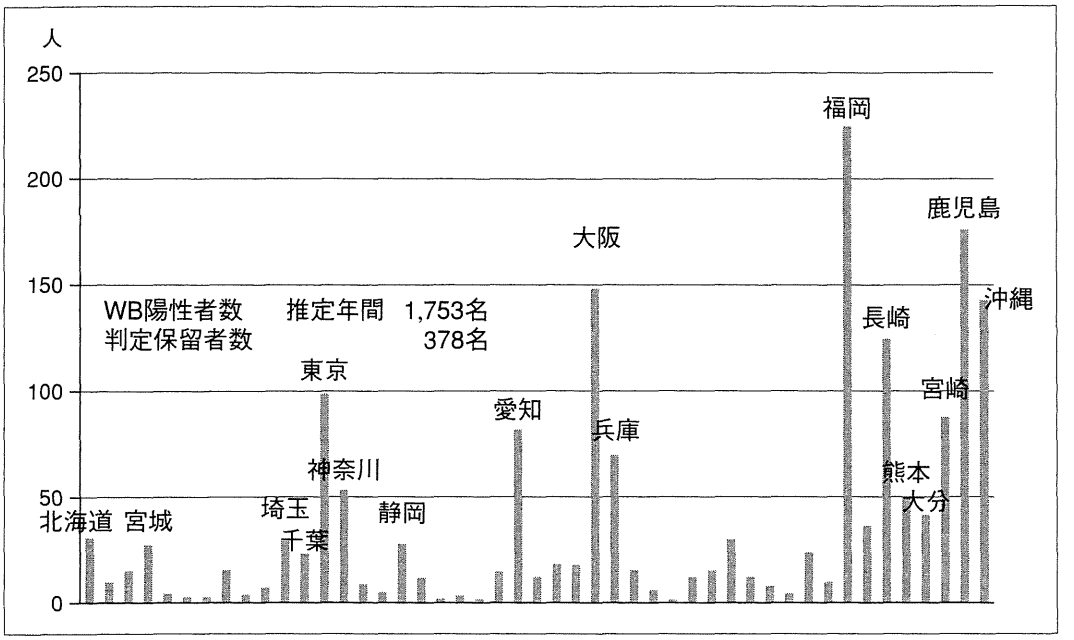


図２ 妊婦健診で判明したHTLV-1感染者数 都道府県別推定値(2011年)
妊婦健診によってウエスタンブロット法の結果抗HTLV-1抗体陽性ないし判定保留の判定を受ける妊婦は全国で年間2,000名を超えると推定される．九州，沖縄のみではなく，東京，大阪など大都市圏では九州地区各県に匹敵する数のキャリア妊婦がいることがわかる．〔日本産婦人科医会，厚生労働科学研究「HTLV-1母子感染予防に関する研究：HTLV-1抗体陽性妊婦からの出生児のコホート研究」(研究代表者・板橋家頭夫)による〕

endemic area出身，あるいはHTLV-1キャリアであるのが約15%で，HTLV-1の主要な感染ルートが母乳を介した母子感染と性感染であることを考慮すると首都圏地区のHTLV-1キャリアの約70%はendemic areaからの人口の移動に関連したキャリアであることがわかり，大都市圏へキャリアの分布が拡散していくことが予想される[3]．一昨年のわが国における妊婦健診により判明したキャリア妊婦数の都道府県別推定値を図２に示す．現在でも九州・沖縄地区の在住者が多いが，大都市圏居住者が目立っていることがわかる[4]．これまでは地域的な偏在からHTLV-1感染症に対する対策は九州地区の地方の問題と考えられてきたが，もはや九州地区のみではなく，国として大都市圏を中心に全国的な対応が必要であることは明らかである．

妊婦健診と献血

わが国においてはHTLV-1の感染の主要ルートは母乳の授乳を介した母子感染である．HTLV-1キャリア妊婦から通常の母乳哺育を受けた児の感染率は約20%であるのに対し，断乳して人工乳哺育を行った児の感染率は約３%と６～７分の１に感染率を低下させることができる．母乳哺育を３か月以内の短期とする，あるいは搾乳した母乳を凍結解凍して必要時に与える凍結母乳法でも断乳とほぼ同様に感染率を低下させると考えられるデータがある[5]．したがって感染予防のためには妊婦の抗HTLV-1抗体のチェックと陽性者に対して授乳の指導を行うことが重要であり，九州地区では長崎県における20年以上にわたるAPP(ATL Prevention Program)など各県で取り組みがあったが，全国的には組織的な取り組みはなかった．九州地区以外でも80%前後の妊婦は抗HTLV-1抗体のチェックを受けていたと推定されるが[5]，適切な授乳に関する指導や，HTLV-1感染症全般についての情報提供が必ずしもなされていなかった．

