Serum level of soluble interleukin-2 receptor correlates with CD25 expression in patients with T lymphoblastic lymphoma

Tomohiro Toji a, Katsuyoshi Takata a, Yasuharu Sato a, Tomoko Miyata-Takata a, Eiko Hayashi b, Toshiyuki Habara c, Yoshinobu Maeda d, Mitsune Tanimoto d, Tadashi Yoshino a

aDepartment of Pathology and dDepartment of Hematology, Oncology and Respiratory Medicine Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Science, Okayama, Japan

bDepartment of Pathology, Iwakuni Medical Center, Iwakuni, Japan

cDepartment of Clinical Laboratory, Chugoku Central Hospital of the Mutual Aid Associations of Public School Teachers, Hiroshima, Japan.

*Corresponding Author: Katsuyoshi Takata, M.D., Ph.D.
Department of Pathology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama City, Okayama 700-8558, Japan

Tel. +81 86 235 7150; Fax. +81 86 235 7156

E-mail: katsuyoshi.t@h5.dion.ne.jp
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Abstract

Lymphoblastic leukemia/lymphoma (ALL/LBL) is an aggressive form of non-Hodgkin lymphoma (NHL) affecting B- or T-cells, respectively. The serum level of soluble interleukin-2 receptor (sIL-2R) is known to reflect the immune activity and tumor volume in aggressive NHL; however, the release of sIL-2R in LBL has not been extensively studied. Further, the relationship between sIL-2R release with the expression level of IL-2R α subunit (CD25) remains unknown. In the present study, we examined the serum level of sIL-2R in 23 patients with T-LBL and compared these to the levels in 20 patient with T acute lymphoblastic leukemia (T-ALL), 40 patients with diffuse large B-cell lymphoma (DLBCL) and 40 patients with peripheral T-cell lymphoma, not otherwise specified (PTCL). The release of sIL-2R into the serum in T-LBL patients was significantly lower than that for T-ALL, DLBCL, and PTCL ($P < 0.001$). Immunohistochemistry revealed that CD25 expression was correlated with the serum level of sIL-2R in T-LBL ($P = 0.0069$), whereas no correlation was found to exist between serum sIL-2R levels and CD25 expression in DLBCL ($P = 0.348$) and PTCL ($P = 0.266$) patients. Furthermore, double immunohistochemical analysis revealed that CD25-positive cells were also found to be Foxp3-positive non-neoplastic T-cells. In conclusion, CD25-positive non-neoplastic T-cells in T-LBL are presumed to be the primary source of sIL-2R, and the low number of cells present results in a lower level of
sIL-2R released into the serum compared to the other aggressive and highly aggressive lymphomas.
1. Introduction

Lymphoblastic leukemia/lymphoma (ALL/LBL) is caused by neoplasm of immature B or T-cells and can affect numerous tissues, including bone marrow, blood, and sometimes nodal and extranodal sites. LBL accounts for approximately 2% of all lymphomas and is a subtype of aggressive non-Hodgkin lymphoma (NHL). Notably, immune activity and tumor volume in NHL has been linked to the serum levels of soluble interleukin-2 receptor (sIL-2R).\textsuperscript{1} Elevation of serum sIL-2R expression has been reported in patients with a variety of hematological malignancies, such as adult T-cell leukemia/lymphoma (ATLL), Hodgkin’s lymphoma (HL), diffuse large B-cell lymphoma (DLBCL), and peripheral T-cell lymphoma not otherwise specified (PTCL),\textsuperscript{2-6} as well as in patients with solid malignancies and autoimmune disorders.\textsuperscript{7-9} sIL-2R consists of three non-covalently associated subunits, $\alpha$ (CD25), $\beta$ (CD122), and $\gamma$ (CD132). To the best of our knowledge, there have been only a few reports on the serum levels of sIL-2R and CD25 expression in malignant lymphoma\textsuperscript{10,11} and only one report on the serum levels of sIL-2R in LBL patients.\textsuperscript{12} Furthermore, the relationship between serum sIL-2R levels and CD25 expression in LBL is still unknown. In the present study, we compared serum levels of CD25 in T-LBL, T-ALL, DLBCL and PTCL, examined CD25 expression in T-LBL, DLBCL, and PTCL patients by immunohistochemical analysis in order
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to determine the relationship between the histological features of these different lymphomas and serum sIL-2R levels.

