

Review Article

The Role of Tumor-Associated Macrophages on Serum Soluble IL-2R Levels in B-Cell Lymphomas

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Interleukin-2 receptor (IL-2R) is comprised of three different subunits (α , β , and γ chain) and is expressed on B cells and NK cells besides T cells. CD25 is also known as the IL-2R α chain on cell membranes, while soluble IL-2R (sIL-2R) is generated by the proteolytic cleavage of the IL-2R α chain. Levels of sIL-2R in sera are monitored as a marker of disease activity in patients with lymphoma. However, elevated serum sIL-2R levels are also found in inflammatory diseases, such as infectious diseases. Levels of sIL-2R in sera are thought to reflect tumor burden in adult T-cell leukemia/lymphoma due to the expression of CD25 on tumor cells. Conversely, sIL-2R is thought to be mainly derived from activated T cells infiltrating tumor tissues in diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) because lymphoma cells except for a subtype of DLBCL are mainly negative for CD25. Matrix metalloproteinase-9, a protease capable of cleaving the membrane bound IL-2R α chain, is mainly produced by tissue-associated macrophages. Increased macrophages in tumor tissues are reported to be associated with poor prognosis, especially in Hodgkin's lymphoma. We found increased macrophages in DLBCL and FL compared with reactive lymphoid hyperplasia as well as a positive correlation between the levels of sIL-2R in sera and the number of macrophages in tumor tissues in FL and extranodal DLBCL. [*J Clin Exp Hematop* 54(1): 49-57, 2014]

Keywords: soluble interleukin-2, tumor-associated macrophage, matrix metalloproteinase

INTRODUCTION

In clinical practice, the levels of soluble interleukin-2 receptor (sIL-2R) in sera are monitored in patients that are suspected of having malignant lymphoma. sIL-2R is generated by the proteolytic cleavage of membrane bound IL-2R α (CD25),¹ and the levels of sIL-2R in sera have proven to be a useful marker of tumor burden in adult T-cell leukemia/lymphoma (ATL/L)^{2,3} caused by human T-cell leukemia virus type 1 infection of CD4⁺ T cells, due to CD25 being expressed mainly on CD4⁺ T cells. Subsequently, the levels of sIL-2R in sera are monitored in B-cell lymphomas as well, because of the theory that sIL-2R levels reflect disease activity.^{4,5} Furthermore, elevated serum sIL-2R levels are

found in inflammatory diseases such as infectious diseases, and are related with disease activity. Recently, the levels of serum sIL-2R have been shown to have predictive value for patients with acute-type and lymphoma type ATL/L.⁶ However, sIL-2R is not included in the international prognostic index (IPI) for diffuse large B-cell lymphoma (DLBCL),⁷ or in the follicular lymphoma international prognostic index 2 (FLIPI2) for follicular lymphoma (FL).⁸ Therefore, it is important to elucidate which cell types release sIL-2R and how the levels of serum sIL-2R correlate with these diseases.

IL-2 AND IL-2R

Interleukin-2 (IL-2) was discovered in 1976 as a T-cell growth factor in the supernatants of activated T cells.⁹ IL-2 is essential in the development of CD4⁺ CD25⁺ Foxp3⁺ regulatory T (Treg) cells,^{10,11} which are induced from naïve T cells by T cell receptor stimulation in the presence of transforming growth factor- β (TGF- β) and IL-2.¹² In the absence of IL-2, Treg cells cannot survive or expand in the thymus or in the peripheral blood.¹³⁻¹⁶ IL-2R comprises three different subunits: α , β , and γ chains (Fig. 1). Among these, the IL-2 receptor α chain (IL-2R α , CD25, Tac antigen) is IL-2-specific and expressed on activated, but not non-activated lymphocytes.¹⁷ IL-2R α binds IL-2 with low affinity and forms a complex with IL-2R β (CD122) and IL-2R γ (common