一方，妊婦健診と並んでHTLV-1感染が判明する機会として多いのが献血である．前出の東京大学医科学研究所附属病院血液内科HTLV-1キャリア専門外来を受診した首都圏のHTLV-1キャリアの背景因子の解析では，キャリアと判明した契機として最も多かったのが献血で，次いで妊

表1　HTLV-1総合対策の骨子(2010年12月策定)

推進体制

国，地方公共団体，医療機関，患者団体などの密接な連携を図り，HTLV-1対策を強力に推進

●厚生労働省：
- HTLV-1対策推進協議会の設置
  患者，専門家などが参画し，協議会での議論を踏まえて，総合対策を推進
- 省内連携体制の確立と，窓口担当者の明確化

●都道府県：HTLV-1母子感染対策協議会

●研究班：HTLV-1・ATL・HAMに関連する研究班の総括的な班会議　研究班の連携強化，研究の戦略的推進

重点施策

1. 感染予防対策
   ○全国的な妊婦のHTLV-1抗体検査と，保健指導の実施体制の整備
   ○保健所におけるHTLV-1抗体検査と，相談指導の実施体制の整備
2. 相談支援(カウンセリング)
   ○HTLV-1キャリアやATL・HAM患者に対する相談体制の整備
   ・相談従事者への研修の実施やマニュアルなどの配布
   ※相談体制の構築や手引きの作成などにおいて，患者団体などの協力も得ながら実施
3. 医療体制の整備
   ○検査制度の向上や発症リスクの解明に向け，標準的なHTLV-1ウイルスのPCR検査方法などの研究の推進
   ○ATL治療に係る医療連携体制などの整備，地域の中核的医療機関を中心としたHAMの診療体制に関する情報提供
   ○ATLおよびHAMの治療法の開発・研究の推進，診療ガイドラインの策定・普及
4. 普及啓発・情報提供
   ○厚労省のホームページの充実など，国民への正しい知識の普及
   ○母子感染予防のため，ポスター，母子健康手帳に挟むリーフレットなどを配布
   ○医療従事者や相談担当者に対して，研修などを通じて正しい知識を普及
5. 研究開発の推進
   ○実態把握，病態解明，診断・治療などの研究を総合的・戦略的に推進
   ○HTLV-1関連疾患研究領域を設け，研究費を大幅に拡充

婦健診であった．初回献血者で抗HTLV-1抗体陽性と判明するのは日赤中央血液研究所の佐竹らの調査によれば年間1,900名程度と推定され[1)6)]，一方前出の妊婦健診で判明したキャリアの年間推定数は約1,700名[4)]である．抗HTLV-1抗体陽性の献血者には，本人が通知を希望していた場合，その結果とHTLV-1感染症について概説したパンフレットが送付され，また必要な場合日赤血液センターの相談窓口で説明を受けることができるが，献血者にとってはまったく突然のことであり，さまざまな不安や疑問を抱えたままになるケースもある．これらのケースに対する相談機能を担う施設が，特にnon-endemic areaでは整備されていないことも大きな問題であった．

HTLV-1総合対策の開始

こういった現状に対応するためHAM(HTLV-1関連脊髄症)の患者団体や，HTLV-1領域の研究者と厚生労働省の意見交換のために2009年から厚生労働省においてHTLV-1有識者会議が継続的に開催され，そこでの議論を踏まえて2010年9月首相官邸にHTLV-1特命チームが設置され，4回の会合を経て12月までに提言をまとめてHTLV-1総合対策として翌2011年から開始されることになった[7)]．HTLV-1総合対策の骨子を表1に示す．HTLV-1キャリア，ATL患者に対する相談機能の観点では推進体制として都道府県に母子感染対策協議会が設置されることになり，重点施策の部分では，第1項に感染予防対策として保健所における抗体検査と相談指導実施体制の整備が記載され，第2項には相談支援(カウンセリング)としてHTLV-1キャリアやATL/HAM患者に対する相談体制の整備があげられている．また第4項に普及啓発・情報提供が重点施策としてあげられている．HTLV-1総合対策で目指しているHTLV-1キャリア対策は，感染予防対策の中心に母子感