2. Materials and methods

2.1. Patient selection

We recruited 23 T-LBL patients (age range, 12 – 77 y; median age, 34 y; 7 males [30%] and 16 females [70%]), who were diagnosed at the Department of Pathology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences between 2004 and 2012. The diagnosis was based on the 2008 World Health Organization classification and the cases in each study were reviewed by 4 hematopathologists (K.T., Y.S., T. M-T. and T.Y.). Patients with greater than 25% bone marrow (BM) involvement or more than 5% of the peripheral blood (PB) contained lymphoma cells were excluded to avoid inclusion of patients with leukemia. Patients in control groups (n= 100) were included 20 patients with T-ALL, 40 patients with DLBCL and 40 patients with PTCL. The sIL-2R serum levels for the control group individuals were measured at the time of initial diagnosis. The patients’ serum sIL-2R levels were determined by a commercial laboratory using a sandwich enzyme-linked immunosorbent assay (ELISA). Clinical data were kindly provided by the referring clinicians or obtained from the medical records of the patients. This study
was conducted with the approval of the Institutional Review Board of Okayama University, Okayama, Japan. All study procedures were conducted in accordance with the guidelines of the Declaration of Helsinki.

2.2. Histological examination and immunohistochemistry

Tissue samples were fixed in 10% formalin and embedded in paraffin. Serial sections of 4 µm were cut from each paraffin-embedded tissue block, and several sections of each block were stained with hematoxylin and eosin. Immunohistochemistry was also performed on paraffin sections with a panel of antibodies. Following antigen retrieval using Bond Epitope Retrieval Solution 1 (pH 6.0) or Solution 2 (pH 9.0) (Leica Biosystems Newcastle Ltd, UK), the tissue sections were automatically stained using a Bond-Max autostainer (Leica Biosystems Melbourne Pvt. Ltd., Australia) with the Bond Polymer Refine and AP Red Detection systems (Leica Biosystems Newcastle Ltd) as described previously. The following primary antibodies (clone, dilutions) were used in this study: CD20 (L26, 1:200); CD3 (LN10, 1:200); CD25 (4C9, 1:100); and terminal deoxynucleotidyl transferase (TdT) (SEN28, 1:500) purchased from Novocastra (Newcastle-upon-Tyne, UK); and Foxp3 (236A/E7, 1:100) purchased from Abcam (UK). Positive and negative control samples were used for each antibody. For all antigens, samples were scored as positive when 30% or more of the cells were positively stained. Cells counts for each tissue were obtained by averaging the absolute
number of immune-reactive cells counted on the immunostained slides in four
high-power fields (HPF; 400 × magnification).\textsuperscript{15}

2.3. Statistical analysis

SPSS software (version 14.0; SPSS Inc., Chicago, IL) was used for statistical
analysis. Correlations between the serum sIL-2R levels in each patient group and tissue
CD25 expression were tested using the Pearson’s correlation coefficient. Statistical
comparisons between the patient groups and the control group were performed using the
paired-sample $t$-test. A $p$-value less than 0.05 was considered statistically significant.

3. Results

3.1. Comparison of the sIL-2R serum levels in T-LBL, T-ALL, DLBCL, and PTCL
patients

The serum levels of each patient were summarized in table 1. The serum levels
of sIL-2R measured in T-LBL patients (302 – 1,467 U/mL; median 593 U/mL), were
significantly lower than those measured for patients with T-ALL (223–4,320 U/mL;
median 1,911 U/mL), DLBCL (450 – 9,090 U/mL; median 2,805 U/mL; $P < 0.001$) and
PTCL (282 – 9890 U/mL; median 3,065 U/mL; $P < 0.001$) (Fig. 1). Notably, 20 of
T-LBL patients (87%) had sIL-2R serum levels below 1,000 U/mL (Table 1).
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3.2. Immunohistochemical detection of CD25 expression