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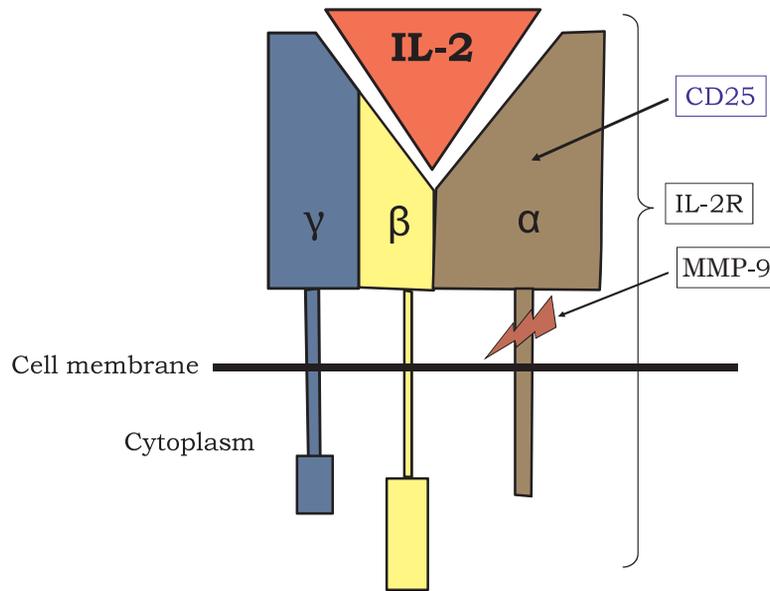


Fig. 1. Structure of the IL-2 receptor. The IL-2 receptor (IL-2R) is comprised of three different subunits: α , β , and γ chains.

cytokine receptor γ chain, γ_c , CD132). The IL-2R β/γ_c complex is mainly expressed on memory T cells and NK cells that bind IL-2 with intermediate affinity. All three receptor chains are co-expressed on activated T cells and Treg cells and bind IL-2 with high affinity. IL-2R β is also a key component of the IL-15 receptor, whereas IL-2R γ is an essential component shared by the receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21.^{18,19} IL-2 itself acts primary on lymphoid populations, including T cells,²⁰ B cells,²¹ and NK cells.²² IL-2 signals, via the heterodimerization of the IL-2R β and IL-2R γ cytoplasmic domains, lead to the activation of at least three major signaling pathways: phosphoinositol 3-kinase (PI3-K)/AKT, Ras-MAP kinase, and the JAK-STAT pathways with JAK1 and JAK3.^{19,23,24}

sIL-2R is generated by enzymatic cleavage and does not appear to be the product of a unique post-transcriptional splicing event.¹ The levels of sIL-2R are elevated in sera and other biologic fluids from patients with a variety of cancers and autoimmune diseases, and sIL-2R levels have proven to be a useful marker of disease activity and progression.^{25,26} Interestingly, sIL-2R is not simply a serum component, as it is known to have a function. Recently, Yang *et al.* demonstrated the biologic relevance of sIL-2R in terms of its effects on IL-2-mediated T-cell function in follicular lymphoma (FL).²⁷ They observed that serum sIL-2R levels were elevated compared with controls and that elevated pre-treatment sIL-2R levels were associated with poor outcome. Furthermore, they determined the effects of sIL-2R on IL-2 signaling and revealed that a sIL-2R-IL-2 complex promoted T-cell differentiation to inhibitory Treg cells, rather than to

T_H1 or T_H17 cells (CD4⁺ T helper (T_H) cells), and inhibited CD8⁺ T cells. Therefore, these findings indicate that sIL-2R plays an active biological role in the microenvironment of FL. In addition to our study,²⁸ Yang *et al.* and others have shown that T cells expressing surface IL-2R α (CD25) are the major source of sIL-2R in B-cell lymphomas including FL.^{29,30}

CLINICAL SIGNIFICANCE OF sIL-2R LEVELS IN B-CELL LYMPHOMAS

It is reasonable to predict disease activity based on serum levels of sIL-2R in CD4⁺ T-cell lymphoma, including ATL/L in which tumor cells are CD25 positive (Fig. 2). However, B cells are mainly negative for CD25. In order to determine whether sIL-2R has prognostic value in B-cell lymphomas, we retrospectively analyzed DLBCL and FL patients (Fig. 3).²⁸ Patients were divided into two groups according to pre-treatment sIL-2R levels (> 1,500 and \leq 1,500). In DLBCL, patients with high sIL-2R showed poor prognosis when compared to patients with low sIL-2R. Conversely, patients with high sIL-2R in FL tended to have poor prognosis with no significant differences observed. We then performed a flow cytometric study to identify the types of cells expressing CD25 in B-cell lymphoma samples (Fig. 4). We found three representative patterns in DLBCL: First, most lymphoma cells (CD19⁺ cells) are positive for CD25, while T cells (CD3⁺ cells) are partially positive for CD25. Second, both lymphoma cells and T cells are partially positive for CD25. Third, most lymphoma cells are negative for CD25 and T cells are partially positive for CD25. In FL, T cells are

