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]

[a]Department of Pathology and [d]Department of Hematology, Oncology and Respiratory Medicine Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Science, Okayama, Japan

[b]Department of Pathology, Iwakuni Medical Center, Iwakuni, Japan

[c]Department 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

Running title: sIL-2R level in T lymphoblastic leukemia/lymphoma

Keywords: T lymphoblastic leukemia/lymphoma, soluble interleukin-2 receptor, CD25

Word count: 1837 words

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).[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),[2-6] as well as in patients with solid malignancies and autoimmune disorders .[7-9]

sIL-2R consists of three non-covalently associated subunits, α (CD25), β (CD122), and γ (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[10,11] and only one report on the serum levels of sIL-2R in LBL patients.[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

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.).[13] 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.[14,15] 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).[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).

Table 1. Serum sIL-2R level and CD25 positive cell counts in each lymphoma/leukemia subtypes

T lymphoblastic lymphoma			T lymphoblastic leukemia		diffuse large B-cell lymphoma			peripheral T-cell lymphoma, not otherwise specified		
No.	sIL-2R (U/ml)	No. of CD25 positive cells	No.	sIL-2R (U/ml)	No.	sIL-2R (U/ml)	CD25 positive cells	No.	sIL-2R (U/ml)	No. of CD25 positive cells
1	1,467	17.5	1	4,530	1	9,090	1,735.75	1	9,890	243.75
2	1,160	21.75	2	4,320	2	8,750	1,028.50	2	9,650	141.5
3	1,076	12.25	3	3,753	3	8,070	205.5	3	8,490	157.5
4	942	0	4	3,567	4	7,590	161.75	4	8,440	88
5	875	18.5	5	2,978	5	6,460	150.75	5	8,420	69.5
6	867	7	6	2,765	6	5,740	305.5	6	8,116	91.5
7	831	6.75	7	2454	7	5,235	76	7	7,540	506.5
8	738	10.25	8	2,234	8	5,030	453.25	8	7,090	353
9	708	6.75	9	1,987	9	4,900	152.75	9	5,380	966
10	607	8	10	1,956	10	4,575	195	10	5,130	145.25
11	597	12.25	11	1,865	11	4,455	453.5	11	4,870	45
12	593	0	12	1,654	12	4,334	592	12	4,680	98
13	589	3	13	1,456	13	4,270	813.5	13	4,654	99.25
14	554	4.5	14	1,421	14	4,215	332	14	4,632	68.5
15	532	4.75	15	1,213	15	4,070	300.75	15	4,590	165.5
16	511	6.5	16	964	16	3,878	540.5	16	4,428	232.75
17	502	3.25	17	754	17	3,420	96.25	17	4,280	157.5
18	480	7	18	432	18	3,330	65.5	18	4,000	88
19	402	6	19	231	19	3,080	267.25	19	3,450	124
20	402	4.25	20	223	20	2,840	85	20	3,120	48.25
21	389	0.25			21	2,770	345.5	21	3,010	23.5
22	327	2.75			22	2,760	454	22	2,340	105
23	302	3.25			23	2,660	795	23	1,950	308.5
					24	2,450	456.25	24	1,865	245
					25	1,870	46.5	25	1,540	101.5
					26	1,584	1,441.25	26	1,432	90.5
					27	1,546	40.5	27	1,235	88.25
					28	1,345	126	28	1,005	340.5
					29	1,272	67.25	29	904	180
					30	1,270	600	30	890	134.5
					31	1,230	266.25	31	823	137.5
					32	1,205	234.5	32	789	98.75
					33	1,187	432	33	655	45
					34	1,170	155.75	34	608	137.75
					35	1,151	199.25	35	505	32.5
					36	650	87.25	36	408	32
					37	554	134	37	390	8
					38	543	453.25	38	376	2
					39	532	102.5	39	332	23
					40	450	235	40	282	130.25

