INTRODUCTION

Adult T-cell leukaemia/lymphoma (ATLL) is a neoplasm originating in mature CD4+ peripheral T cells. However, rare cases of CD20+ ATLL have been reported. Here, we describe six cases of CD20+ ATLL diagnosed in our department. The median age was 79 years (range, 54–90 years); two patients were men, and four were women. Elevated lactate dehydrogenase was observed in four cases. All cases were lymphoma type and positive for human T-lymphotropic virus-1 (HTLV-1). HTLV-1 proviral DNA was detected in four cases. The Ann Arbor stage was I, II, or IV in one patient each and III in three patients. The clinical course was poor in almost all cases. Tumour cells were large in all cases, and flow cytometry revealed CD20+ lymphoma cells in five of six cases. Immunohistochemistry revealed lymphoma cells positive for CD20, CD3, CD4, and CCR4 and negative for CD8, CD79a, and PAX5 in all cases. CD20 expression was lower than that in normal B cells. One case was initially misdiagnosed as diffuse large B-cell lymphoma. Thus, combined use of an antibody panel and molecular genetic studies is important to avoid misdiagnosing ATLL as B-cell lymphoma. (J Clin Exp Hematop 56(2):119-125, 2016)

Keywords: adult T-cell leukaemia/lymphoma, CD20, human T-lymphotropic virus 1

Six Cases of CD20-Positive Adult T-Cell Leukemia

Riko Kawano,1) Daisuke Niino,2) and Koichi Ohshima1)

MATERIAL AND METHODS

Cases

 Several studies have examined the markers of ATLL cells. Typically, ATLL cells exhibit the helper inducer T-cell phenotype, i.e., CD2+CD3+CD4+CD8-CD5+CD25+HLA-DR+. Some rare cases have shown the phenotypes of CD4-CD8+, CD4+CD8+, or CD4-CD8+. Moreover, it is critical to differentiate between T cells and B cells when diagnosing malignant lymphoma. Therefore, in the diagnosis of malignant lymphoma, CD20 and CD3 immunostaining should be routinely performed. Notably, CD20 is a common marker of B cells, and ATLL cells are generally negative for CD20. However, CD20+ ATLL has been reported in rare cases. These cases were also shown to express CD3, CD4, and CD25.

In the study, we identified six cases of CD20+ ATLL diagnosed at our department, and analysed the clinical and pathological features.

Received: March 25, 2016
Revised: July 15, 2016
Accepted: July 19, 2016

1)Second Department of Pathology, Kurume University School of Medicine, Fukuoka, Japan
Corresponding author: Professor Koichi Ohshima, Second Department of Pathology, Kurume University School of Medicine, 67 Asahi-machi, Fukuoka, 830-0011, Japan
E-mail: ohshima_kouichi@med.kurume-u.ac.jp
Histological and immunohistochemical studies

Tissue samples were fixed in 10% buffered formalin, embedded in paraffin, and processed using conventional histological and immunohistochemical methods. Sections (5 μm) were stained with hematoxylin and eosin (H&E) for histological evaluation. The remaining serial unstained sections were used for immunohistochemistry. All specimens were histologically diagnosed according to the World Health Organization criteria.

Immunohistochemical imaging was performed on paraffin-embedded sections following heat-induced antigen retrieval, staining with the appropriate antibodies, and signal detection using the immunoperoxidase methods (ChemMate ENVISION kit/HRP [DAB]; DakoCytomation; Dako, Glostrup, Denmark) using an autostainer (Dako Autostainer Universal Staining System). We performed immunostaining using monoclonal antibodies against human CD20 (dilution 1:5; IR604; Dako), human CD3 (dilution 1:50; M7254; Dako), human CD4 (dilution 1:30; 790-4423; Ventana, Tucson, AZ, USA), human CD8 (dilution 1:50; NCL-L-CD8-295; Leica, Newcastle Upon Tyne, UK), CD45RO (UCHL-1; dilution 1:200; NCL-UCHL1; Leica), human CD79a (dilution 1:400; M7050; Dako), human PAX5 (dilution 1:5; IR604; Dako), human CD3 (dilution 1:50; M7254; Dako), human CD20, human CD3, human CD4, human CD5, human CD7, human CD8, human CD10, human CD11c, human CD16, human CD25, human CD30, human CD34, and human CD56; and (iii) double positive or double negative for CD4/CD8.

