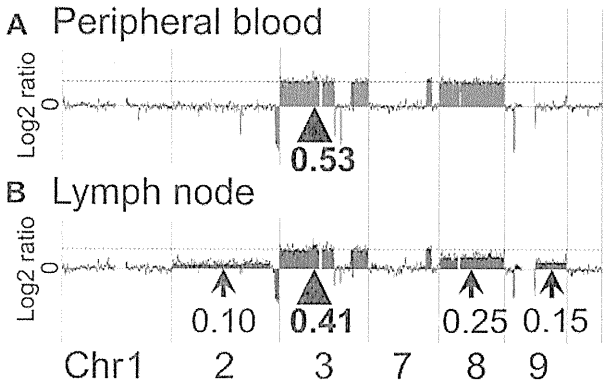

Figure 1. Representative profiles of the PB and LN samples of case 1. Array CGH results for case 1 are shown. (A) In the PB sample of case 1, regions of gain were detected. The log2 ratio of chromosome 3 was 0.53 (arrowhead). The log2 ratios of chromosomes 7 and 8 were the same as for chromosome 3 (dotted line). (B) In the LN sample of case 1, a log2 ratio imbalance was found. Log2 ratios among chromosomes 2, 3, 7, 8, and 9 differed. The log2 ratios of chromosome 3 and 7 were 0.41 (arrowhead and dotted line). Arrows show different log2 ratios: chromosome 2 = 0.10, chromosome 8 = 0.25, and chromosome 9 = 0.15.

57 years (range, 32-74 years). Detailed patient information is provided in supplemental Table 1 (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article).

Four cell lines, SP-49,[9] HANK1,[10] ATN-1,[11] and Jurkat,[12] were also analyzed. SP-49 is a mantle cell lymphoma cell line, HANK1 is a natural killer/T-cell lymphoma line, ATN-1 is an ATLL cell line, and Jurkat is a T-cell lymphoblast–like cell line.

Peripheral blood samples were obtained from the blood of 8 healthy male donors. PBMCs were isolated by Ficoll-Paque PLUS centrifugation (GE Healthcare).

DNA extraction

$CD4^+$ cells in PB samples were purified using a magnetic-activated cell-sorting protocol (Miltenyi Biotec). High–molecular-weight DNA was extracted from $CD4^+$ cells, frozen LNs, and from the SP-49, HANK1, ATN-1, and Jurkat cell lines using standard proteinase K treatment and phenol-chloroform extraction.[13] Normal DNA was obtained from PBMC samples of 8 healthy male donors.

Oligo-array CGH

Characterization of the genomic aberrations was performed using Agilent 44K Whole Human Genome CGH arrays (Agilent Technologies) containing 44 000 probes. Procedures for DNA digestion, labeling, hybridization, scanning, and data analyses were performed according to the manufacturer's protocol (Agilent Technologies).

CGH data analysis

CGH data were extracted from scanned images using Feature Extraction software (version 10.3; Agilent Technologies). Raw data were transferred to the Genomic Workbench v5.0 software (Agilent Technologies) for further analysis. We defined gains and losses over a continuous 15-probe dataset as a linear log2 ratio average of ≥ 0.05 or ≤ -0.05, respectively, and microdeletion for a range of 3-15 probes as a linear log2 ratio average of ≤ -0.4. A detailed explanation of the log2 ratio is available in the supplemental data. The array CGH data have been deposited in ArrayExpress under the accession number E-MEXP-3042.

Southern blot analysis of HTLV-1 integration and TCRγ rearrangement

Integration of the HTLV-1 provirus genome and TCRγ rearrangement were assayed as described previously.[5,14] In brief, DNA samples (5 μg) of LNs were digested with restriction enzymes (PstI) and electrophoresed through 0.7% agarose gels. The DNA was then transferred onto a Hybond N^+ membrane (Amersham Pharmacia Biotech) and hybridized to randomly primed ^{32}P-labeled DNA probes specific for the HTLV-1 and TCRγ genes. Blots were then washed at the appropriate stringency and visualized by autoradiography. The HTLV-1 probe comprised a 1.0-kb fragment of the pX region, which was PCR amplified using the primers 5′-ccacttcccagggtttggacag-3′ and 5′-tctgcctctttttcgttaaaaagtagagaaatggg-3′, and the TCRγ probe comprised a 0.6-kb fragment of Jγ2.1.[14]

Results

Oligo-array CGH analysis against paired samples obtained from the PB and LNs

In all of the 13 acute-type ATLL cases, genomic aberrations were detected by oligo-array CGH. Representative profiles of the paired samples obtained from the PB and LNs in cases 1 and 2 are shown in Figure 1A and B and Figure 2A and B, respectively.

In the PB sample of case 1, genomic aberration regions showed a constant log2 ratio. Regions of gain were detected on chromosomes 3, 7, and 8. The log2 ratios corresponding to these regions were 0.53, suggesting that there was no imbalance (Figure 1A arrowhead). On the other hand, imbalance of the log2 ratio among chromosomes was found for the LN sample of case 1. Genomic aberrations of the case 1 LN sample were similar to

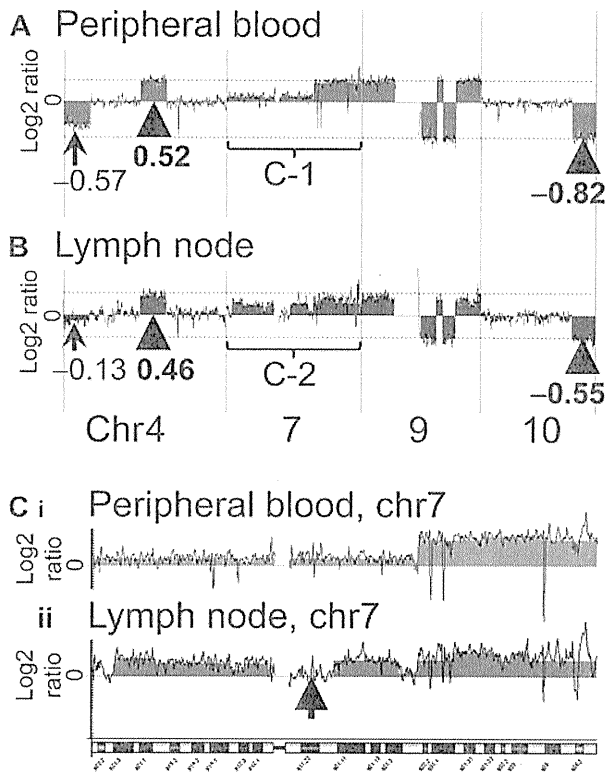

Figure 2. Representative profiles of the PB and LN samples of case 2. The results for case 2 were more complex than those for case 1. In both the PB and the LN samples of case 2, a log2 ratio imbalance was found. (A) In the PB sample, the arrowhead and dotted line indicate the majority of log2 ratios of gain and loss regions. Log2 ratios of the majority of loss regions were −0.82. The log2 ratio of chromosome 4 was −0.57. (B) In the LN sample, the arrowhead and dotted line indicate the majority of log2 ratios of gain and loss regions. Log2 ratios of the majority of loss regions were −0.55. The log2 ratio of chromosome 4 was −0.13. Chromosome 7 regions of PB and LN samples are magnified as Ci and Cii, respectively. (C) Chromosome 7 of the case 2 PB sample shows complex aberrations (i). This result also indicates a log2 ratio imbalance. Chromosome 7 of the case 2 LN sample shows more complex aberrations (ii). An arrow indicates a region (7q11.21-11q.23) without genomic aberration. Ci and Cii suggest that the genomic profiles of the PB and LN samples differ.

Table 1. Array CGH results of paired samples of acute-type ATLL

Case no.	Genome aberrations	Log2 imbalance		Genomic profiles of PB and LN	Common aberration regions between PB and LN	ATLL clones
		PB	LN			
1	+	−	+	different	+	Multiple subclones
2	+	+	+	different	+	Multiple subclones
3	+	−	−	same	+	Monoclone
4	+	+	+	different	+	Multiple subclones
5	+	+	−	different	+	Multiple subclones
6	+	−	−	same	+	Monoclone
7	+	+	+	different	+	Multiple subclones
8	+	−	+	different	+	Multiple subclones
9	+	−	+	different	+	Multiple subclones
10	+	−	+	different	+	Multiple subclones
11	+	−	−	same	+	Monoclone
12	+	−	−	same	+	Monoclone
13	+	+	+	different	+	Multiple subclones
Total	13 (100%)	5 (38.4%)	8 (61.5%)	9 (69.2%)	13 (100%)	9 (69.2%)

+ indicates present; and −, absent.

that of the PB sample. However, the log2 ratios among chromosomes 2, 3, 7, 8, and 9 differed as follows. Regions of gain were detected on chromosomes 2, 3, 7, 8, and 9, as shown by the log2 ratios: chromosome 2 = 0.10, chromosomes 3 and 7 = 0.41, chromosome 8 = 0.25, and chromosome 9 = 0.15 (Figure 1B arrowhead and arrows). The log2 ratio of chromosome 8 was lower than that of chromosomes 3 and 7. Gains of chromosomes 2 and 9 were detected in the LN sample, but not in the PB sample. These results indicated that a log2 ratio imbalance occurred in the LN sample.