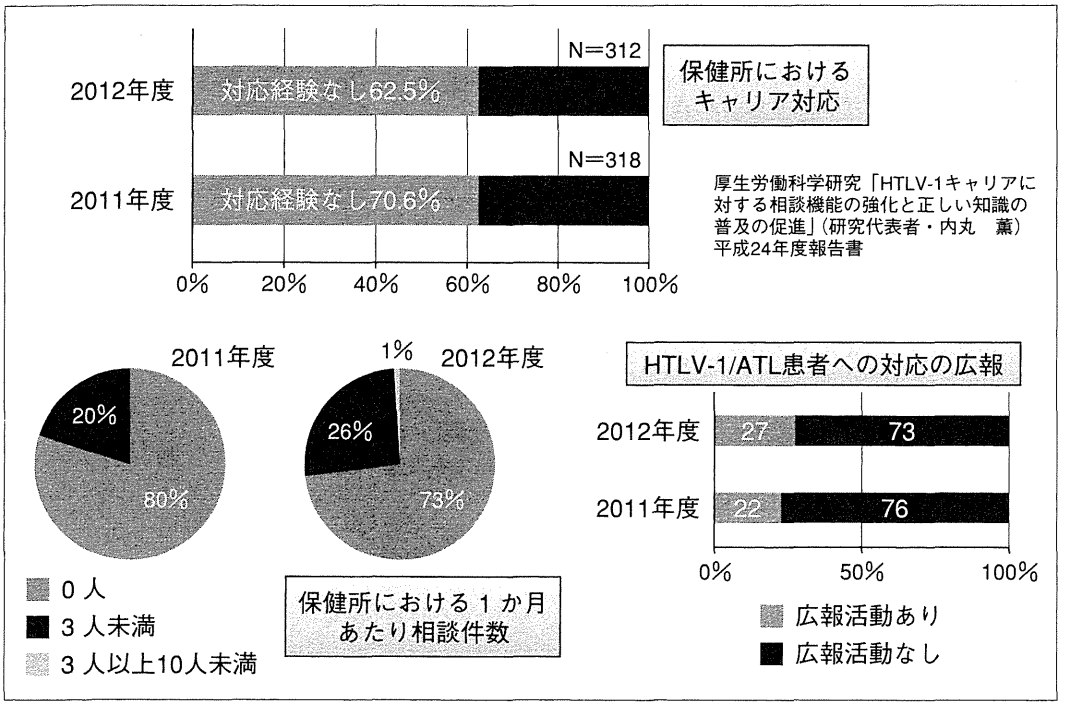


図3　保健所の現状と活性化への課題
2011年，2012年に厚生労働科学研究「HTLV-1キャリア・ATL患者に対する相談機能の強化と正しい知識の普及の促進」班(研究代表者・内丸　薫)によって行われた全国の保健所におけるHTLV-1キャリアに対する相談対応の実態調査の結果のまとめ．

染予防対策を置き，妊婦の抗HTLV-1抗体の検査を公費で全例施行とした．その円滑な遂行のために都道府県母子感染対策協議会を設置し，都道府県単位で母子感染予防対策を検討するとされている．一方，妊婦の抗HTLV-1抗体スクリーニング全例化により，キャリアと判明した妊婦に対する相談体制を充実させることは必須であり，これは総合対策では全国の保健所が担当することが想定され，合わせて献血など，他の理由で判明したキャリアに対する相談にも対応することが想定されている．相談支援(カウンセリング)の記載は主にATLをはじめとするHTLV-1関連疾患をすでに発症した患者に対する相談支援を念頭に置いており，こちらは全国のがん診療連携拠点病院の相談支援センターが当たることを想定している．これらの保健所，がん診療連携拠点病院相談支援センターのネットワークで全国をカバーするというのがHTLV-1総合対策で想定された相談機能の枠組みである．