Tissue morphology of the samples isolated from T-LBL patients is shown in Fig. 2a. Lymphoblasts were observed to be small to medium-sized with scant cytoplasm, condensed nuclear chromatin, and indistinct nucleoli. Further, all of lymphoblasts were positive for TdT (Fig. 2b) and the T-cell marker CD3 (Fig. 2c). CD25 expression in these tissues is highlighted in Fig. 2d-2f. The distribution of CD25-positive cells was scattered in the T-LBL patients (Fig. 2d), whereas a stronger, ubiquitous expression pattern was observed in 33% and 25% of the DLBCL (Fig. 2e) and PTCL patients (Fig. 2f), respectively. Similarly, the number of CD25-positive cells in the T-LBL patients (0 – 21.75; median 6.5) was significantly lower than that for both the DLBCL patients (40.5 – 1735.75; median 251; \( P < 0.001 \)) and the PTCL patients (2 – 966; median 103.25; \( P < 0.001 \)) (Table 1).

Furthermore, double immunohistochemical analysis revealed that CD25-positive cells were TdT negative, CD3 positive, Foxp3 positive, and CD20 negative (Fig. 3a-3d, respectively).

3.3. Serum sIL-2R level correlates with CD25 expression

The relationship between serum sIL-2R levels and CD25 expression was examined among the three lymphoma subtypes (T-LBL, DLBCL and PTCL). Fig. 4a shows the correlation curve in T-LBL and, for this subtype, the level of serum sIL-2R and the number of CD25-positive cells were found to be correlated with each other (\( P = \)
0.0069). On the other hand, no correlation was found to exist between serum sIL-2R levels and CD25 expression in DLBCL (Fig. 4b; $P = 0.348$) and PTCL (Fig. 4c; $P = 0.266$) patients.

4. Discussion

sIL-2R is a soluble form of the IL-2R $\alpha$ chain. The $\alpha$ chain is expressed on the cell surface of activated lymphocytes and, after secretion, forms sIL-2R. It has been demonstrated that IL-2R is released from the cell surface in a soluble form under particular conditions in vitro and in vivo, and while it is not clear whether neoplastic or non-neoplastic activated T-cells produce sIL-2R, both cell types are thought to have the capacity to release it into the serum. Furthermore, the considerable elevation in serum sIL-2R in malignant lymphoma observed in this study may be caused by the release of this factor from CD25-positive neoplastic cells. In the present study, extremely high levels of serum sIL-2R were observed in several DLBCL patients that also diffusely expressed CD25 in tumor cells, suggesting that these cells could be releasing sIL-2R from their cell membranes in this context. And same cases of DLBCL and PTCL lacking immunohistological expression of CD25 showed high levels of serum sIL-2R. Nakase et al. discussed the possibility that sIL-2R released from hematological neoplastic cells may be due to not only the shedding of the cell membrane of the CD25-positive neoplastic cells but also active secretion by the
CD25-negative inducible neoplastic cells. Therefore, we suspect that both non-neoplastic and neoplastic cells release sIL-2R in DLBCL and PTCL.

We also observed that the serum sIL-2R levels in patients with T-LBL were significantly lower than those of patients with T-ALL, DLBCL and PTCL ($P < 0.001$). In T-LBL, the CD25-positive T-cells were determined to be non-neoplastic cells. Moreover, these cells were Foxp3-positive, suggesting that they may be non-neoplastic regulatory T-cells. The Foxp3 transcription factor is considered to be the master regulator of regulatory T-cells (T$_{reg}$) and, thus, the most specific T$_{reg}$ marker. Furthermore, in the T-LBL patients, the number of CD25-positive cells was correlated with serum sIL-2R levels ($P = 0.0069$). Yang et al. have also reported that T-cells expressing CD25 are highly represented in B-cell NHL, suggesting that CD25-positive T-cells were the major source of sIL-2R in this type of NHL. In a similar fashion, CD25-positive non-neoplastic T-cells in T-LBL are presumed to be the primary source of sIL-2R, and the low number of cells present results in a lower level of sIL-2R released into the serum compared to the other lymphomas studied here.

In numerous other studies, high levels of sIL-2Rs have been observed in ALL patients. For example, Moon et al. have reported that serum sIL-2R levels were approximately 2,561 ± 2,194 U/ml in ALL patients (n = 39) and the median sIL-2R level in childhood ALL was 2,007 U/mL (267 to 80,000 U/mL; n = 344). Further, Lee et al. also reported that serum sIL-2R levels in ALL patients with CNS invasion
averaged 2,987 ± 1,944 U/mL. In the present study, T-ALL patients had statistically significant higher serum sIL-2R levels than T-LBL patients (\(P < 0.001\), Fig. 1). This discrepancy may be caused by the localization and tumor cell volume between ALL and LBL or the existence of BM/PB invasion.