Case 2 had a log2 ratio imbalance in both the PB and LN samples (Figure 2A). The genomic aberrations of the case 2 PB sample differed from those of the LN sample, as was also found with case 1. In the case 2 PB sample, regions of loss were detected on chromosomes 4, 9, and 10, as shown by the log2 ratios: chromosome 4 = −0.57 (Figure 2A arrow) and chromosomes 9 and 10 = −0.82 (Figure 2A arrowhead and dotted line). In the case 2 LN sample, regions of loss were also detected on chromosomes 4, 9, and 10, as shown by the log2 ratios: chromosome 4 = −0.13 (Figure 2B arrow) and, chromosomes 9 and 10 = −0.55 (Figure 2B arrowhead and dotted line). These data indicated that both samples had a log2 ratio imbalance. Complex genome aberrations were found for chromosome 7 in the paired samples of case 2. Consecutive gain regions were found in the whole of chromosome 7 of the PB sample (Figure 2Ci, and a region (7q11.21-11q.23) without genomic aberrations was found in chromosome 7 of the LN sample (Figure 2Cii arrow).

A log2 ratio imbalance among chromosomes was present in many other samples of acute-type ATLL, as summarized in Table 1.

Confirmation of log2 ratio imbalance among chromosomes

A log2 ratio imbalance among chromosomes was found in many ATLL clinical samples. We expected that a log2 ratio imbalance would indicate the presence of clones with different genomic aberrations. Therefore, we prepared 2 cell lines, SP-49 and HANK1, which possess different genomic aberrations. The genomic DNA of SP-49 was mixed with that of HANK1. We then conducted oligo-array CGH using the mixed-genomic DNA samples at various ratios.

Array CGH analysis of the SP-49 genome showed some genomic aberration regions, which were consistent with the G-band result that had been reported.[9] Log2 ratios of all 1-copy gain regions were 0.55, and log2 ratios of all 1-copy loss regions were −0.80. Imbalance of the log2 ratio among the chromosomes was not found. The same was true for HANK1, in which genomic aberration regions were consistent with the G-band result that had been reported and an imbalance of the log2 ratio among the chromosomes was not found.[10]

A representative array CGH result using a mixed-DNA sample at a ratio of 7:3 (SP-49:HANK1) is shown in Figure 3. The results showed an imbalance of the log2 ratio among chromosomes. It was possible to reproduce the log2 ratio imbalance. For example, the log2 ratios of chromosomes 2p14-pter, 2q14.3-qter, and 7p were 0.55, 0.15, and 0.46, respectively. These log2 ratios clearly differed. Furthermore, additional regions with different log2 ratios were found.

These results indicated that some of the clones present in the sample that had different genome profiles caused a log2 ratio imbalance in the array CGH result. The log2 ratio did not differ in chromosome 2p, which had a copy region identical to both SP-49 and HANK1.

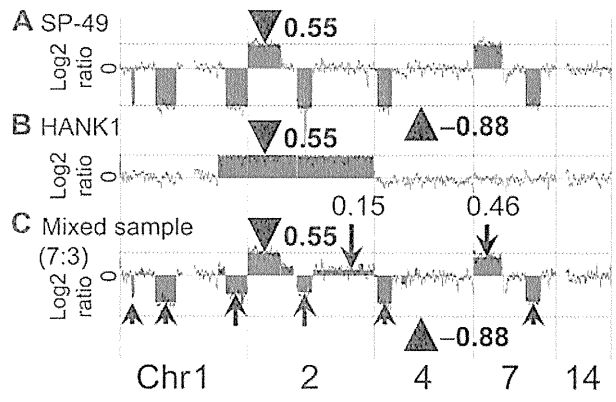

Figure 3. Confirmation of log2 ratio imbalance among chromosomes. The manner in which the log2 ratio imbalance occurred was confirmed. (A) SP-49 showed no imbalance. Log2 ratios of gain regions were 0.55 (arrowhead and dotted line). Log2 ratios of loss regions were −0.88 (arrowhead and dotted line). (B) HANK1 showed no imbalance. Log2 ratios of gain regions were 0.55 (arrowhead and dotted line). (C) Mixed-genomic DNA at a ratio of 7:3 reproduced the log2 ratio imbalance. The log2 ratio of chromosome 2p14-pter of the mixed DNA sample was 0.55 (arrowhead). Chromosome 2p had a copy region identical to both SP-49 and HANK1. Arrows indicate the log2 ratio imbalance.

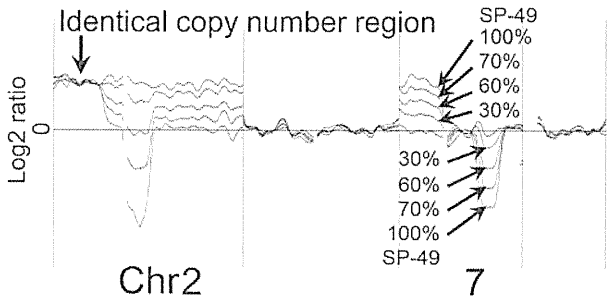

Figure 4. Log2 ratio reflects the ratio of tumor. The genome profiles of mixed-DNA samples comprising various ratios were superimposed. Gain was detected in chromosome 7 of all mixed samples, as shown by the log2 ratio: SP-49 = 0.55; 100%, 7:3 = 0.46; 70%, 6:4 = 0.32; 60%, 3:7 = 0.20; 30%. Loss was also detected in chromosome 7 of all mixed samples as shown by the log2 ratio: Sp-49 = −0.88; 100%, 7:3 = −0.62; 70%, 6:4 = −0.39; 60%, 3:7 = −0.14; 30%. Chromosome 2p had a copy region identical to both SP-49 and HANK1. The log2 ratios never changed in these regions.

Log2 ratios reflect the ratio of tumor

The genome profiles of mixed-DNA samples comprising various ratios were superimposed (Figure 4). The ratios of SP-49 to HANK1 were 7:3, 6:4, and 3:7. These results clearly revealed that the log2 ratio reflected the ratio of the tumor. When tumors included in a sample had identical genomic aberration regions, the log2 ratio never changed in these regions.

Southern blot analysis of HTLV-1 integration and TCRγ rearrangement

HTLV-1 integration

HTLV-1 integration was examined using Southern blot analysis, and the results showed HTLV-1 integration in all of the 11 cases examined. Eight of the 11 cases examined comprised a mono-integration band, whereas the others showed multi-integration bands. (Figure 5A)

TCRγ rearrangement

Southern blot analysis of TCR Jγ rearrangement was also conducted and evaluated as described previously by Moreau et al.[14] The results indicated that all samples were monoclonal (Figure 5B). Five of the 11 cases examined had a 6.8-kb rearrangement band, and 2 had a 2.9-kb rearrangement band. The others showed loss of germinal bands. In case 2, one allele of TCRγ was rearranged, because the germinal band of 8.0 kb was weaker than that of 4.9 kb. Case 7 lost all germinal bands, such as ATN-1, which is an ATLL cell line. This result indicated that both alleles of TCRγ were rearranged at Jγ2.3, because no deletion was found in case 7 by array CGH. Given that 3 or more TCR rearrangement bands were not found, no cases showed definite multi-clonality in tumor cells. These results indicated that the acute-type ATLL examined represented a monoclonal tumor comprising TCR rearrangements and with some possessing multiple integrations of HTLV-1.

Appearance of LN subclones before PB subclones

Array CGH analysis revealed that PB samples from 5 of 13 cases had homozygous loss regions that were not found in the corresponding LN samples of each case. In case 2, 1p12-1p13.1 of the PB sample was seen to represent homozygous loss, unlike the case with the LN sample (Figure 6). However, log2 ratios of same region in the LN sample seemed to be slightly lower than those of neighboring regions. This raised the possibility that a minor

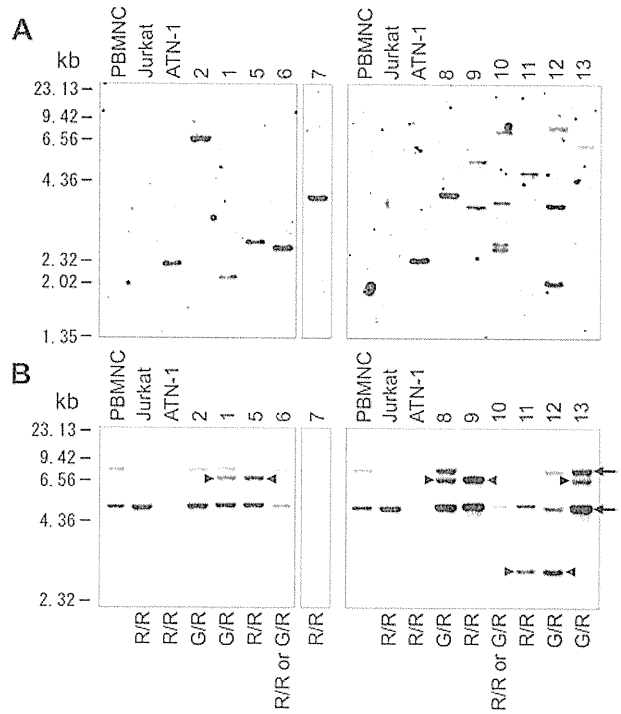

Figure 5. Southern blot analysis. (A) Southern blot analysis of HTLV-1 integration in 11 of 13 cases. (B) Southern blot analysis of TCRγ rearrangement. Arrows indicate the 8.00- and 4.9-kb germline bands. Arrowheads indicate the 6.8- and 2.9-kb rearrangement bands. G indicates a germinal allele; R, rearrangement allele; G/R, rearrangement of one allele; R/R, rearrangement of both alleles.

subclone was present. The PB samples from cases 1, 4, 8, and 10 also had homozygous loss regions that were not clearly found in the corresponding LN samples (Table 2).

No cases had a homozygous loss region in the LN samples when the PB samples had a heterozygous loss in the same regions. Array CGH and Southern blotting results indicated that multiple subclones had developed from one clone. Therefore, when 2 clones were found in a patient, the clone with homozygous loss must have developed from the clone with heterozygous loss. The homozygous loss analysis revealed that in about 40% of ATLL patients, subclones that had appeared in the PB were derived from LN subclones.