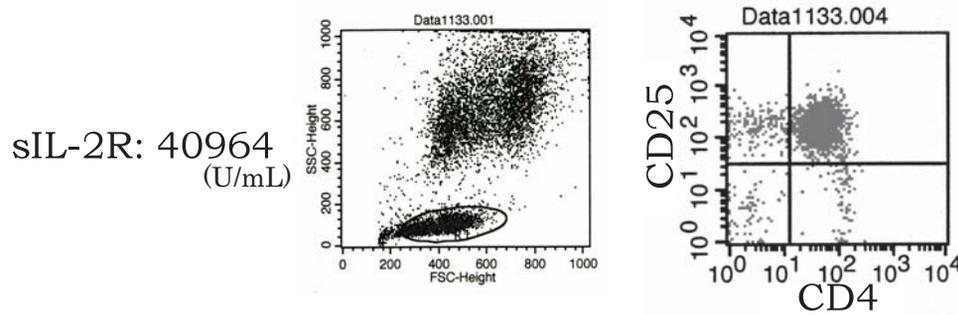


Fig. 2. Two-color staining analysis using flow cytometry. Tumor cells in peripheral blood of patients with ATL are CD4⁺/CD25⁺.

partially positive for CD25 and lymphoma cells are partially positive for CD25 or mostly negative for CD25. In reactive lymphoid hyperplasia (RLH), T cells are partially positive for CD25 and B cells (CD19⁺ cells) are mostly negative for CD25. On the other hand, tumor cells in mantle cell lymphoma (MCL) are positive for CD25. Subsequently, analysis of the relationship between serum level of sIL-2R and the degree of CD25⁺ lymphoma cells or T cells and other clinical data showed that sIL-2R levels in sera do not precisely indicate tumor burden in DLBCL and FL.²⁸

MECHANISM OF sIL-2R PRODUCTION

sIL-2R is generated by the enzymatic cleavage of IL-2R α (CD25).^{1,31} However, expression of CD25 in tumor cells does not clarify the mechanisms responsible for the elevation of sIL-2R in DLBCL and FL.²⁸ One of the matrix metalloproteinases (MMPs), MMP-9, attracts attention as a protease capable of cleaving IL-2R α chains on cell membranes (Fig. 1). Additionally, MMP-2 is a gelatinase-containing gelatin binding repeats similar to MMP-9 and is thought to have the ability to cleave IL-2R α chains. Furthermore, these MMPs are produced by macrophages, neutrophils, and dendritic cells, such as inflammation related cells.^{32,33} We also confirmed that MMP-9 is able to cleave IL-2R α chains depending on its concentration, using an ATL cell line (MT4) that does not express endogenous MMP-9.²⁸ However, the detection of sIL-2R in the supernatants of MT4 cells in the absence recombinant MMP-9 treatment raised the possibility that factors in addition to MMP-9 are involved in the cleavage of IL-2R α chains.²⁸

Therefore, it is important to determine which cells generate and release MMP-9. According to previous studies,³⁴⁻³⁹ we speculated that the cleavage of IL-2R α chains on lymphoma cells and activated T cells by MMP-9 produced in tumor microenvironments of lymphoma patients leads to the elevation of sIL-2R levels in sera. MMP-9 activity was detected in the supernatant of purified lymphoma cells. However, expression of MMP-9 was not detected in lymphoma cells and

activated T cells, except for tumor cells in ATL/L, using immunohistochemistry (IHC) (Fig. 5).²⁸ It is noted that termed tumor-associated macrophages (TAMs) were positive for MMP-9 in IHC experiments (Fig. 5).²⁸ However, the mechanism involved is not simple (Fig. 6); the number of TAMs, identified as CD68 (KP1)⁺ or CD163⁺ macrophages, in DLBCL and FL was significantly higher than in RLH.²⁸ Evaluation of the correlation between levels of sIL-2R and MMP-9 in sera showed a positive correlation in FL but not in DLBCL.²⁸ However, evaluation of the correlation between levels of sIL-2R in sera and number of TAMs showed a positive correlation in FL and extranodal DLBCL but not in all cases with DLBCL.²⁸