An increased number of infiltrating T\(_{\text{reg}}\) in tumor tissues has been reported in both carcinoma and lymphoid malignancies and previous studies consistently demonstrate that the number of tumor-infiltrating T\(_{\text{reg}}\) can be used as a predictor for survival. However, the influence of T\(_{\text{reg}}\) on tumor and patients outcome is inconsistent and the tumor-infiltrating T\(_{\text{reg}}\) might suppress cytotoxic T-cells related to the anti-tumor immune response, in addition to the neoplastic lymphoid cells, resulting in an unfavorable clinical behavior in hematological malignancies.

During a median follow-up of 20 months (7-70 months), clinical data was available for 11 of the T-LBL patients (supplementary table 1). Two patients died of primary disease, while all of the surviving patients remained free of disease. The Kaplan-Meier analysis was shown in the supplementary figure 1. When the serum sIL-2R level cut-off was set at 1,000 U/mL, there was a significant difference of 3-year over-all survival (\(P = 0.017\)). This finding suggests that the serum sIL-2R level reflects the activity and microenvironment of the T-LBL tumor. However, the number of patients studied for clinicopathological analysis was too small and patients of T-LBL in the present study were underwent several treatments, further
investigations including accumulation of cases will be needed.

In conclusion, the release of sIL-2R into the serum in T-LBL patients was significantly lower than in T-ALL, DLBCL and PTCL patients and it correlated with the number of CD25-positive cells. We suspect that the cells secreting sIL-2R in patients with T-LBL might be T_{reg}. Investigation into the detailed role of sIL-2R and T_{reg} and to accumulate more cases will provide us further important about significance of sIL-2R in LBL.
Acknowledgements

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Conflicts of Interest: The authors have not conflicts of interest to disclose.

Take home messages

1. The release of sIL-2R into the serum in T-lymphoblastic lymphoma patients was significantly lower than that for T-acute lymphoblastic leukemia, diffuse large B-cell lymphoma, and peripheral T-cell lymphoma.

2. CD25 expression was correlated with the serum level of sIL-2R in T-lymphoblastic lymphoma.

3. CD25-positive cells were also found to be Foxp3-positive non-neoplastic T-cells.
References


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Oncol 1987; 5, 1262-1274.


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**Figure legends**

**Fig. 1** The serum sIL-2R levels measured in patients with T-LBL were significantly lower than that found for patients with T-ALL, DLBCL ($P < 0.001$) and PTCL ($P < 0.001$).

**Fig. 2** Morphological and immunohistochemical analyses. (a) Hematoxylin and eosin staining results showing the small-to-medium–sized lymphoblasts with scant cytoplasm, condensed nuclear chromatin, and indistinct nucleoli (images from patient number 3). Lymphoma cells were positive for TdT (b) and CD3 (c). CD25 expression in T-LBL (d), DLBCL (e), and PTCL (f) tissue samples. Each image is a $40 \times$ magnification.

**Fig. 3** Immunohistochemical analysis of CD25-expressing cells in T-LBL patients. CD25-expressing cells are TdT negative (a), CD3 positive (b), Foxp3-positive (c), and CD20 negative (d). Each images is a $40 \times$ magnification.

**Fig. 4** (a) Correlation between the serum sIL-2R levels (IU/mL) and the number of CD25-positive cells in T-LBL patients ($P = 0.0069$). No correlation was observed between the serum sIL-2R levels and the number of CD25-positive cells in DLBCL ($P = 0.348$) (b) or PTCL ($P = 0.266$) (c) patients.
Figure 3

a. CD25 (Red)/ TdT (Brown)
b. CD25 (Red)/ CD3 (Brown)
c. CD25 (Red)/ Foxp3 (Brown)
d. CD25 (Red)/ CD20 (Brown)
Figure 4

a: T-LBL

\[ P = 0.0069 \]

b: DLBCL

\[ P = 0.348 \]

c: PTCL, NOS

\[ P = 0.266 \]