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 α chain. The α 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,[16] 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.[1,17] 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.[10,18] 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.[2] 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,[19] suggesting that CD25-positive T-cells were the major source of sIL-2R in this type of NHL.[20] 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) [21]and the median sIL-2R level in childhood ALL was 2,007 U/mL (267 to 80,000 U/mL; n = 344).[22] Further, Lee et al. also reported that serum sIL-2R levels in ALL patients with CNS invasion

averaged 2,987 ± 1,944 U/mL.[23] 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_{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_{reg} can be used as a predictor for survival.[24-26] However, the influence of T_{reg} on tumor and patients outcome is inconsistent and the tumor-infiltrating T_{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

We express our appreciation to Drs. Tadayoshi Kunitomo, Shuji Yohehara, Koichi Mizobuchi, Ichiro Yamadori, Kunihiro Omonishi, Satoko Nakamura, Yoko Shinno, Toshiaki Morito, and Midori Ando for providing samples and clinical information. We also thank the pathologist and the staff at the Okayama University Hospital for their help in the diagnosis of the patients of this study.

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

1. Wasik MA, Sioutos N, Tuttle M, Butmarc JR, Kaplan WD, Kadin ME. Constitutive secretion of soluble interleukin-2 receptor by human T cell lymphoma xenografted into SCID mice. Correlation of tumor volume with concentration of tumor-derived soluble interleukin-2 receptor in body fluids of the host mice. Am J Pathol 1994; 144, 1089-1097.

2. Nakase K, Tsuji K, Tamaki S, Tanigawa M, Ikeda T, Miyanishi E, Shiku H. Elevated levels of soluble interleukin-2 receptor in serum of patients with hematological or non-hematological malignancies. Cancer Detect Prev 2005; 29, 256-259.

3. Kamihira S, Atogami S, Sohda H, Momita S, Yamada Y, Tomonaga M. Significance of soluble interleukin-2 receptor levels for evaluation of the progression of adult T-cell leukemia. Cancer 1994; 73, 2753-2758.

4. Pizzolo G, Chilosi M, Vinante F, Dazzi F, Lestani M, Perona G, Benedetti F, Todeschini G, Vincenzi C, Trentin L. Soluble interleukin-2 receptors in the serum of patients with Hodgkin's disease. Br J Cancer 1987; 55, 427-428.

5. Goto H, Tsurumi H, Takemura M, Ino-Shimomura Y, Kasahara S, Sawada M, Yamada T, Hara T, Fukuno K, Goto N, Okuno M, Takami T, Seishima M, Moriwaki H. Serum-soluble interleukin-2 receptor (sIL-2R) level determines clinical outcome in patients with aggressive non-Hodgkin's lymphoma: in combination with the International Prognostic Index. J Cancer Res Clin Oncol 2005; 131, 73-79.

6. Kitagawa J, Hara T, Tsurumi H, Goto N, Kanemura N, Yoshikawa T, Kasahara S, Yamada T, Sawada M, Takahashi T, Shimizu M, Takami T, Moriwaki H. Serum-soluble interleukin-2 receptor (sIL-2R) is an extremely strong prognostic factor for patients with peripheral T-cell lymphoma, unspecified (PTCL-U). J Cancer Res Clin Oncol 2009; 135, 53-59.

7. Wang LS, Chow KC, Li WY, Liu CC, Wu YC, Huang MH. Clinical significance of serum soluble interleukin 2 receptor-alpha in esophageal squamous cell carcinoma. Clin Cancer Res 2000; 6, 1445-1451.

8. Bien E, Balcerska A. Serum soluble interleukin 2 receptor alpha in human cancer of adults and children: a review. Biomarkers 2008; 13, 1-26.

9. Jiskra J, Antosová M, Límanová Z, Telicka Z, Lacinová Z. The relationship between thyroid function, serum monokine induced by interferon gamma and soluble interleukin-2 receptor in thyroid autoimmune diseases. Clin Exp Immunol 2009; 156, 211-216.

10. Yoshida N, Oda M, Kuroda Y, Katayama Y, Okikawa Y, Masunari T, Fujiwara M, Nishisaka T, Sasaki N, Sadahira Y, Mihara K, Asaoku H, Matsui H, Seto M, Kimura A, Arihiro K, Sakai A. Clinical Significance of sIL-2R Levels in B-Cell Lymphomas. PLoS One 2013; 8, e78730.