TUMOR-ASSOCIATED MACROPHAGES (TAMs)

Macrophages that infiltrate tumor tissues are referred to as TAMs.^{40,41} Classically activated macrophages (M1) have tumoricidal activity and elicit tissue destructive effects following exposure to interferon γ (IFN γ). Alternatively activated macrophages (M2), in response to IL-4 and IL-13, have cancer related inflammatory (CRI) activity.⁴²⁻⁴⁴ In particular, studies in solid tumors reported that TAMs play critical roles in tumor growth, progression, invasion, metastasis, and angiogenesis.⁴⁴ TAMs are identified as CD68⁺ or CD163⁺ cells. Although the KP1 clone for CD68 has been reported to be a less specific marker for macrophages,⁴⁵ there are some studies that predict prognosis by analyzing the number of macrophages in tumor tissues.⁴⁶⁻⁵¹ Interestingly, a study in classic Hodgkin's lymphoma revealed that an increased number of CD68⁺ macrophages was correlated with a decreased progression-free survival as well as with an increased likelihood of relapse after autologous hematopoietic stem-cell transplantation, resulting in shortened disease-specific survival.⁴⁸ Furthermore, a study using tissue microarray multivariate analysis showed increased CD68 or CD163 expression to be significant independent predictors of inferior failure free survival or overall survival (OS).⁵⁰ On the other hand, analysis of increased CD31⁺ angiogenic sprouting in poor