HTLV-1キャリア/ATL患者に対する相談機能の現状

それでは実際にこれらの相談体制はうまく機能しているのであろうか．厚生労働科学研究「HTLV-1キャリア・ATL患者に対する相談機能の強化と正しい知識の普及の促進」班(研究代表者・内丸　薫)では2011年度末に全国の保健所におけるHTLV-1キャリアに対する相談対応の実態調査を行った．全国495か所の保健所を対象に郵送法による調査票を用いた調査で318施設から回答を得て回収率は64%であった．結果の一部を図3に示すが，全国の保健所の約70%がキャリア対応の経験がないと答えており，1か月あたりの対応件数は0件と回答した施設が80%に達した．この保健所での相談対応件数の低さの原因はいろいろ考えられるが，これらの保健所のうちHTLV-1に対する相談対応を行っていることを広報紙やホームページ，ポスターなどで広報していると回答した施設は全体の20%程度であり，保健所で相談対応が行われていることが周知されていないことが原因の一つと考えられた[8]．2012年は

●患者会を介したATL患者対象調査　n＝28

がん診療連携拠点病院（がん診療連携拠点病院）に相談支援センターがあるのを知っていますか

選択項目	回答件数	回答率
知っている	7	25%
知らない	19	68%

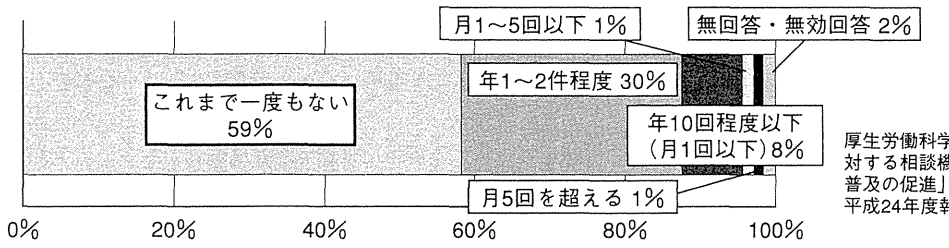
相談支援センターではATLについても相談にのってくれることを知っていますか

選択項目	回答件数	回答率
知っている	5	18%
知らない	21	75%

厚生労働科学研究「HTLV-1キャリアに対する相談機能の強化と正しい知識の普及の促進」（研究代表者・内丸　薫）2013未発表データ

●がん診療連携拠点病院相談支援センターでの対応の実態調査　n＝246

最近1年間におけるATL患者，HTLV-1キャリアに対する相談件数



厚生労働科学研究「HTLV-1キャリアに対する相談機能の強化と正しい知識の普及の促進」（研究代表者・内丸　薫）平成24年度報告書　渡邊清高による

図4　がん診療連携拠点病院 がん相談支援センターの現状と活性化への課題

2012年に厚生労働科学研究「HTLV-1キャリア・ATL患者に対する相談機能の強化と正しい知識の普及の促進」班（研究代表者・内丸　薫）によって行われた全国がん診療連携拠点病院相談支援センターの実態調査，および同研究班による2013年患者団体を対象としたATL患者意識調査の結果．

改善傾向がみられるが，さらなる対策が必要であろう[9]．保健所でHTLV-1キャリア相談対応を行う上での問題点として，相談対応を行うための研修などが行われているものの専門知識の不足などの不安と，その後のフォローアップなどを含めた二次対応が必要な場合の専門施設との連携，情報がないことなどがあげられており，今後保健所における相談機能を活性化していくためには血液内科などの専門施設との連携，バックアップ体制の組織化などの体制を構築していくことが重要であろうと考えられる[8]．