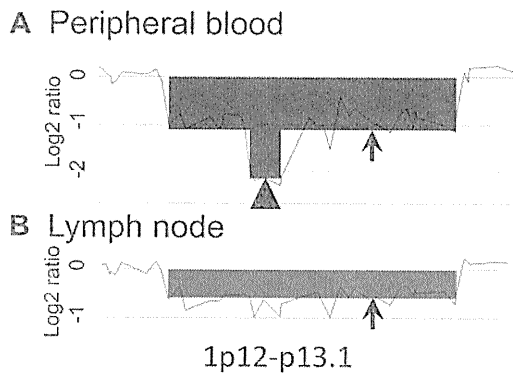

Figure 6. Homozygous loss region analysis. A representative homozygous loss region of case 2 is shown (1p12-p13.1). The total scale of the figure is approximately 2 Mb. The arrowhead indicates a homozygous loss region; arrows indicate heterozygous loss regions. Homozygous loss was found only in the PB sample. The log2 ratio of this region in the LN sample was slightly lower than that in the neighboring regions, suggesting the possibility that a minor subclone may exist in the LNs.

Table 2. PB samples of 5 of 13 cases only had homozygous loss regions that were not found in the LN samples

Case no.	Homozygous loss only in PB	Homozygous loss only in LN	Locus	Gene
1	+	–	3q22.3	*PCCB, STAG1*
2	+	–	1p12-p13.1	*IGSF3*
3	–	–		
4	+	–	6p22.3	*ATXN1*
5	–	–		
6	–	–		
7	–	–		
8	+	–	4q31.21	*INPP4B*
9	–	–		
10	+	–	9q31.2	*KLF4*
11	–	–		
12	–	–		
13	–	–		
Total	5	0		

+ indicates present; and –, absent.

Selected subclone of LNs in the PB

Tumor cells in the PB samples of some cases (eg, cases 1 and 9) appeared to have been selected from multiple subclones. In these cases, a log2 ratio imbalance was not found in the PB sample but was found in the LN sample. This indicated that PB samples were monoclonal and that the LN samples contained multiple subclones. Both samples from each case had common aberrations, and the LN samples had aberrations that were not found in the PB samples. These results may indicate that the LNs contain multiple subclones with different genomic aberrations, and that one of these subclones then appears in the PB (Figure 7).

Discussion

The imbalance and differing genomic profiles of PB and LN samples indicate that acute-type ATLL comprises multiple subclones

In this study, we revealed the presence of a log2 ratio imbalance among chromosomes of LN samples in many patients with acute-type ATLL. Most of the genomic profiles were found to differ from those of the PB samples. Although monoclonal proliferation of acute-type ATLL is referred to in the World Health Organization classification,[15] these data clearly show that acute-type ATLL contains multiple subclones that originate as a result of clonal evolution in ATLL patients.

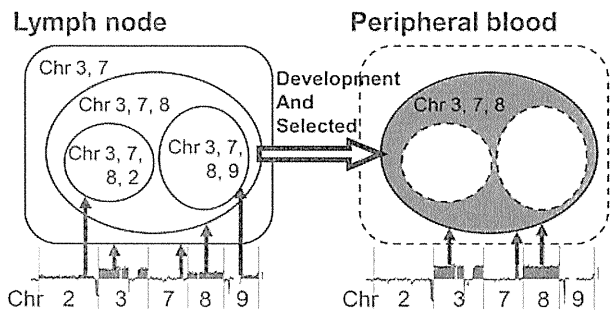

Figure 7. Selected subclone from the LN in the PB. Shown is a schematic representation of a selected subclone from the LN sample in the PB of case 1. In the LN sample of case 1, at least 4 subclones exist: a subclone with chromosome 3 and 7 aberrations; a subclone with additional chromosome 8 aberrations; a subclone with chromosome 3, 7, 8, and 2 aberrations; and a subclone with chromosome 3, 7, 8, and 9 aberrations. Among these subclones, a subclone with chromosome 3, 7, and 8 aberrations appeared in the PB sample.

Shinawi et al[16] reported a case of pediatric AML in which 2 clones with different chromosome aberrations showed a log2 ratio imbalance as detected by array CGH. We were able to reproduce a log2 ratio imbalance among chromosomes by mixing different ratios of DNA prepared from 2 different cell lines. The log2 ratio reflected the ratio of tumor clones. Based on these data, we analyzed the acute-type ATLL data and identified that a log2 ratio imbalance indicated the presence of multiple subclones in a sample. Minority clones with low log2 ratios could be found in this experiment by taking advantage of the high sensitivity associated with the use of array CGH. As a result, the presence of multiple subclones was unambiguously determined.

Cases showing different genomic profiles between PB and LN samples reached as high as 69%. We reported previously that paired samples obtained from different sites had different chromosomal aberrations in some cases.[17] We also reported that sequential samples at chronic and crisis or acute onset and relapse in each case showed different chromosome aberrations or integrations as determined by chromosomal CGH or Southern blot analysis.[17] Similar clonal change has been reported previously in some cases of B-cell lymphoma.[18] Although analysis of sequential samples is important when examining the stability of multiple subclones, it is difficult to acquire sequential samples from acute-type ATLL patients because these patients require immediate chemotherapy. However, chronic-type ATLL can be treated with "watchful waiting," so the clonal stability of ATLL may be explored in these patients.

Our data indicate that acute-type ATLL comprises multiple subclones with differing genomic aberrations. Several morphologic variants of ATLL have been described,[15] and the presence of a mixture of cells of different sizes has been reported. However, the histological type does not correspond to the clinical subtype.[19] Therefore, it is reasonable to postulate that the histological type does not always reflect the clinical features because the tumor subclones may differ at various sites.

HTLV-1 integration and TCRγ rearrangement determined by Southern blotting

We focused on the cell origin of the multiple subclones in each patient. Southern blot analysis revealed a monoclonal band of HTLV-1 integration or monoclonal rearrangement of TCRγ in all samples examined. These data indicated that the ATLL clones in each case had a common tumor cell origin. ATLL research and treatment utilize the Shimoyama classification. Acute-type ATLL represents one subtype in the classification, and is considered to be a monoclonal tumor. Our data are also consistent with this classification. However, it is possible that multiple subclones in the LNs possess a diversity that may account for the variable clinical manifestations and drug resistance that can occur during the treatment of ATLL.

Selection of leukemic clone and diversity in LNs

Array CGH suggested that the subclones in the PB and LNs differed even though they are derived from an identical monoclonal tumor cell, as determined by in Southern blot analysis. Given that the clones are derived from one clone, theoretically the clone with heterozygous loss is never derived from a cell with homozygous loss. Homozygous loss regions were only present in the PB samples examined at a frequency of 38% (5 of 13 cases examined). None of the 5 samples showed homozygous loss

regions found in the LN samples, indicating that in these cases, subclones present in the LNs were not derived from those in the PB. These results suggested that the selected subclones appeared in the PB after subclones developed in the LNs. However, it remains to be determined how these clones in the PB become stable during the course of disease. It is also important to determine whether the tumor cells in the PB can proliferate at the level of tumor cells in the LNs.

In conclusion, the results of the present study showed that there are multiple subclones in acute-type ATLL, all of which possess a common TCR rearrangement and the genomic profiles of which often differ between the PB and LNs. Cases were identified in which a selected subclone from multiple subclones in the LN samples was also identified in the PB samples. ATLL was clinically classified into 4 subtypes by Shimoyama. However, the specific genes that characterize acute-type ATLL have not been identified. Our results reveal that acute-type ATLL is a genetically heterogeneous neoplasm and that clonal evolution of ATLL takes place in the LNs.

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Authorship

Contribution: A. Umino performed the experiments, analyzed data, and wrote the paper; M.N. analyzed data and performed experiments; A. Utsunomiya provided advice, discussed clinical data, and treated patients; K.T. provided advice, discussed clinical data, treated patients, and wrote the paper; N.T. analyzed and discussed clinical data; N.K. discussed clinical data; and M.S. supervised the research, discussed clinical data, analyzed data, and wrote the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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Current status of HTLV-1 infection

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Abstract It is 30 years since human T-cell leukemia virus type 1 (HTLV-1) was identified as the first human retrovirus. To assess the implications of the virus for human health it is very important to know the past and present prevalence. Most of the estimates of HTLV-1 prevalence are based on serological screening of blood donors, pregnant women and other selected population groups. The widely cited estimate that the number of HTLV-1 carriers in Japan is 1.2 million was calculated from data that are now more than 25 years old. Here I summarize previous reports of prevalence studies in the world and Japan. Then, a recent analysis of seroprevalence of healthy blood donors in Japan will be described in comparison with that of 1988. A decrease in the number of HTLV-1 carriers in Japan was demonstrated, however, it is still more than one million. The number has increased in the metropolitan areas, probably reflecting the migration of Japanese population. I conclude that there is a paucity of general population data in countries where HTLV-1 is endemic, and re-evaluation of HTLV-1 infection is required to understand the virus burden on the human health.

Keywords Seroprevalence of HTLV-1 · Vertical and horizontal transmission · Prevention of transmission

1 Introduction

Discovery of adult T-cell leukemia (ATL) by Takatsuki's group [1] was followed by the discovery of the first human retrovirus human T-cell leukemia virus (HTLV) and adult T-cell leukemia virus (ATLV) by research groups of the United State and Japan, respectively [2, 3]. In 1980, Poiesz et al. [2] identified HTLV in a T-cell line from a patient with cutaneous T-cell lymphoma. Independently of this, Hinuma and Miyoshi found specific antibodies against ATL cells in the patients' sera [3] and type C retrovirus particles produced by a T-cell line established from peripheral blood of ATL patient in 1981 [4]. In 1982, Yoshida et al. [5] identified ATLV as a human retrovirus. Soon, HTLV and ATLV were shown to be identical at the sequence level and were named HTLV type 1 (HTLV-1) [6, 7].