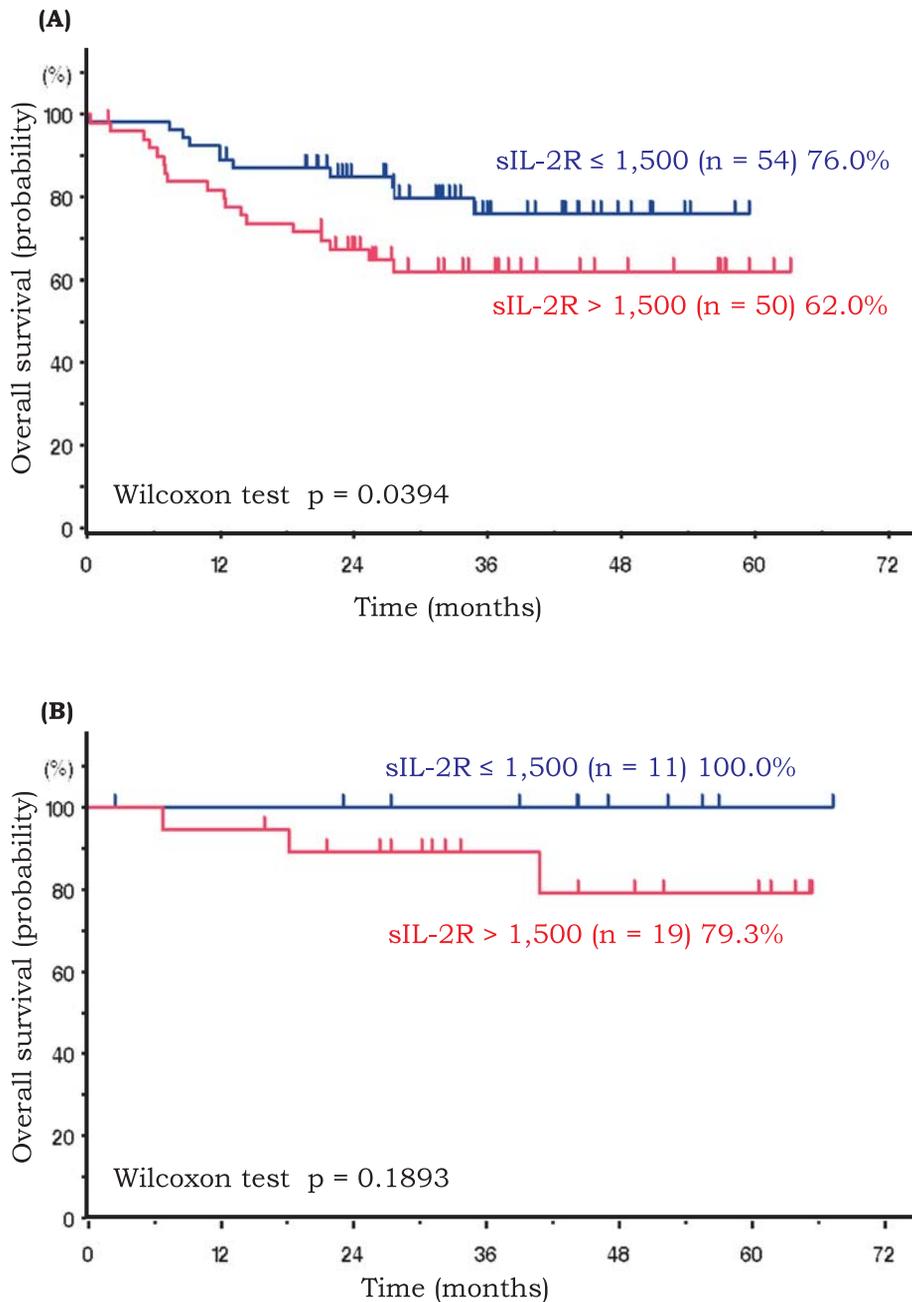


Fig. 3. Overall survival stratified according to soluble interleukin-2 receptor (sIL-2R) levels ($\leq 1,500$ vs. $> 1,500$) in diffuse large B-cell lymphoma (DLBCL) (3A) and follicular lymphoma (FL) (3B). Serum sIL-2R levels were analyzed in previously untreated patients with DLBCL (n = 104) or FL (n = 30). The 5-year overall survival rates for patients with sIL-2R levels of $\leq 1,500$ U/mL and $> 1,500$ U/mL were 76% and 62%, respectively ($p < 0.05$) in DLBCL, and 100% and 79.3%, respectively ($p = 0.189$) in FL.