一方，ATL患者に対する相談体制に関しては，いくつかの視点が必要である．2010年度にHTLV-1総合対策を前倒しする形で厚生労働科学研究「成人T細胞白血病のがん幹細胞の同定とそれを標的とした革新的予防・診断・治療法の確立」班（研究代表者・東京大学・渡邉俊樹）の追加研究課題として全国のATL診療の実態調査が行われた．その結果，indolent ATLに対する治療方針，aggressive ATLに対する造血細胞移植の適応などを中心に，施設によって大きく治療方針が異なっている実態が明らかになった[10]．したがって，ATLと診断されたとき，患者が提示された治療方針以外に選択肢があるのか，など治療方針に対しての相談対応の場が現時点では必要になる．もう一つの視点は，いわゆるがん患者に対する相談支援であり，療養上の不安，医療費などの相談などの支援である．2011年（平成23年）3月からがん診療連携拠点病院相談支援センターの業務に「HTLV-1関連疾患であるATLに関する医療相談」の項目が追加され，ATL患者に対する相談機能はがん診療連携拠点病院の指定用件にもなっており，ATL患者相談にがん診療連携拠点病院で対応するという基本の構図が明確に打ち出されている．前出の厚生労働科学研究「HTLV-1キャリア・ATL患者に対する相談機能の強化と正しい知識の普及の促進」班では2012年に全国397のがん診療連携拠点病院相談支援室を対象に実態調査を行った．自記式質問紙による郵送法による調査で246施設から回答を得て，回収率は62%であった．その結果，図4に示すように約60%の相談支援センターではこれまでATL患者・家族に

対する相談・支援の実績がなく，年に1～2件という施設まで合わせると全体の90％に達することが判明し，がん診療連携拠点病院においても必ずしもATL患者に対する相談機能が十分に果たされていないことが推察された[9]．その原因についてもいろいろあると考えられるが，同調査で院内掲示やホームページなどで相談支援センターがATL患者に対する相談窓口であることを周知しているかという問いに対して87％がしていないと回答し，相談支援センターがATL患者の相談窓口になることが院内職員においてもあまり認知されていないと回答した施設が80％に上っていることから，相談支援センターが認知されていないことが最大の問題点の一つであろうと推定された．そのことを裏づけるものとして，少数例の調査であるが，患者会を対象としたATL患者の調査で70％程度の患者が相談支援センターがATL患者の相談に乗ることを知らず，そもそも相談支援センターそのものを認知していないという結果であった(図4)．相談支援センターに必要な情報として80％の施設がATLの専門医や専門医療機関の情報をあげており，希少疾患であり必ずしもすべての施設が対応できるとは限らないATL診療に関する情報提供を行うための連携体制の構築の必要性が示唆された．

情報提供という観点のみならず，正しい知識の普及という観点からウェブサイトの充実が図られ，厚生労働省のホームページ上にも情報提供サイトが整備されるとともに[11]，厚生労働科学研究「HTLV-1キャリア・ATL患者に対する相談機能の強化と正しい知識の普及の促進」班では「HTLV-1情報サービス」[12]というウェブサイトを運営し，HTLV-1ウイルスおよび関連疾患に関する正しい知識の普及と診療対応施設や臨床試験の情報の提供を行っている．本ウェブサイトには2011年度18,487件，2012年度40,110件のアクセスがあり需要の高さをうかがわせる．利用者居住地のトップは2年連続で東京都であった[9]．

今後の課題

相談機能の現状から今後の課題を考えると拠点化と連携というキーワードが浮かびあがってくる．上記の「HTLV-1情報サービス」の医療機関検索に昨年まで掲載されていた対応可能施設に対する再調査の結果，検査などのみではなく相談対応まで可能と回答した施設は全体の40％しかなく，血液内科であればどこでも相談対応が可能というわけではない．一方で，保健所，がん診療連携拠点病院相談支援センターの活性化のためには，専門的な対応が可能な血液内科施設との連携体制を地域ごとに構築していくことが重要である．また，図2のデータから推定される都道府県ごとのキャリアの分布から考えても，全国で一律に同じ基準で体制を整備することが適切とは必ずしも考えられない．保健所，がん診療連携拠点病院などで一次対応の上，必要に応じ二次対応を行う拠点施設を地域ごとに設定し連携を取る体制を組織的に構築することが必要であろう．母子感染対策協議会は妊婦の抗体スクリーニングの円滑な実施について協議するのが目的であるが，陽性と判明した妊婦への相談対応の体制構築も重要な課題である．都道府県によっては母子感染対策協議会を妊婦健診に限らずHTLV-1感染対策全体の対応体制を協議するHTLV-1感染対策協議会へと発展的に改組する動きもみられ，産科医療施設，血液内科，がん診療連携拠点病院，赤十字血液センター，保健所などの地域ごとの連携体制構築を目指している．地域ごとに行政と連携して組織的体制の構築を目指していくことが必要であろう．