After the discovery of HTLV-1, related viruses have been isolated and HTLV is now composed of 4 related HTLVs, HTLV-1 to HTLV-4 [8]. However, only HTLV-1 has been convincingly linked to human diseases at present. HTLV-1 has six reported subtypes (subtypes A–F). Diverse studies have been performed on HTLV-1 subtyping but present a minor role in the epidemiological status of the virus. The great majority of infections are caused by the cosmopolitan subtype A, and there is no report of subtype influence on the pathogenic potential of HTLV-1 [9].

2 HTLV-1 infection in the world

Approximately 20 million people worldwide are estimated to be infected with HTLV-1 [10]. Among them, more than 90% remain asymptomatic carriers during their lives. Since 1986, HTLV-1 screening has been developed and was slowly implemented worldwide [11]. In 1993, HTLV-1 screening of blood donors was already performed in all developed countries and in many developing countries where HTLV-1 is endemic.

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About the geographic distribution of the virus, a lot of studies have been done in these 30 years. Results indicate that Japan, Africa, the Caribbean islands, and Central and South America are the areas of highest prevalence in the world (reviewed in [12], [13]). However, the data from international prevalence studies should be interpreted and compared with caution as to the population selection criteria, because any difference in the diagnostic strategies can interfere with the final result. Data of the serological screening of healthy blood donors mainly provide basis for the estimation of the global prevalence of HTLV-1, which tends to underestimate the prevalence in the population. The geographic distribution of HTLV-1 infection is shown in Fig. 1 [13].

In addition to Japan, high rates of HTLV-1 infection have been reported for some Caribbean islands in studies of blood donors or segments of the general population. In Jamaica, the prevalence is around 5%. In Africa, the seroprevalence increases from the north to the south, varying from 0.6% in Morocco to greater than 5% in several sub-Saharan African countries, for example, Benin, Cameroon, and Guinea-Bissau, however, more studies are clearly required about these regions in detail. In Europe and North America, the prevalence is low and limited to groups that emigrated from endemic areas. For blood donors, very low rates were found in France (0.0039%) and the United States (0.025%). In South America, the virus was found in all countries, but more studies of the general population are needed to ascertain the real prevalence of HTLV-1. Medium prevalence was found in blood donors from Chile (0.73%) and Argentina (0.07%). In Australia, a prevalence of 14% was reported in a cluster among Aborigines in the Northern Territory, even though the prevalence in blood donors is low. The prevalence of HTLV-1 was highest in the two studies of Japanese islands (36.4%) and lowest in studies from Mongolia, Malaysia and India. In Haiti the prevalence was 3.8%; in Africa between 6.6 and 8.5% in Gabon, and 1.05% in Guinea. Only three studies were from West Africa and none were from the South; the only study from India was from the north of the country. It has to be

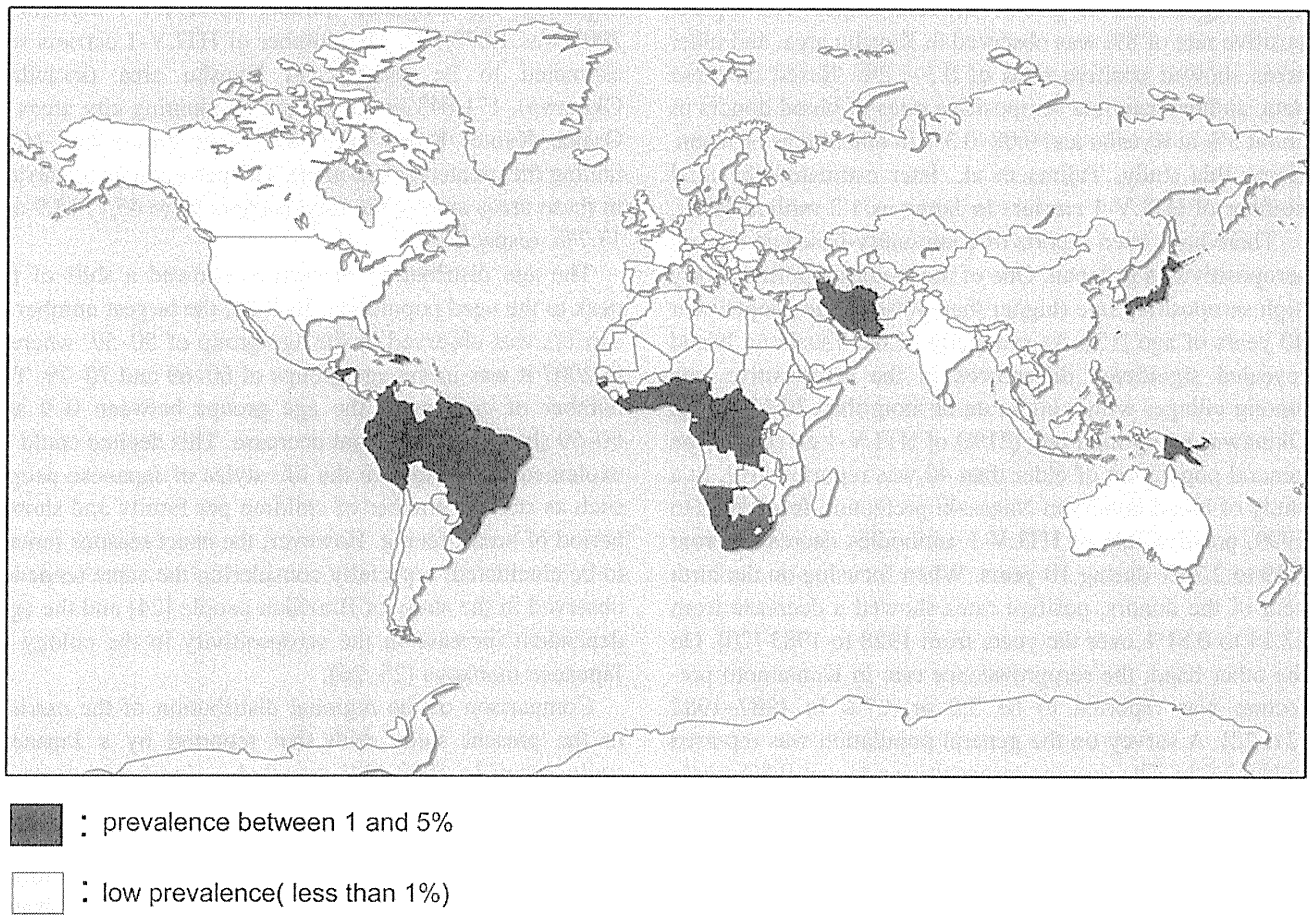

Fig. 1 Countries with endemic HTLV-I, defined as prevalence between 1 and 5% in some populations, are shown in *red*. Countries with reports of low prevalence (less than 1% in some groups), due mainly to immigration from endemic areas, are shown in *yellow*. It should be noted that HTLV-I endemic areas do not correspond exactly to the country boundaries shown in the map, for example, Brazil, Japan and Iran, where HTLV-I is limited to residents of certain areas of each country (modified from the reference [13])

concluded that there is a paucity of general population data from countries in which HTLV-1 is endemic, and that new studies are required to reevaluate the global burden of infection (reviewed in ref. [12] and [13]).

3 HTLV-1 Infection in Japan

3.1 Past studies of HTLV-1 carriers

Many efforts have been made to know the number of HTLV-1 carriers since the discovery of the virus in Japan. An example of early nationwide studies is the report of seropositive rates in the 15 blood centers of Japanese Red Cross [14]. It was reported that among 15 blood centers, 7 showed a higher positive rates between 6 and 30%, tested by indirect immunofluorescence assays (IFA). The other report is based on the data of all blood centers in Japan, which was the only study of all areas of Japan before the resent survey by Satake et al. [15]. They studied by IFA about 15,000 samples composed of 200 samples of blood donors aged from 40 to 64 from each center. The highest positive rate of 8% was observed in Kyushu area, and other areas showed positive rates of 0.3–1.2%. Based on these data, authors estimate seropositive rates of blood donors as about 3% in Kyushu and 0.08–0.3% in other areas of Japan. Using this study, Tajima et al., later estimated the total number of HTLV-1 carriers in Japan as 1.2 million [16].

There have been reports of community-based studies on seropositivities in Japan. One of the studies reported a very high seropositive rate (higher than 40%) in the people over 40 years of age [17]. An old study of the Tsushima Island revealed significant differences in the seropositive rate among villages with a high rate of more than 30% [18]. In Okinawa, a very high rate (21%) of HTLV-1 carriers in the general population of older than 40 was reported [19]. In a study of blood donors in Nagasaki prefecture from 1990 to 1999, positive rate of HTLV-1 antibodies decreased from 3.39 to 2.78% during 10 years. When focusing on the birth year of the donors, positive rates showed a decrease from 13.14 to 0.81% over the years from 1928 to 1983 [20]. On the other hand, the seroprevalence rate in Kumamoto prefecture was reported to be 3.6 or 4.7% in 1987–1988 [21, 22]. A survey on the general population was reported in Hokkaido. The average seropositive rate was 0.8% (male 0.6% and female 0.9%), with some regions showing higher seroprevalence rates as much as 5.2% [23].

Taken together, studies in 1980s and 1990s were mostly community-based ones using sera of blood donors. The oldest nationwide survey of the seroprevalence of HTLV-1 in blood donors and estimation of the number of HTLV-1 carriers [15, 16] had been referred to as the only published information until recently.