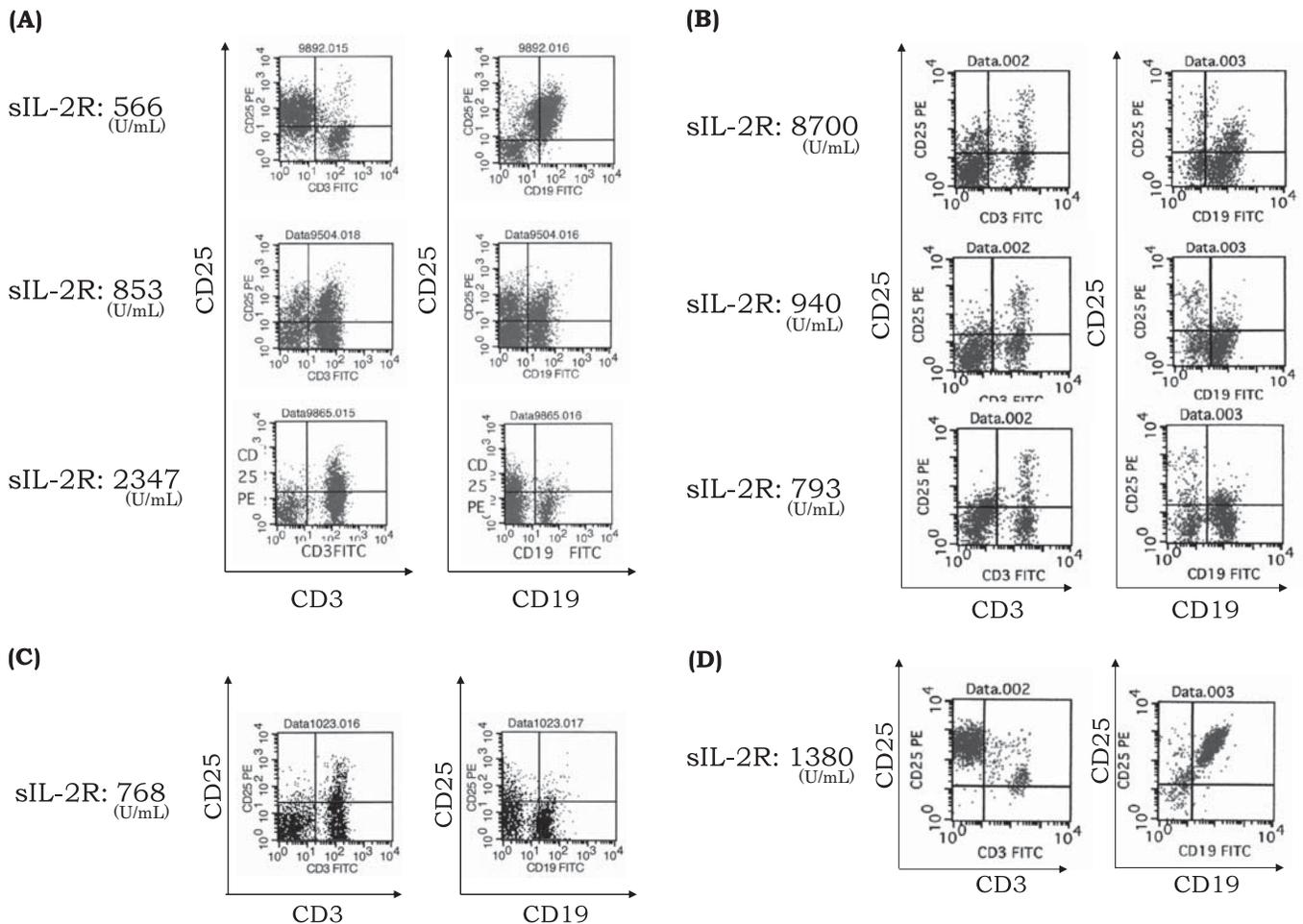


Fig. 4. Two-color staining analysis using flow cytometry. Expression of CD25 (interleukin-2 receptor α : IL-2R α) on B cells (CD19-positive) and T cells (CD3-positive) in diffuse large B-cell lymphoma (4A), follicular lymphoma (4B), reactive lymph node hyperplasia (4C), and mantle cell lymphoma (4D). There is no apparent association between CD25 expression on B or T cells and the levels of serum sIL-2R in these cases.

prognosis FL demonstrated an association between increased sprouting and elevated numbers of infiltrating CD163⁺ macrophages.⁴⁶ Furthermore, elevated CD68⁺ TAMs and an increased proportion of CD163⁺ TAMs were associated with a worse clinical prognosis in ATL/L.⁵¹

Based on the molecular features, using cDNA microarray, of tumor-infiltrating immune cells in FL, T cells-related gene expression was associated with favorable prognosis, whereas monocyte-related gene expression was associated with poor prognosis.⁵² Therefore, increased infiltration of macrophages in the tumor microenvironment is thought to be associated with poor prognosis, however, the mechanism remains unclear. TAMs produce various cytokines and chemokines. In particular, vascular endothelial growth factor (VEGF) and MMPs, including MMP-9, are also produced by TAMs and enhance angiogenesis, blood vessel permeability, and destruc-

tion of the extracellular matrix (ECM) in the tumor microenvironment.^{33,44} In mice study, IL-10 released from macrophages acts in a paracrine manner on Treg to maintain Foxp3 expression.⁵³ Therefore, Treg might increase in the microenvironment where macrophages increase (Fig. 6). Altogether, TAMs might produce a permissive environment for lymphoma cell growth and survival.

FUTURE PERSPECTIVES

First, to elucidate the mechanism of sIL-2R production in lymphoma, it is necessary to reanalyze the relationship between lymphoma cells and the tumor microenvironment or tumor infiltrating cells. To date, primary therapeutic strategies target lymphoma cells according to phenotypic analyses and the histopathological classification. Furthermore, many