In situ hybridization

In situ hybridization for the detection of Epstein-Barr Virus-encoded nuclear RNA (EBER) was performed on paraffin-embedded sections using standard procedures and commercially available reagents (dilution 1:50; Y5200; Dako).

Flow cytometry

Flow cytometry was performed on a FACSCalibur flow cytometer (Becton-Dickinson) using Cell Quest software (Becton-Dickinson) and conventional methods described previously. Briefly, cells were stained with fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-labelled monoclonal antibodies using the following combinations: CD2 (FITC), CD3 (FITC), CD4 (FITC), CD5 (PE), CD7 (PE), CD8 (PE), CD10 (PE), CD11c (PE), CD16 (FITC), CD19 (PE), CD20 (FITC), CD25 (PE), CD30 (FITC), CD34 (FITC), and CD56 (PE). Monoclonal antibodies targeting CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD16, CD19, CD34, and CD56 were obtained from Coulter-Immunotech (Hialeah, FL, USA); those targeting CD11c, CD20, and CD25 as well as monoclonal kappa and lambda immunoglobulins were obtained from Becton-Dickinson; and those targeting CD23 and CD30 were from Coulter-Immunotech and Dako Cytomation, respectively. B-cell clonality was confirmed when evaluation of surface immunoglobulin expression showed bright monocytic light chain expression. In contrast, T-cell clonality was confirmed when the expression pattern was considered abnormal and to reflect a tumour cell pattern when one of the following three conditions was met: (i) partly positive or negative for at least a single pan-T-cell marker (CD2, CD3, CD5, and CD7); (ii) positive or partly positive for at least a single aberrant marker (CD10, CD11c, CD16, CD25, CD30, CD34, and CD56); and (iii) double positive or double negative for CD4/CD8.

Polymerase chain reaction (PCR) for the detection of TCRγ rearrangements

The TCRγ locus consists of 14 variable (Vγ) genes that can be organized into four subgroups and five join (Jγ) genes. Because the VγI (VγI–8) subgroup genes and the Jγ gene segments are preferentially used in the majority (approximately 70%) of TCRγ rearrangements in lymphoid malignancies, we restricted our study to the analysis of TCRγ rearrangements involving these segments. The high homology between the genes of the VγI subgroup allowed the construction of a consensus primer (TCR-GV1). For the Jγ region, the consensus J12, JP12, and JP primers were designed to anneal to a completely homologous region of published sequences (Jγ1 and Jγ2, JγP1 and JγP2, and JγP, respectively). These primers were used as a mixed primer J-Mix containing J12, JP12, and JP primers. The primer sequences were as follows: TCR-GV1, 5'-CACCGAGGAGGGAGGAGCCTT-3'; J12, 5'-GACAAC(A/C)ACGGTGGTTCTCCACTGCA-3'; JP12, 5'-TTACCGGG(T/G)TACCAGC-3'; and JγP, 5'-TAATGATAAGCCTTGGACCC-3'. PCR amplification was performed with AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA) using the primers TCR-GV1 and J-Mix. PCR cycling conditions were as follows: an initial denaturation at 95°C for 10 min; followed by 25 cycles of 95°C for 30 sec, 65°C for 30 sec, and 72°C for 30 sec; and a final extension at 72°C for 10 min. The amplified products were evaluated by 3% agarose gel electrophoresis and visualized by ethidium bromide staining under ultraviolet light. PCR products of TCRγ rearrangements appeared in the size range between 230 and 250 bp.

PCR for the detection of IgH rearrangements

Amplification of the IgH gene from the framework two part of the V segment to the J region was carried out using

Kawano R, et al.
the consensus primers complementary to the framework two portion of the VH region (FR2B) and the JH region (CFW1) from genomic DNA. The sequences of the primers used were as follows: FR2B, 5’-GTCCCTGAGGG(C/T)(C/T) CC(G/A)AA(A/G)(A/G)GTCTGGAGTG-3’; CFW1, 5’-ACCTGAGGAGC(GTTGACCAGGT-3’. The PCR conditions were as follows: initial denaturation at 95°C for 10 min; five cycles of 95°C for 30 sec, 63°C for 30 sec, and 72°C for 30 sec; 45 cycles of 95°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec; and a final extension at 72°C for 10 min. Analysis of PCR products was performed as described above for TCRγ amplification. The size of IgH rearrangement fragments was usually between 250 and 300 bp.