3.2 Recent studies of HTLV-1 infection in Japan

Based on the numbers of seropositive blood donors, Satake et al. have estimated the number of HTLV-1 carriers in Japan [15]. They analyzed data of blood donors who donated for the first time in 2006 and 2007, because Japanese Red Cross Blood center has notified the donors with the results of screening tests since 2000. This notification would have caused a bias in the population of total blood donors reducing the number of HTLV-1 carriers. In Satake's study, the total of number of tested was 1,196,321 (M: 704,074; F: 492,247), among them, HTLV-1 antibody was confirmed to be positive in 37,787 (M: 2,115; F: 1,672). Thus, the positive ratio was 0.32% for both male and female. Since the ages of blood donors were limited between 16 and 64, they estimated the seropositive rates of the peoples of younger than 15 or older than 65 by an assumption that the positive rate will increase exponentially in the young population, and for the aged people, by adding the average increase in the percentage in each age group in 20 years comparing with the data in 1988. Consequently, the estimated number of HTLV-1 carriers in 2007 was 1,078,722. The number of HTLV-1 carriers was estimated to be 492,582 in Kyushu area (including Okinawa), 171,843 in Kinki area (containing city areas of Osaka, Kyoto, Kobe) and 190,609 in Kanto area (containing the greater Tokyo area). The percentages of carriers in these areas among the total carriers were 45.7, 15.9 and 17.7%, respectively.

The age distribution of carriers showed a shift of the peak to the aged population. In 1988, the largest number of carriers was observed in the age group of 50–59, whereas in 2007 it was in the age groups of 60–69 and 70–79. The number of carriers in the age groups between 0–9 and 50–59 showed a significant decrease. This decline could be explained by changes in the life styles of Japanese people such as smaller number of children per family and shorter period of breast feeing. However, the exact reasons remain to be elucidated, especially considering the same tendency observed in the study of Brazilian people [24] and the age-dependent increase in the seropositivity in the colony of Japanese monkeys [25, 26].

Comparison of the regional distribution of the carriers in the present study with that reported by a Japanese study group in 1990 [27] revealed a significant decrease of the HTLV-1 carriers in Kyushu area (50.9 to 45.7%) and an increase in Kanto area (10.8 to 17.7%). The observed changes were considered to be mainly due to the migration of Japanese people from the Kyushu/Okinawa area to the metropolitan areas (Fig. 2). This interpretation is supported by the observation of Uchimaru et al. [28], who studied HTLV-1 carriers in Tokyo area and revealed that many of HTLV-1 carriers in Tokyo are either born in

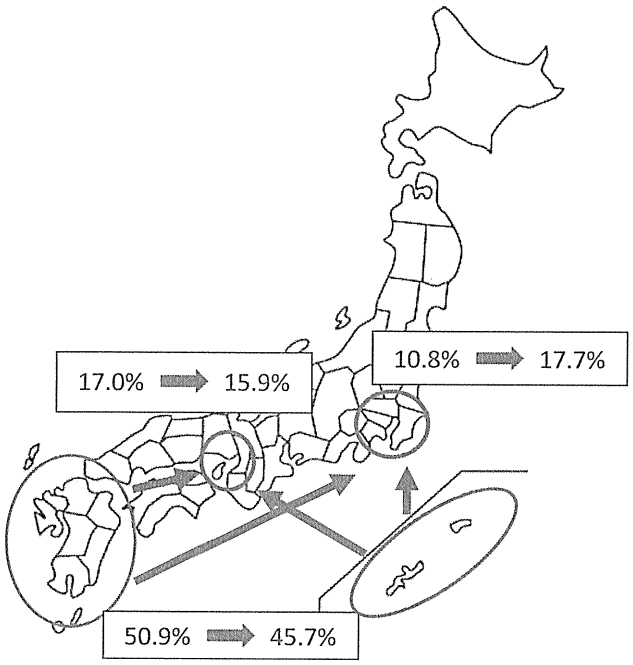

Fig. 2 Distribution of HTLV-1 carriers in Japan. Migration to the metropolitan areas is apparent. The number of HTLV-1 carriers in the endemic areas is still the largest, however, those in the great Tokyo area is significantly increasing

the endemic areas or the descendants of migrants from those areas.

4 Remaining problems and future directions

We have attributed the decrease in the HTLV-1 prevalence in Japan to the modernization and westernization of life styles of Japanese people. However, when we consider the same tendency in Brazil and age-dependent increase of seropositive rates in Japanese monkeys, we have to be cautious about interpretation of the observed data and may have to re-evaluate the meaning of the age-dependent carrier rates.

Another point that was raised by Satake's study is unexpectedly high increase in the positive rates in 20 years in the age-cohort [15]. This indicates the presence of horizontal transmission of the virus, probably through sexual contacts. This mode of infection should have contributed, at least to some extent, to the age-dependent increase in the positive rates. Thus, epidemiological studies on the horizontal transmission are definitely required; however, no such studies are now under way in Japan.

Taken together, we have to realize that we do not have enough data about the prevalence of HTLV-1 even in Japan, where serological data of blood donors are the only information to estimate the prevalence. Serological screening of the pregnant women that started in 2011 will provide valuable information about young females in Japan. Since the number of carriers who develop ATL is estimated about 1,200 per year in Japan, we have to expect more than 20,000 ATL patients from the present carriers in the future. In addition to the screening for the blood donors, prevention of mother-to-child infection by stopping breast feeding will greatly reduce the vertical transmission, nonetheless, there still remain other modalities of HTLV-1 infection, that are sexual transmission and possible trans-uterine infection. Neutralizing antibodies are often observed in carriers of HTLV-1 [29–32]. Furthermore, previous reports suggest that a primed immune response can be protective or prevent infection postviral exposure and challenge. It was shown that maternally acquired antibody protect infants from HTLV-1 infection in the early months of life [33]. A vaccine candidate based on an envelope expressing vaccinia virus provides protection to experimentally challenged primates [34, 35], and an attenuated viral strain provides long-term protection against the closely related bovine leukemia virus [36]. Taking all these into consideration, a costeffective vaccine may be a viable objective for prophylactic intervention in HTLV-1-endemic areas.

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感染に由来するヒトの腫瘍―その現状と対策

成人T細胞白血病ウイルスと白血病/リンパ腫

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要　旨　HTLV-1のキャリアは我が国の人口の約1%に相当する．ATLはいまだに治療抵抗性で予後不良の白血病/リンパ腫であり，母子感染予防，発症予防および新規治療法開発の3者が緊急の課題である．昨年末に策定された「HTLV-1感染総合対策」に基づき，これらの課題に取り組み，早急に成果を上げることが期待される．既存の薬剤の検証，抗体療法の開発，分子病態を基盤とした分子標的薬の開発が必要である．

はじめに

1977年に京都大学の高月らが「成人T細胞白血病（ATL）」を新たな疾患概念として報告してから30年以上が過ぎた．その後，ATLの研究からヒトで初めての白血病ウイルスが我が国と米国でほぼ同時に同定され，我が国ではATLV，米国ではHTLVとして報告された．後に，ウイルスの名称はHTLVに統一され，ATLの原因ウイルスであることが実験的に証明されて現在に至っている[1,2]．現在では，ATLは「HTLV-1感染細胞が腫瘍性増殖を示したもの」と定義されている[3]．本稿では，ウイルス感染による発癌のモデルとしてATL発症を位置づけ，最初に我が国におけるHTLV-1感染の現状，HTLV-1のウイルス分子の機能から感染宿主細胞の腫瘍化に与える影響について概説し，次に，50年にわたる時間の中で腫瘍化イベント蓄積した結果であるATL細胞内での分子病態解析結果の概略を整理し，最後に，ATL治療の現状と新たな試みに触れることにする．

■我が国におけるHTLV-1感染の現状

1980年代に多くの疫学研究がなされたが，それ以後20年にわたって感染の実態把握がなされていなかった．最近，厚生労働省科学研究費の研究班「本邦におけるHTLV-1感染及び関連疾患の実態調査と総合対策」が，2007年の献血者の抗体陽性率に基づく推計値を明らかにした[1]．その結果，全国で約107万9千人のキャリアがいるとの結果が得られた．同時に，同様のデータが得られる1988年の推定感染者数は130万5千人であった．全国を8ブロックに分けた地域分布から見ると，全感染者に占める割合は，九州・沖縄地区が45.7%と半数を下回り，首都圏を含む関東甲信越の割合が17.7%，大阪等の近畿地方が15.9%であり，大都市圏へのキャリアの移動が推察された．平成22年9月，菅　直人首相が首相官邸に「HTLV-1特命チーム」を設置し，感染予防，啓発活動，研究開発の促進等からなる「HTLV-1総合対策」を決定した．これによって，全国で妊

婦の抗体スクリーニングが実施され，母子感染予防に本格的に取り組む体制ができた．また，これまで継続性と総合性に欠ける点があったHTLV-1/ATLの研究開発体制も整備されるものと期待される．

■ATLの臨床疫学

ATLは20歳以上の成人に発症し，発症平均年齢は約60歳である．男女比では1.2：1と男性が多い．臨床的には，急性型（acute type），リンパ腫型（lymphoma type），慢性型（chronic type），くすぶり型（smoldering type）の4病型と，慢性型あるいはくすぶり型から急性型あるいはリンパ腫型へ変化する急性転化（blast crisis）という病態に分けられる．白血病で発症する症例が7割で残りの2〜3割がリンパ腫型である[2]．臨床的な特徴としては，皮膚病変，臓器浸潤，高カルシウム血症に加え，免疫不全による日和見感染症があげられる（図1）．厚生労働省の人口動態統計の死因から見ると，2000年代になって年間1,150人前後がATLで死亡している．厚生労働省の研究班の2009年度の実態調査によると，年間のATL患者数は1,146名と推定された[3]．