キャリア，ATL患者のみではなく一般へのHTLV-1の知識の普及を図っていくことも重要である．キャリア妊婦に対する社会の理解が進むことで，必要以上に不安，苦痛を与えることが避けられる．また，HTLV-1は性感染症でもある．性感染キャリアはATLを発症しないと考えられていることもあって，現時点でHTLV-1の性感染に対して積極的な介入はなされていないが，正しい知識を適切に社会に広めないと，特に若い世代のキャリアが誤った偏見にさらされる危険性もある．ウェブサイトなどによる広報では，検索されない限り情報発信にならないという欠点があり，より積極的な知識の広報手段を講じていく必要がある．

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【特集】産婦人科感染症

HTLV-1
—その発見から母子感染対策事業となるまで—

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キーワード：抗体検査，HTLV-1母子感染対策協議会，PCR，Western Blot法

はじめに

1977年に高月　清(京都大学)らにより従来の白血病と異なる成人T細胞白血病(ATL)という新たな白血病が報告された[1]．ATLは中高年齢者に発症し，極めて難治性であり，かつ西日本，とくに九州・沖縄地区に多いことが特徴である．1980年に三好　勇夫(高知医大)らはATL発症患者のリンパ球と，臍帯血を共培養したところ，臍帯血T細胞株が樹立されたため，何らかの病原体(おそらくはレトロウイルス)がATLに関与していることが示唆された[2]．翌年の1981年に日沼　頼夫(京都大学)によりHTLV-1ウイルスがATLの病原ウイルスであることが報告された[3]．これは，ヒトでのレトロウイルスによる発癌を証明した初めてのケースとなる．重要なことに，HTLV-1キャリアのすべてがATLを発症するのではなく，生涯ATL発症リスクは男性キャリアで4～7%，女性キャリアで2～5%であり，その他，キャリアには地域偏在があることがわかった．1982年には吉田　光昭(東京大学)らにより，HTLV-1ウイルスの全構造が明らかとなった．1984年には一條　元彦(奈良医大)らにより，母乳中ならびに精液中にHTLV-1感染細胞が検出され，母乳を介した母子感染，精液を介した性行為感染の可能性が指摘され[4]，1985年に日野　茂男らにより，マーモセットにHTLV-1キャリア婦人の母乳を飲ませることにより，感染が生じることが報告され[5]，HTLV-1が母乳を介して母親から子供へ母子感染することが実験的に証明された．HTLV-1ウイルス発見から10年後に厚生省心身障害研究重松班により，キャリアが全国で120万人存在し，九州・沖縄に多いことが報告され，母乳を介した母子感染を防ぐため人工乳を推奨した．また，HTLV-1キャリアへの告知は精神的負担が大きく，新しい差別の対象とならないため，キャリア率の高い地域以外では，HTLV-1母子感染予防対策不要と提言した[6]．国はこの提言を受け，その後20年間，HTLV-1母子感染については全くの空白期間となってしまった．2009年に厚生労働研究山口班報告で，HTLV-1キャリアが全国に拡散していることが明らかとなったため[7]，2010年に厚生労働特別研究齋藤班で，最新のデータを交えてHTLV-1母子感染につきまとめた[8]．その後，厚生労働研究森内班により，保健指導マニュアルが作成され[9]，板橋班により，HTLV-1キャリア妊婦からの出生