RESULTS

Patients

We examined six cases of CD20+ ATLL. The median age of patients was 79 years (range, 54–90 years). Two patients were men, and four were women. Laboratory data showed an elevation of lactate dehydrogenase (LDH) in four of six cases. In all cases, serum test results were positive for HTLV-1, and HTLV-1 proviral DNA was detected in four of the six cases (i.e., cases 3, 4, 5, and 6). The Ann Arbor stage was I, II, III, or IV in one, one, four, and zero patients, respectively. Liver dysfunction was detected in case 1 (Table 1). We investigated the clinical course in cases 1, 2, 4, 5, and 6 (Table 1) and found that the survival time ranged from 10 days to 9 mon.

Pathological histology

Lymph nodes showed diffuse proliferation of large lymphoid cells (Fig. 1).

Immunostaining

As shown in Table 2 and Fig. 1, CD20 was weak to strongly positive (20–90%) in all cases, and CD45RO was weak to strongly positive (20–100%) in tumour cells. CD3 was strongly positive in all cases, and CD4 was positive in cases 1, 2, 3, 4, and 6. CD8 was negative in all cases, whereas CD79a was positive in only one case (case 6). PAX5 was negative in all cases, and CCR4 was positive in all cases. FOXP3 was positive in cases 2, 3, and 5. In cases 4 and 5, EBER-positive cells were scattered in the background (Fig. 1).

Flow cytometry

Flow cytometry revealed that all five of the analysed cases (cases 2, 3, 4, 5, and 6) were positive for CD20 (Table 3).

Table 1. Clinical data

| No. | 1 | 2 | 3 | 4 | 5 | 6 |
|-----|---|---|---|---|---|---|
| Age | 54 | 88 | 65 | 90 | 73 | 84 |
| Sex | Female | Female | Male | Female | Male | Female |
| Site | Neck Clavicle superior fovea | Neck | Skin Eyelid | Neck Abdominal cavity | Axilla Neck | Neck Abdominal cavity Inguinal region Mediastinum | Neck Axilla Inguinal Abdominal cavity |
| Fever | + | - | - | - | - | - |
| Hepatosplenomegaly | Liver dysfunction (AST 69, ALT 120, γGTP 191) | - | - | - | - | - |
| LDH | 1,018 | 192 | 188 | 345 | 609 | 1,226 |
| PS | 1 | 1 | 0 | 0 | 0 | 1 |
| Stage | III | I | III | II | III | III |
| Extranodal region | - | - | + | - | - | - |
| HTLV-1 antibody | + | + | + | + | + | + |
| sIL-2R | 23,100 | 5,130 | 1,490 |
| Survival time | 6 mon | 9 mon | - | 6 mon | 6 mon | 10 days |

AST, aspartate aminotransferase; ALT, alanine transaminase; γGTP, γ-glutamyl transpeptidase; HTLV, human T-lymphotropic virus
Fig. 1. Histological and immunohistochemical studies (cases 4 & 5). CD20 was weak to strongly positive, and CD3 was strongly positive. Epstein-Barr virus-encoded small RNA (EBER)-positive cells were scattered in the background.

Fig. 2. TCRγ rearrangement and IgVH gene rearrangement on polymerase chain reaction (PCR).

Table 2. Morphological and immunohistochemical features

| No. | Neck Lymph node | Neck Lymph node | Neck Lymph node | Axillary lymph node | Neck lymph node | Lymph node |
|-----|-----------------|-----------------|-----------------|--------------------|----------------|-----------|
| Biopsy site | Diffuse | Diffuse | Diffuse | Diffuse | Diffuse | Diffuse |
| Growth pattern | Large | Large | Large | Large | Large | Large |
| Cell size | Pleomorphic | Pleomorphic | Pleomorphic | Pleomorphic | Pleomorphic | Pleomorphic |
| Nucleus | + | + | + | + | + | + |
| Mitosis | CD20/CD3 (%) | 50 | 80 | 50 | 90 | 80 | 20 |
| CD20 | + | + | + | + | + | + |
| CD3 | + | + | + | + | + | + |
| CD4 | - | - | - | - | - | - |
| CD8 | - | - | - | - | - | - |
| CD45RO/CD3 (%) | 20 | 70 | 20 | 20 | 90-100 | 60 |
| CD79a | - | - | - | - | - | + |
| PAX5 | - | - | - | - | - | - |
| CCR4 | + | + | + | + | + | + |
| FOXP3 | - | + | + | - | + | - |
| EBV-ISH | - | - | - | (+) | (+) | - |

(+), positive cells in the background; EBV-ISH, Epstein-Barr virus-in situ hybridization
**HTLV-1 proviral DNA**

HTLV-1 proviral DNA was analysed and found to be positive in four cases (data not shown).