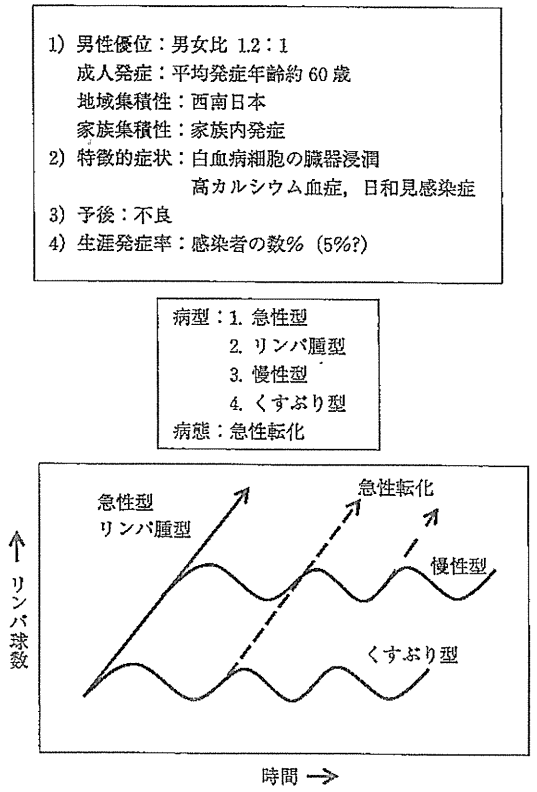

図1　ATLの臨床疫学的特徴

■HTLV-1による感染Tリンパ球の腫瘍化機構

HTLV-1がコードする蛋白質には，レトロウイルスに共通のGag, Pol, Envの構造蛋白質に加えて，TaxおよびRexという制御蛋白質，およびp12^{I}，p13^{II}，p30^{II}（I，IIはORFを表す）のアクセサリー蛋白質，およびアンチセンスから転写されるHBZが存在する（図2）．これらのウイルス蛋白質は，ウイルス粒子の構成成分に加えて，本来，ウイルスの効率的な自己複製とライフサイクルの完結に必要なものである．一方，これらの蛋白質が感染細胞内で発現すると，それが宿主細胞に対して様々な作用を及ぼす．Taxは分子量40kDaのリン酸化蛋白質であり，主に核内に局在する．ウイルス複製の観点から見ると，Taxはウイルス遺伝子の強力な転写活性化因子である．一方，感染細胞の不死化・腫瘍化に決定的な意義を持つと考えられているHTLV-1の制御蛋白質Taxの機能について，現在の知見を整理すると図4のようになる．その機能は，細胞性遺伝子発現の脱制御とシグナル伝達系の活性化を通じた細胞増殖とアポトーシス抑制，DNA修復機構の抑制と染色体異常の誘発によるゲノム不安定性の誘導という，共通の腫瘍化過程にかかわるものであり，腫瘍化のイニシエーションにかかわると考えられる（図3，4）．Taxの機能の詳細は，他の総説を参照されたい[4]．

最近では，他の非構造蛋白質であるp12^{I}，p13^{II}，p30^{II}の3つのアクセサリー蛋白質，およびアンチセンス転写産物とその翻訳産物HBZの機能が注目されているが，細胞の癌化への関与は明らかでない．アンチセンスから発現するHBZ

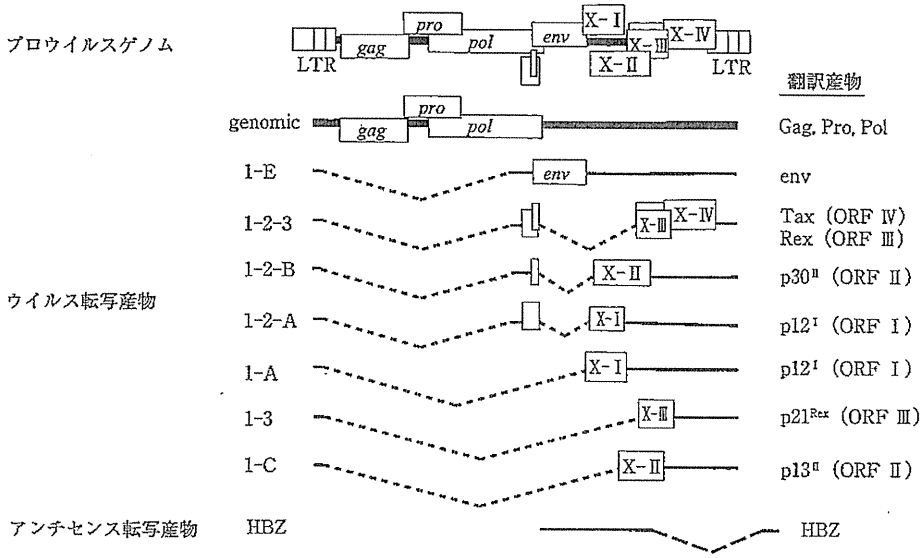

図2　HTLV-1プロウイルスの遺伝子構造と転写・翻訳産物

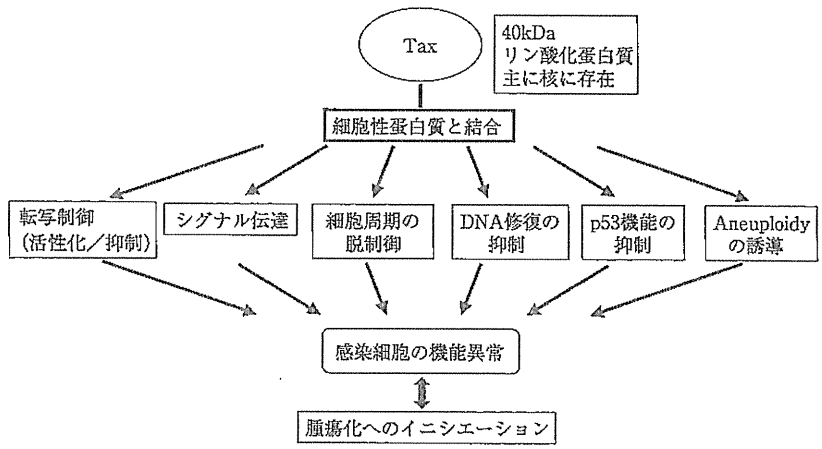

図3　Taxの多彩な機能

は，キャリアの感染細胞あるいはATL細胞で常に発現しているとの報告がある．HBZ蛋白質は約25kDaの核蛋白質であり，機能的にはTaxによる転写活性化に対して拮抗的に働き，生体内での感染効率を高める，あるいはHTLV-1で腫瘍化した細胞の増殖に促進的に機能し，この作用はRNAレベルでも認められるという報告もある[5]．

■ATL細胞の特徴

末梢血におけるATL細胞は典型的には「花細胞（flower cell）」と呼ばれ，過分葉してクロマチンの濃縮した核を持ち，普通明確な核小体を示さない．この特有の核の形態は，ATL細胞のAILIM/ICOSシグナルによるPI3-kinaseの活性化によることが報告されている[6]．ATL細胞に

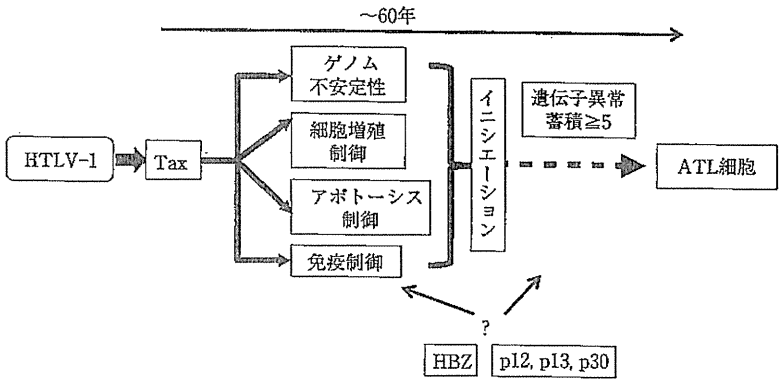

図4　HTLV-1の細胞腫瘍化機構

HTLV-1感染で発症するATLは多段階発癌機構によって発症する．ウイルスの癌遺伝子Taxは強力かつ多彩な機能をもち，免疫制御，アポトーシス制御，細胞増殖，ゲノム不安定性のすべてに関与するが，ATL細胞ではウイルスゲノムが欠損・変異したり，エピジェネティックに抑制されており，発現は認められない．したがって，イニシエーションに関与すると考えられている．HBZの作用に関する分子機序は明らかでない．

発現する膜抗原は，典型的にはCD3dullCD4$^+$CD8$^-$CD25$^+$HLA$^-$DR$^+$とされるが，CD25やHLA-DRの発現は全例に認められるわけではない．CD4$^+$CD8$^+$の例は約7%であり，CD4$^-$CD8$^-$も7%，CD4$^-$CD8$^+$の症例も4%の割合であると報告されている[7]．最近の報告では，急性型ATL患者の末梢血リンパ球をmulti-color FACSで検討し，腫瘍細胞がCD3(dim) CD7(low)のCD4$^+$Tリンパ球分画に対応することが確認された[8]．末梢血中の腫瘍細胞集団を同定し，その細胞生物学的表現型を解析する上で有用な情報であると考えられる．ATL細胞でFoxP3およびCTLA-4の発現があることから，一部には「ATLが制御性T細胞(Treg)が腫瘍化したものである」との考えがある．しかし，多くの報告から，FoxP3およびCTLA-4の発現が半数以下の例に限られること，*in vitro*でのCD4$^+$あるいはCD8$^+$Tリンパ球の増殖抑制能を持つのはさらにその一部であることが確認されている[9~12]．したがって，ATL細胞におけるFoxP3等の発現は，制御性T細胞の腫瘍化を示すものではなく，腫瘍化過程で獲得された形質の一つに過ぎないと考えるのが妥当であろう．