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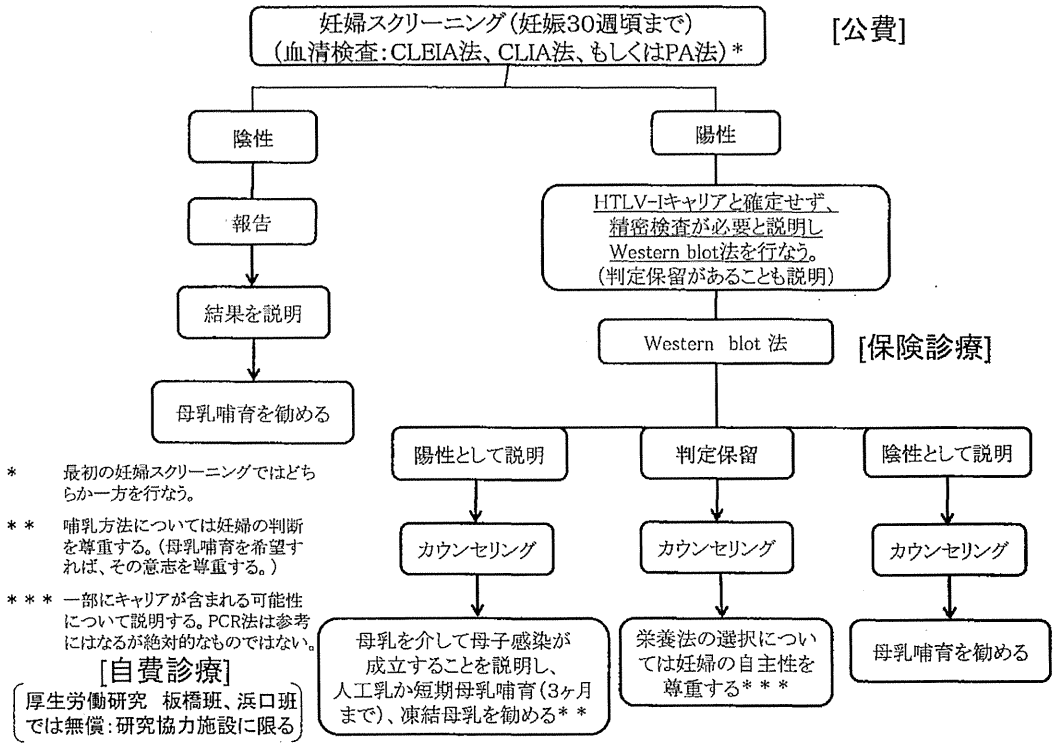

図1 HTLV-I スクリーニングの進め方

児コホート研究が開始された[10]. HTLV-1 母子感染については拙著[11]を参考にしていただき，本論文では重松班報告以降，変更された点，ならびに新たな問題となってきた点を中心に解説する.

1. HTLV-1 スクリーニング法

これまでは任意で，HTLV-1 スクリーニングが妊婦に対して行われていたが，2011 年からは公費でスクリーニングが行われるようになったため，産婦人科診療ガイドラインにおいても妊婦における HTLV-1 スクリーニング検査の推奨レベルが A となった．図1に HTLV-1 スクリーニングの進め方を示すが，まずは抗体検査により，スクリーニングを行う．重要なこととして，スクリーニング陽性であっても，偽陽性例が多く含まれるため，必ず確認検査である Western blot(WB)法を保険診療で行う必要がある．特に偽陽性例は九州・沖縄以外の地域で多い[8)12)13)]．これは一次スクリーニングには一定の率で偽陽性が生じるため，偽陽性率より真の陽性率が高い九州・沖縄地域では WB 法陽性率は高くなるが，その他の地域では，偽陽性率が真の陽性率を上回るため，WB 法陽性率は低くなる．日本産婦人科医会が行った調査では，全国で 707,711 例にスクリーニングが行われ，陽性者が 2,259 例，そのうち WB 法が 1,894 例(83.8%)に行われていた(図2)．初回の妊娠時に WB 法を施行し陽性であったため，今回省略した例も含まれるが，全例に WB 法が施行されることを切望する．WB 法施行例では，陽性が 49.7%，陰性が 34.8%，判定保留が 11.2%，不明が 4.2% であった．方針として WB 法陽性者は HTLV-I キャリアと診断し，その後の栄養法を指導する．WB 法陰性者は一次検査の偽陽性と判断し，キャリアでなかった事を説明し，母乳哺育を推奨する．最も苦慮するのが判定保留である．WB 法では HTLV-1 の gag 部分，env 部分の両方に抗体を有する場合に陽性と判定するが，いずれか一方のみ反応する抗体を有する場合，判定保留となる．そ