**Table 3. Flow cytometry analysis**

| No. | 1   | 2   | 3   | 4   | 5   | 6   |
|-----|-----|-----|-----|-----|-----|-----|
| CD2 | ND  | 66.2| 94.1| 51.9| 5.7 | 97.4|
| CD3 | ND  | 18.0| 11.3| 49.0| 4.1 | 30.3|
| CD4 | ND  | 20.9| 93.5| 38.4| 2.9 | 88.8|
| CD5 | ND  | 79.0| 77.2| 54.0| 99.3| 17.2|
| CD7 | ND  | 71.7| 9.3 | 47.8| 80.1| 15.9|
| CD8 | ND  | 3.7 | 1.5 | 11.6| 12.9| 10.5|
| CD20| ND  | 71.6| 49.0| 55.9| 81.4| 70.7|
| CD25| ND  | 24.6| 64.5| 16.0| 38.5| 71.3|
| CD30| ND  | 10.1| 3.9 | 1.4 | 30.9| 16.6|
| ND, not done |

**DISCUSSION**

ATLL cells are usually negative for CD20. However, we encountered six cases of CD20+ ATLL in our department. All cases showed a poor clinical course with CD20, CD45RO, and CD3 expression. PAX5, a transcription factor expressed in pro- and premature B cells, was negative in all cases. TCRγ rearrangements were observed in all cases, whereas no IgH rearrangements were observed.

**Chromosome test**

As shown in Table 4, samples from five patients were subjected to chromosome examination (cases 2, 3, 4, 5, and 6). A complex karyotype was observed in all five cases.

**Gene rearrangement**

TCR rearrangement and IgH rearrangement were analysed in all cases. TCR rearrangement was found in all cases, whereas IgH rearrangement was not detected in any case (Fig.2).

**Table 4. Chromosome test**

| No. | 1 | Not done |
|-----|---|----------|
| 2   | 47,X,- | 47,X,- X\(_y\),add(1)(q42),add(2)(q31)x2,dup(3)(p21p25),+add(4)(p11),add(4)(q21),del(5)(q?),add(10)(p11.2),add(12)(q13),add(22)(p11.2)+mar1(19)/46,XX[1] |
| 3   | 46,XY,add(5)(q33), add(10)(p11.2), -14, add(21)(p11.2), +mar1[1]/46.idem,-14,+mar2[7]/46.idem,ins(11;?) (q13;?) -14,+mar3[4] |
| 4   | 48,XX,add(4)(q31), -5,add(5)(p11), -9, -9, add(12)(p11.2), -16, -17, +7mar[1]/46,XX[18] |
| 5   | 60<2n>,X,- | 60<2n>,X,- Y\(_y\),+add(X)(p22.1), +add(1)(p13), add(1)(q11), add(2)(p11.2), add(2)(p23), add(3)(q11.2), add(3)(q27), del(5)(q?), -6, del(6)(q?), +add(7)(q11.2), +add(8)(q22), +add(11)(p11.2), add(12)(q24.1), -13, add(14)(q32), add(14)(q32), 15, 17, 17, 18, -22, +18mar[1]/46,XY[8] |
| 6   | 87,XX,-X,-X,add(1)(p13),add(1)(p32)x2,-2,-3,add(3)(q11.2)ins(3;?) (p21;?) x2,add(4)(q31), -7, -8, del(10)(p13)x2,-13,-15,+17,-18,-20,-20,+5mar[1]/46,XX[5] |
findings also suggested a T-cell origin. These rare findings provide important insights into the pathogenesis of CD20+ ATL.

The EBER+ lymphocytes were scattered in the background in two cases; however, no tumour cells showed EBV infection. Generally, EBV infects normal B lymphocytes, and when the percentage of EBV+ cells increases, B lymphocytes are also increased. In this study, the EBER+ lymphocytes detected in the background were thought to be B cells. Moreover, there were no correlations between CD20+ tumour cells and EBER+ cells. Thus, the CD20+ tumour cells in this study were shown to originate from T cells, and CD20 was aberrantly expressed by tumour cells.