■ATL細胞の腫瘍化とプログレッション

岡本らは，ATL患者の年齢分布からWeibullのモデルを用いて，ATLの発症は典型的な多段階発癌モデルに一致すること，腫瘍化に至るには5つあるいはそれ以上のgenetic eventの蓄積があると考えられることを示した[13]．ATL細胞のサザンブロット法での解析結果から，ATLは「HTLV-1感染細胞が腫瘍化してモノクローナルに増殖したもの」と考えられている．しかし，複数のクローンが多段発癌のプロセスをたどり，ある時点では同時に2つ以上のクローンが腫瘍細胞として共存しうることを示唆する報告がある．最近のSetoらの報告では，ATL細胞がリンパ節内でprogressionして末梢血に出現することを示唆している[14]．したがって，生体内では，多段階のステップを種々のレベルまでたどったいくつかのクローンがオリゴクローナルに存在しており，そのうちの1あるいは数個のクローンが急速に増殖を示して顕在化したものが，臨床的に認知されるATLであり，さらに腫瘍化後もリンパ節においてclonal progressionを継続していると考えるのが妥当であると思われる．

■包括的なゲノム異常解析と遺伝子発現解析の現状

ATL細胞における遺伝子異常は，ある特定の遺伝子一つで説明できるものではないことが明らかである．そこで，ATL細胞におけるゲノム異常と遺伝子発現以上を包括的に解析し，確実なデータベースを構築して次の展開を図ることが必須である．

1. microarrayによる発現解析

最近，ATL細胞の遺伝子発現解析の結果が論文として報告されているが，その内容は，感染細胞株を対象にしたものと新鮮ATL細胞を解析したものに分かれる．*In vitro*で培養された細胞株を用いたデータの意義付けは限界があると考えられる．検体の解析も複数報告されているが，対象検体数が少ないため，一般性には限界があると考えられる．筆者らは，全国共同研究組織JSPFADによって構築されたマテリアルバンクを用い，50例のATL検体を対象にしたデータベースを構築し解析中である． 現在詳細は検討中であるが，全体的な特徴としては以下の点が指摘できる．①すでに発現異常が報告されている遺伝子の発現異常が確認された．②Tリンパ球以外の臓器組織特異的な遺伝子群の異所性過剰発現が認められる．③過剰発現遺伝子中に，機能未知遺伝子が多く含まれる．

ATL細胞におけるmiRNAの発現解析を報告した論文は3報ある[15~17]．これらの報告間では共通の結果が少なく，いずれがより正確な情報か判定できない．筆者らはATL 41検体について，CD4陽性細胞を対照として比較したデータベースを作成した．その結果，有意に発現レベルの異なる55の遺伝子が同定されている．その特徴は，①発現異常の実体は大多数で発現低下である．②ATL検体中で共通の発現異常を示すことが多い．③発現の欠損を示すmiRNAの標的遺伝子候補に，T細胞での重要な機能が知られている遺伝子が含まれる．現在，これらのmiRNA発現異常の機能的な意義の解析が進められている．

2. ゲノム異常の解析

筆者らは，これまでJSPFADのマテリアルバンクのATL170検体を用いて，SNPアレイチップと解析ソフトCNAGを用いてゲノムコピー数解析を進めてきた．その結果，ATL細胞における染色体異常の包括的な情報が得られたが，その特徴はコピー数の増幅，LOH，unipairental disomy（UPD）異常が染色体の一定の領域を単位として起こっていること，増幅や欠損領域にはTリンパ球で機能する重要な遺伝子が多数含まれ，増幅あるいは欠損を示していることなどが明らかになった．現在，これら約200遺伝子に注目し，その機能的意義の解析を進めている（未発表データ）．

■ATLの治療の現状と課題

1. 治療の現状と基本的方針

1）化学療法

従来の多剤併用化学療法の臨床治験の試みと成績を取りまとめて表1に示した．現時点では急性型，リンパ腫型およびハイリスクの慢性型ATLの治療の第1選択はLSG15（あるいはmodified LSG15）プロトコールであると考えられる．しかし，このような細胞毒性の強い治療法を適用できない患者が大多数であり，CHOP療法あるいは経口剤による対症療法で対処せざるを得ない例が多いのが実態である[18]．現在の診断と治療の流れを図5に示す．2007年に筆者が会長として箱根で開催した第13回「HTLV-1国際会議」での議論を元に，ATLの治療法に関する国際的な合意の形成が長崎大学の塚崎らによって取りまとめられた（表2）[19]．

2）血液幹細胞移植療法（SCT）

現在も臨床治験が進められている．予後不良のATLに対して行われたSCTのこれまでの成績を総括すると，生存期間中央値は延長し，30～40%

表1　JCOG-LSGによるATLの臨床治験

名称	期間	プロトコール名	症例数	CR (%)	PR (%)	MST (月)	生存率 (%)
JCOG 7801	1978～1980	LSG1(VEPA)	18	16.7	N/A	5	N/A
JCOG 8101	1981～1983	LSG1(VEPA)	24	16.7	N/A	7.5	8.3 (4年)
		LSG2(VEPA-M)	30	36.7	N/A		
JCOG 8701	1987～1990	LSG4	43	41.9	N/A	8	12 (4年)
							15.5 (2年)
JCOG 9109	1991～1993	LSG11	62	28.3	23.3	7.4	10.3 (5年)
JCOG 9303	1994～1996	LSG15	96	35.5	45.2	13	31.3 (2年)
JCOG 9801	1998～2003	mLSG15	57	40	32	12.7	24 (3年)
		mLSG19	61	25	41	10.9	13 (3年)

CR：complete response, PR：partial response, MST：生存期間中央値, N/A：not available

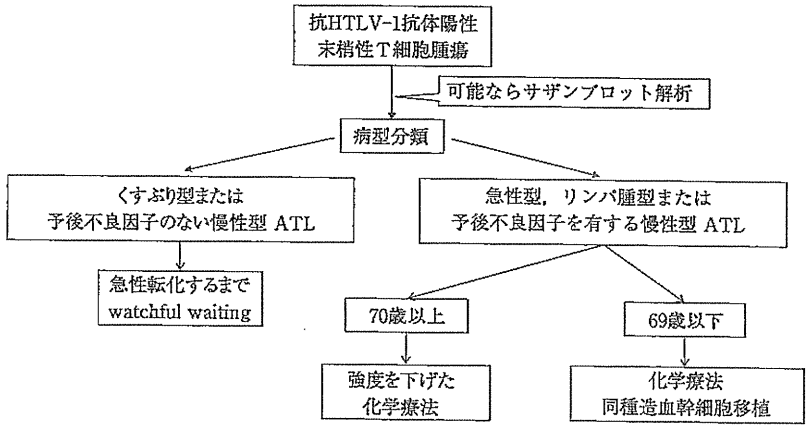

図5　ATLの診断と治療の流れ

の例で長期生存が期待できるが，治療関連死が多いということになる．ATLのSCTにはいくつかの問題・制約があると考えられる．患者の年齢が高いことで，一般のSCTの適応が限られること，HTLV-1非感染donorが得られにくいこと等である．このような背景から，前処置を軽減したreduced-intensity conditioning stem cell transplantation (RIST) が注目されており，その有効性に関する検討も進められている．また，臍帯血幹細胞移植 (UCBT) の有効性に関してはまだ明確なエビデンスが得られていない．また，HLAが適合したdonorが得られない場合の，HLA-haploidentical donorからのallo-SCTも検討課題であると思われる[18]．

2. 新たな治療法の模索

1) 化学療法

既存の治療法の現状が上記のように満足できる状態ではないので，新たな治療法，特に化学療法剤の開発が求められている[18]．Zidovudine (AZT) とinterferonの併用療法は，欧米のグループから多数の報告があるが，我が国でのデータは，平成22年度から開始された塚崎らの班研究で臨床研究の結果を待たなくてはならない．他の例としては，急性前骨髄球性白血病 (APL) での有効性が知られている亜ヒ酸がATLに対して

表2　ATLの治療戦略についての国際的合意による指針

1．くすぶり型，あるいは予後不良因子を有さない慢性型ATL
　臨床試験への参加
　有症候の場合(皮膚病変，日和見感染症など)：
　　interferon/zidovudine療法あるいはwatch and wait
　無症候の場合：watch and wait
2．予後不良因子を有する慢性型，あるいは急性型ATL
　臨床試験への参加
　化学療法（VCAP-AMP-VECP療法など）±同種造血幹細胞移植
　interferon/zidovudine療法
3．リンパ腫型ATL
　臨床試験への参加
　化学療法（VCAP-AMP-VECP療法など）±同種造血幹細胞移植
4．再発・難治のATL
　同種造血幹細胞移植の検討
　新薬開発の臨床試験への参加

参加国：日米英仏伯レバノン　　文献19)

も有効との報告があるが，我が国での追試はまだ行われていない．また，all-trans retinoic acid（ATRA）も，亜ヒ酸との併用効果に関しては検討の余地がある．新たな合成レチノイドとして，NIK333やAm80に関しても検討がなされている．ATL細胞ではNF-κBが恒常的に活性化されていることから，NF-κB阻害剤については，IKK阻害剤であるBay11-7082およびNF-κB核移行阻害薬DHMEQ等の前臨床のデータが報告されている．これらの臨床治験はまだ行われていない．プロテアソーム阻害剤であるbortezomibはNF-κBも阻害することが報告されており，臨床治験では非ホジキンリンパ腫の一部での有効性が報告されている．ATLに関しては有効性を示す前臨床レベルの報告がある．HDAC阻害剤では，一部の薬剤が皮膚T細胞リンパ腫（CTCL）に有効であることが報告されている．*In vitro*の実験ではHTLV-1感染細胞株および新鮮ATL細胞に対しての有効性が確認されており，臨床治験による検証が必要である．