11. Wasik MA, Vonderheid EC, Bigler RD, Marti R, Lessin SR, Polansky M, Kadin ME. Increased serum concentration of the soluble interleukin-2 receptor in cutaneous T-cell lymphoma. Clinical and prognostic implications. Arch Dermatol 1996; 132, 42-47.

12. Wagner DK, Kiwanuka J, Edwards BK, Rubin LA, Nelson DL, Magrath IT. Soluble interleukin-2 receptor levels in patients with undifferentiated and lymphoblastic lymphomas: correlation with survival. J Clin

Oncol 1987; 5, 1262-1274.

13. Swerdlow SH, Campo E, Harris NL, Stein H, Jaffe ES, Pileri SA, et al. (2008) WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: World Health Organization.

14. Larousserie F, Bardel E, Pflanz S, Arnulf B, Lome-Maldonado C, Hermine O, Brégeaud L, Perennec M, Brousse N, Kastelein R, Devergne O. Analysis of interleukin-27 (EBI3/p28) expression in Epstein-Barr virus- and human T-cell leukemia virus type 1-associated lymphomas: heterogeneous expression of EBI3 subunit by tumoral cells. Am J Pathol 2005; 166, 1217-1228.

15. Jones D, Ibrahim S, Patel K, Luthra R, Duvic M, Medeiros LJ. Degree of CD25 expression in T-cell lymphoma is dependent on tissue site: implications for targeted therapy. Clin Cancer Res 2004; 10, 5587-5594.

16. Rubin LA, Kurman CC, Fritz ME, Biddison WE, Boutin B, Yarchoan R, Nelson DL. Soluble interleukin 2 receptors are released from activated human lymphoid cells in vitro. J Immunol 1985; 135, 3172-3177.

17. Lindqvist CA, Christiansson LH, Simonsson B, Enblad G, Olsson-Strömberg U, Loskog AS. T regulatory cells control T-cell proliferation partly by the release of soluble CD25 in patients with B-cell malignancies. Immunology 2010; 131, 371-376.

18. Janik JE, Morris JC, Pittaluga S, McDonald K, Raffeld M, Jaffe ES, Grant N, Gutierrez M, Waldmann TA, Wilson WH. Elevated serum-soluble interleukin-2 receptor levels in patients with anaplastic large cell lymphoma. Blood 2004; 104, 3355-3357.

19. Yang ZZ, Novak AJ, Stenson MJ, Witzig TE, Ansell SM. Intratumoral CD4+CD25+ regulatory T-cell-mediated suppression of infiltrating CD4+ T cells in B-cell non-Hodgkin lymphoma. Blood 2006; 107, 3639-3646.

20. Yang ZZ, Grote DM, Ziesmer SC, Manske MK, Witzig TE, Novak AJ, Ansell SM. Soluble IL-2Rα facilitates IL-2-mediated immune responses and predicts reduced survival in follicular B-cell non-Hodgkin lymphoma. Blood 2011; 118, 2809-2820.

21. Moon Y, Kim Y, Kim M, Lim J, Kang CS, Kim WI, Shim SI, Chung NG, Park YH, Min WS, Han K. Plasma soluble interleukin-2 receptor (sIL-2R) levels in patients with acute leukemia. Ann Clin Lab Sci 2004; 34, 410-415.

22. Pui CH, Ip SH, Iflah S, Behm FG, Grose BH, Dodge RK, Crist WM, Furman WL, Murphy SB, Rivera GK. Serum interleukin 2 receptor levels in childhood acute lymphoblastic leukemia. Blood 1988; 71, 1135-1137.

23. Lee W, Kim SJ, Lee S, Kim J, Kim M, Lim J, Kim Y, Cho B, Lee EJ, Han K. Significance of cerebrospinal fluid sIL-2R level as a marker of CNS involvement in acute lymphoblastic leukemia. Ann Clin Lab Sci 2005; 35, 407-412.

24. Xu L, Xu W, Qiu S, Xiong S. Enrichment of CCR6+Foxp3+ regulatory T cells in the tumor mass correlates with impaired CD8+ T cell function and poor prognosis of breast cancer. Clin Immunol 2010; 135, 466-475.