Notably, when diagnosing ATLL, in order to prevent misdiagnosis, it is necessary to carefully analyse the histopathological features of the samples. For immunostaining of suspected malignant lymphoma, routine analyses include CD20 and CD3 immunostaining. CD3 is a T-cell marker; however, CD3+ cases have been reported on occasion. Additionally, CD3+ B-cell lymphomas have been reported. We recommend immunostaining for CD3, CD4, and CD8, additional T-cell markers, as well as CD79a and PAX5, which are B-cell markers. Further analysis of surface markers of lymphocytes is also necessary for diagnosing malignant lymphoma, and flow cytometry is a useful method in this regard. CD20 is only weakly expressed in a subset of normal peripheral blood T cells. Additionally, based on the expression of CD20 in T-cell lymphomas, the possibility of transformation of neoplastic CD20+ T cells has been reported. In our cases, CD20+ cells were also positive for CD3, CD4, and CCR4; therefore, we concluded that CD20 was expressed in ATLL cells.

In a report by Rahemtullah et al., CD20 expression in T-cell lymphomas was shown to be weaker than that in normal B cells, suggesting neoplastic transformation of a normal CD20dim T-cell subset. Twenty-six case reports of CD20+ T-cell lymphomas have been published, and CD20+ ATLL have been reported, with substitution of liposomal doxorubicin for conventional chemotherapy. However, the patient relapsed within 1 month. Thus, these data suggested that rituximab may not be effective for the treatment of CD20+ T-cell lymphoma.

Finally, when diagnosing ATL, in order to prevent misdiagnosis, it is necessary to carefully analyse and confirm immunostaining, flow cytometry, and histopathological findings.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Takatsuki K, Yamaguchi K, Kawano F, Hattori T, Nishimura H, et al.: Clinical diversity in adult T-cell leukemia-lymphoma. Cancer Res 45(9 Suppl):4644s-4645s, 1985
2. Tajima K, Hinuma Y: Epidemiology of HTLV-I/II in Japan and the world. Gann Mono Can Res 39:129-149, 1992
3. Kress AK, Grassmann R, Fleckenstein B: Cell surface markers in HTLV-I pathogenesis. Viruses 3:1439-1459, 2011
4. Yasukawa M, Arai J, Kikimoto M, Sakai I, Kohno H, et al.: CD20-positive adult T-cell leukemia. Am J Hematol 66:39-41, 2001
5. Buckner CL, Christiansen LR, Bourgeois D, Lazarchick JJ, Lazarchick J: CD20 positive T-cell lymphoma/leukemia: a rare entity with potential diagnostic pitfalls. Ann Clin Lab Sci 37:263-267, 2007
6. Ohshima K, Haraoka S, Suzumiya J, Kawasaki C, Kanda M, et al.: Cytoplasmic cytokines in lymphoproliferative disorders: multiple cytokine production in angioimmunoblastic lymphadenopathy with dysproteinemia. Leuk Lymphoma 38:541-545, 2000
7. Rahemtullah A, Longtine JA, Harris NL, Dorn M, Szwajkoszcz A, et al.: CD20+ T-cell lymphoma: clinicopathologic analysis of 9 cases and a review of the literature. Am J Surg Pathol 32:1593-1607, 2008
8. Storic I, Wilson GA, Granger V, Barnett D, Reilly JT: Circulating CD20dim T-lymphocytes increase with age: evidence for a memory cytotoxic phenotype. Clin Lab Haematol 17:323-328, 1995
9. Quintanilla-Martinez L, Preffer F, Rubin D, Ferry JA, Harris NL: CD20+ T-cell lymphoma. Neoplastic transformation of a normal T-cell subset. Am J Clin Pathol 102:483-489, 1994
10. Matnani RG, Stewart RL, Pulliam J, Jennings CD, Kesler M:...
Peripheral T-cell lymphoma with aberrant expression of CD19, CD20, and CD79a: case report and literature review. Case Rep Hematol 2013:183134, 2013 [doi: 10.1155/2013/183134]

Kamata M, Sugaya M, Miyagaki T, Sonoda K, Ichimura Y, et al.: A case of CD20-positive peripheral T-cell lymphoma treated with rituximab and multiagent chemotherapy. Int J Dermatol 53:e24-e26, 2014