2）抗体療法

現在，第Ⅱ相の臨床治験が進んでいる脱フコシル化CCR4抗体(KW-0761)は，早期に承認されて，臨床現場での使用が可能になることが期待される[19]．他にも，CD25, CD2, CD52, CD30およびトランスフェリンレセプター(CD71)に対する単クローン抗体を用いた抗体療法が検討中であり，一部は臨床治験の段階にある．ただ，ATLに対しての有効性を検証する臨床治験が十分行われていない．

おわりに

ATLはウイルス感染によって感染細胞そのものが多段階発癌の機構で腫瘍化する特異な癌である．したがって，ATL研究の方向性も，ウイルス側と腫瘍細胞側の2方向からのアプローチが必要である．しかし，ATL細胞ではHTLV-1の癌遺伝子に相当するTaxは発現していない．したがって，遺伝子異常を蓄積して腫瘍化したATL細胞を対象とした詳細な解析から，腫瘍化にかかわる分子機構を明らかにする作業が不可欠である．癌の発症機構の理解には，腫瘍細胞内に蓄積された遺伝子異常の実態と，発現する遺伝子の包括的把握が必須である．今後は，包括的なゲノム異常および遺伝子発現異常のデータベースを基盤とした知見を整理して，腫瘍化にかかわる遺伝子異常の実体を解明することが可能になってきた．治療法に関しては，画期的抗体療法の臨床応用が現実になりつつあるが，すでに検討されている多くの薬物に関してATLについての有効性を組織的に検証するとともに，新たな視点に基づく低分子化合物のスクリーニングも進める必要があると考える．これらの解析を通じて，腫瘍化を特徴づけるバイオマーカーの同定と，それに基づく早期診断，発症予防および新たな分子標的治療法の開発が期待される．

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特集 分子病態からみた血液疾患診療の進歩

ATLの分子病態と治療の新展開*

渡邉俊樹**

Key Words：micro RNA, signal transduction, molecular targeted therapy, antibody therapy

はじめに

2007年の献血者の抗体陽性率に基づく推定によると，わが国のHTLV-1感染者数は人口の約1％に近い108万人にのぼる[1]．また，厚生労働省の人口動態統計によると毎年1,000人以上が成人T細胞白血病(ATL)で死亡しており，同省研究班の全国調査によると，ATLの患者数は1,146例/年と推計された[1]．これらの事実は，ATLは疾患概念の確立および原因ウイルスHTLV-1の同定から30年を過ぎても，いまだに，わが国において，深刻ながんの一つであることを示している．ウイルス感染細胞のがん化の分子機構は「多段階発がん機構」によることは示されているが，それに関与する具体的な遺伝子の実態に関してはいまだに不明である(図1)．発症を予防する「発症予防法」の研究も進んでいない[1]．

ATL研究は，他のがんと同様に，その発症機構解明を通じた発症予防法・新規治療法開発による患者の救済を究極の目標とする．しかし，ATL研究の独自性は，そのアプローチに2つの道があることである．まず，HTLV-1感染細胞がウイルスの作用によって，最終的にはがん化するという事実から，ウイルスの遺伝子産物の作用という視点からの病原性発現機構解析である．この視点からは他の関連疾患の発症機構も視野に入ってくるであろう．もう一方の視点は，他のがん研究と共通するものであり，腫瘍細胞＝ATL細胞の解析を通じたアプローチである．ATL細胞の種々の分子細胞生物学的な表現型は，遺伝子

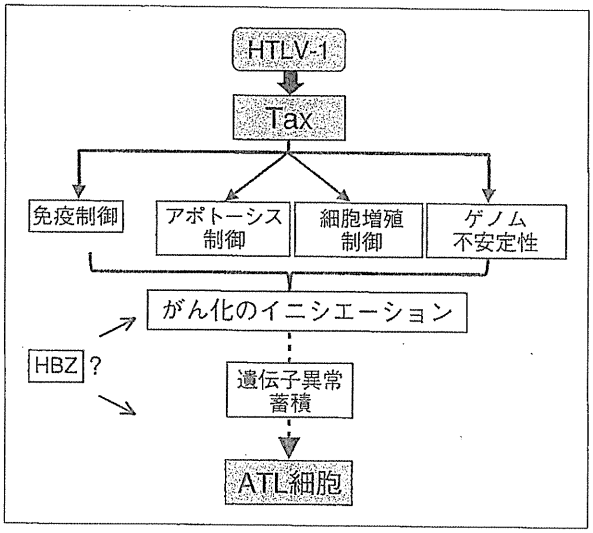

図1　HTLV-1の細胞腫瘍化機構
HTLV-1感染で発症するATLは多段階発がん機構によって発症する．ウイルスのがん遺伝子Taxは強力かつ多彩な機能を持ち，免疫制御，アポトーシス制御，細胞増殖，ゲノム不安定性のすべてに関与するが，ATL細胞ではウイルスゲノムが欠損・変異したり，エピジェネティックに抑制されており，発現は認められない．したがって，イニシエーションに関与すると考えられている．HBZの作用に関する分子機序は明らかではない．

* Recent advances in molecular pathology and therapy of ATL.

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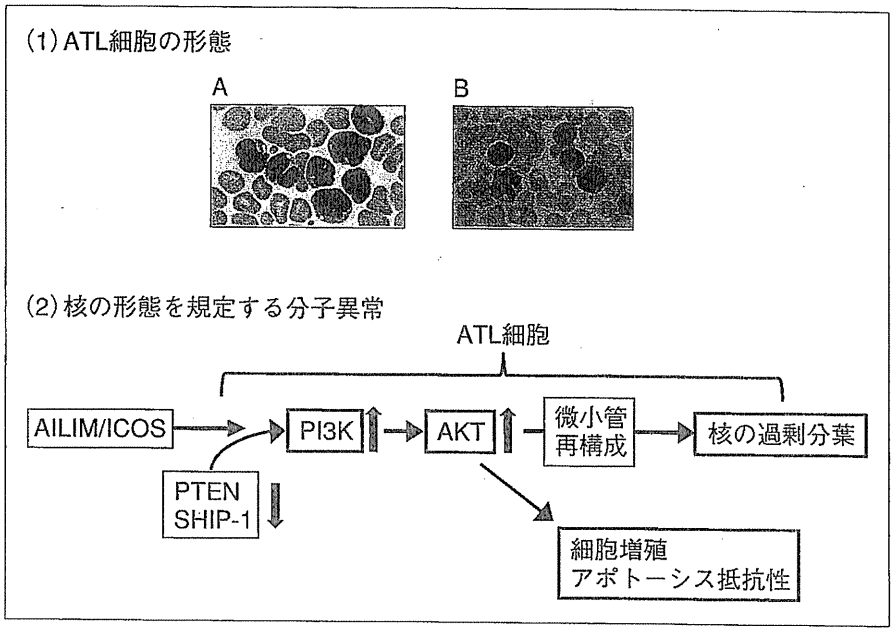

図2　末梢血のATL細胞と「花細胞」の分子基盤
(1) ATL患者末梢血像(今村病院分院 宇都宮與博士提供)．A：急性型ATLの末梢血．ATL細胞は核の変形が著明である．B：慢性型ATLの末梢血．やや小型であるが核の分葉やくびれを認める．(2) PTENやSHIP-1の抑制によるPI3-kinase, AKTの恒常的活性化が微小管の再構成を介して特徴的な分葉核を形成している．

異常の集積の結果を反映している．したがって，ATL細胞の分子病態を理解することは，単に診断のみならず腫瘍化機構の理解，さらには発症予防や治療を考える上で重要かつ必須の作業である[2]．

本稿では，ウイルス感染後約50年の臨床的潜伏期に腫瘍化イベントを蓄積した結果であるATL細胞の分子病態解析の現状を整理するとともに，ATL治療研究のまとめと，新規治療法の試みについて紹介することにする．

ATL細胞の分子病態

ATL細胞の細胞学的あるいは分子生物学的な特徴についてはこれまでに多数の報告が蓄積されている．以下にその概略をまとめる．

1．ATL細胞の細胞生物学的特徴

(1) 花細胞の分子基盤

末梢血におけるATL細胞は典型的には「花細胞＝flower cell」と呼ばれ，過分葉してクロマチンの濃縮した核を持ち，普通明確な核小体を示さない(図2)．この特有の核の形態は，ATL細胞のAILIM/ICOSシグナルによるPI3-kinaseの活性化によることが報告されている[3]．

(2) ATL細胞の膜抗原の発現

典型的には$CD3^{dull}CD4^{+}CD8^{-}CD25^{+}HLA\text{-}DR^{+}$とされるが，CD25やHLA-DRの発現は全例に認められるわけではない．$CD4^{+}CD8^{+}$の例は約7％であり，$CD4^{-}CD8^{-}$も7％，$CD4^{-}CD8^{+}$の症例も4％の割合であると報告されている[4]．最近の報告では，急性型ATL患者の末梢血リンパ球をmulti-color FACSで検討し，腫瘍細胞が$CD3^{dim}CD7^{low}CD4^{+}$Tリンパ球分画に対応することが確認された[5]．末梢血中の腫瘍細胞集団を同定しその細胞生物学的表現型を解析する上で有用な情報であると考えられる．

(3) ATL細胞はTregか？

ATL細胞で，FoxP3およびCTLA-4の発現があることから，一部には「ATLは制御性T細胞(Treg)が腫瘍化したものである」との考えがある．しかし，このようなとらえ方は早計であると判断せざるを得ない．多くの報告から，FoxP3およびCTLA-4の発現が半数あるいはそれ以下の例に限られること，in vitroでの$CD4^{+}$あるいは$CD8^{+}$Tリンパ球の増殖抑制能を持つのはさらにその一部であることが確認されている[6)~9)]．したがって，ATL細胞におけるFoxP3等の発現は，制御性T細