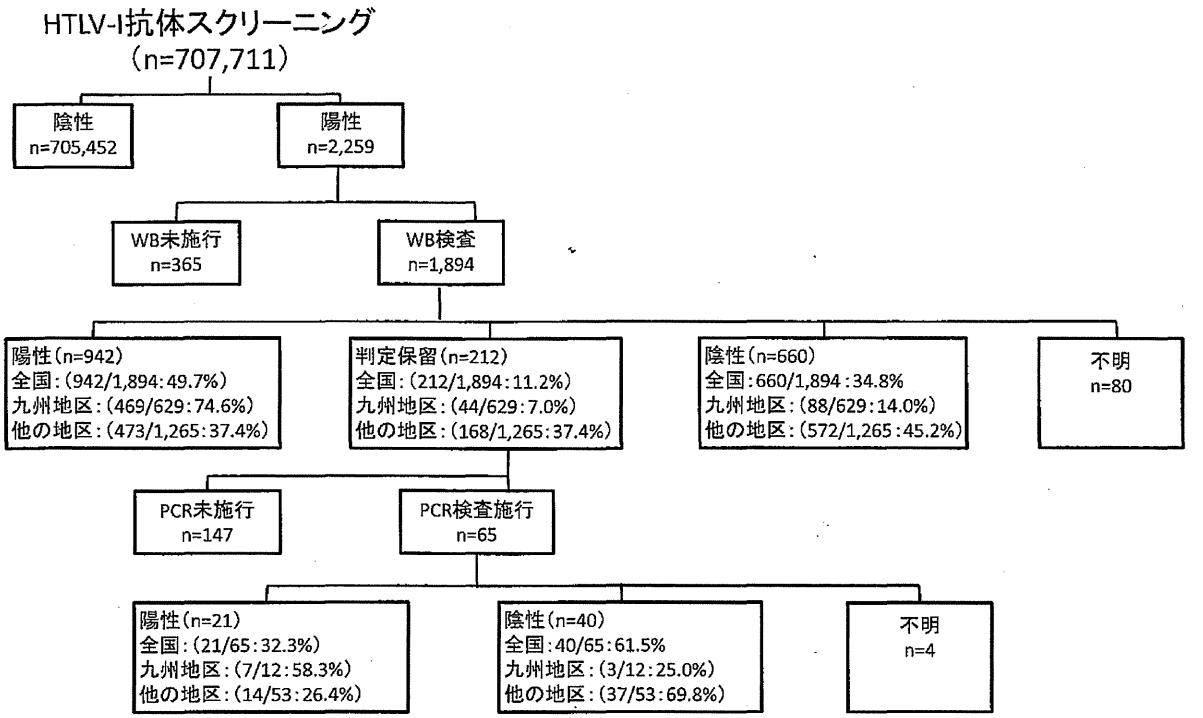

図2 日本の妊婦のHTLV-1抗体スクリーニング，WB法，PCR法の現状（文献12より作成）

のため希望者には保険診療外検査であるが，PCR法を施行するが，日本産婦人科医会のデータでは，WB法判定保留者の約1/3のみしかPCR法陽性とならなかった(図2)．現在，厚生労働研究板橋班と浜口班でWB法判定保留例に対してPCR法を施行しているが，63例中13例(20.6%)にしかPCR法が陽性とならず，陽性者のプロウイルス量も平均値0.01%と極めて微量であった．このためWB法判定保留者に対してPCR法を施行することは，有益であり，約2/3の症例でPCR法陰性となり，不必要な不安を払拭できる．また後述するが，母子感染率が高くなるのは，プロウイルス量が高い例であり，PCR法陽性者であってもプロウイルス量が少ないため安心感を与える．またATLやHAM(HTLV-1関連脊髄症)などの発症リスクが高まるのはプロウイルス量が4%以上であることから[14]，たとえキャリアであってもWB判定保留例では発病のリスクが低いと説明できる．ATL発症にかかわるリスク因子として，母子感染，男性，加齢，特定のHLA保持者，可溶性IL-2R上昇などが報告されてきたが，HTLV-1プロウイルス量の増加は，感染細胞数を反映していると考えられ，ATLのみならずHAMのリスク因子でもある．また母子感染のリスク因子でもある[15][16]．WB法判定保留者はPCR法陽性であってもATL，HAMのハイリスクではなく，また母子感染率も低いと説明できる．

2. HTLV-1母子感染予防法

HTLV-1母子感染は母乳中の感染リンパ球により生じることから，これまでは人工乳の推奨が行われ，大きな成果を得てきた．しかし，母乳中にはIgAを含む多くの免疫物質や成長ホルモン，サイトカイン等の生理活性を有する因子が多く含まれること，母子間の愛情形成に有用であることから，完全人工栄養に替わるHTLV-1感染防止対策が切望されてきた．

HTLV-1の感染は感染細胞が直接，非感染細胞