25. Liotta F, Gacci M, Frosali F, Querci V, Vittori G, Lapini A, Santarlasci V, Serni S, Cosmi L, Maggi L,

Angeli R, Mazzinghi B, Romagnani P, Maggi E, Carini M, Romagnani S, Annunziato F. Frequency of regulatory T cells in peripheral blood and in tumour-infiltrating lymphocytes correlates with poor prognosis in renal cell carcinoma. BJU Int 2011; 107, 1500-1506.

26. Ichihara F, Kono K, Takahashi A, Kawaida H, Sugai H, Fujii H. Increased populations of regulatory T cells in peripheral blood and tumor-infiltrating lymphocytes in patients with gastric and esophageal cancers. Clin Cancer Res 2003; 9, 4404-4408.

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 × 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 × 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 1

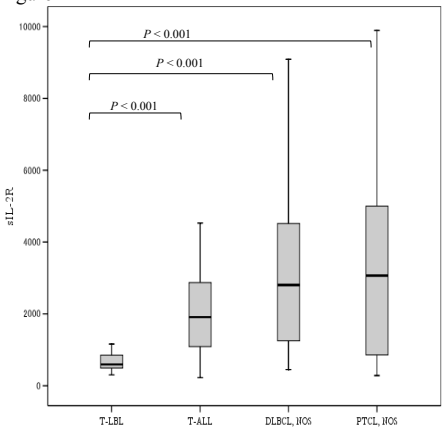

P < 0.001
P < 0.001
P < 0.001
sIL-2R
10000
8000
6000
4000
2000
0
T-LBL
T-ALL
DLBCL, NOS
PTCL, NOS

Figure 2

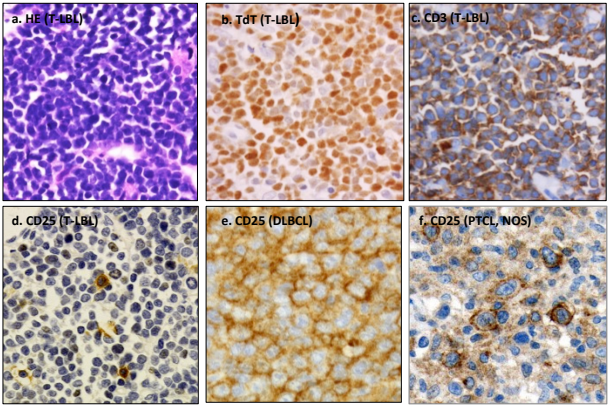

a. HE (T-LBL)
b. TdT (T-LBL)
c. CD3 (T-LBL)
d. CD25 (T-LBL)
e. CD25 (DLBCL)
f. CD25 (PTCL, NOS)

Figure 3

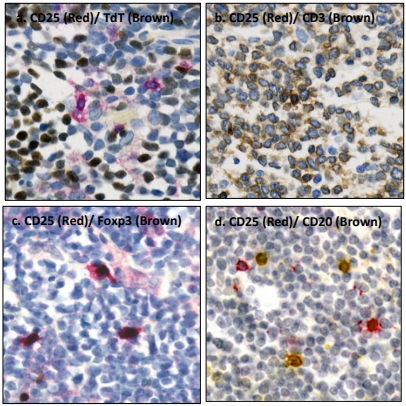
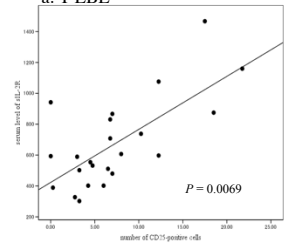
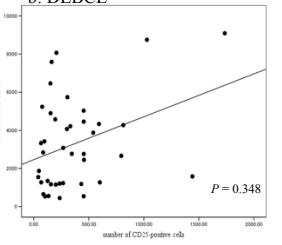

a. CD25 (Red)/ TdT (Brown)
b. CD25 (Red)/ CD3 (Brown)
c. CD25 (Red)/ Foxp3 (Brown)
d. CD25 (Red)/ CD20 (Brown)

Figure 4

a: T-LBL



b: DLBCL



c: PTCL, NOS