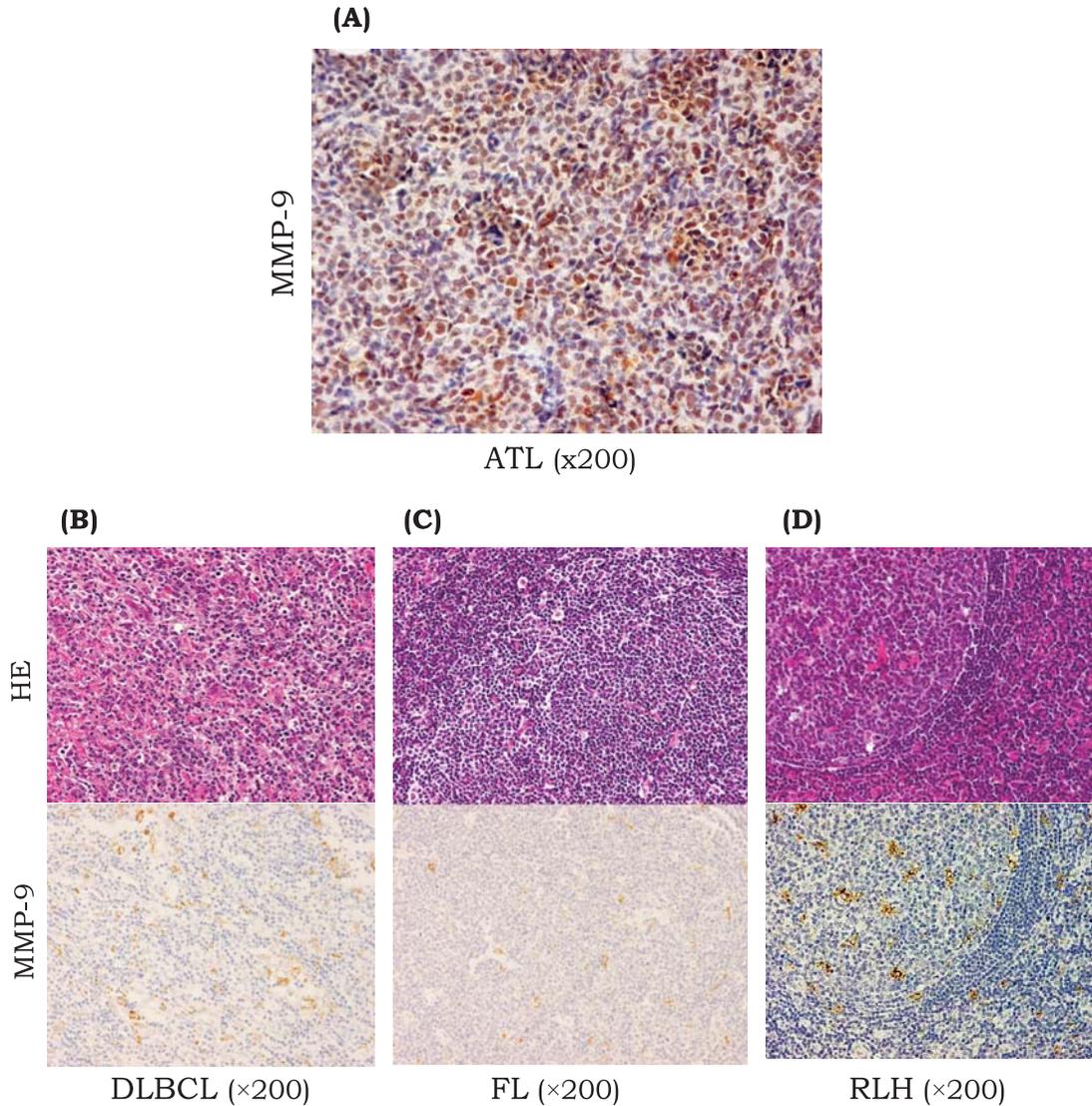


Fig. 5. Immunohistochemical staining using anti-matrix metalloproteinase (MMP)-9 antibody. Adult T-cell leukemia (ATL) cells are diffusely positive for MMP-9 (5A). Lymphocytes including lymphoma cells in diffuse large B-cell lymphoma (DLBCL) (5B), follicular lymphoma (FL) (5C), and reactive lymph node hyperplasia (RLH) (5D) are negative for MMP-9, whereas macrophages are positive.

researchers focus on lymphomagenesis induced by genetic or chromosomal aberrations. However, given that the tumor microenvironment is a reservoir for minimal residual disease (MRD), which is a crucial problem associated with relapse disease, combination of drugs that kill lymphoma cells and modulate bystander cells might be promising. Therapies based on monoclonal antibodies to MMPs have already been attempted.³⁴ We also found that down-regulation of MMP-9 activity by shRNA transfection decreases the matrigel invasion activity in MMP-9 producing ATL cell lines (not published data).

Second, we suggest that CD25⁺ DLBCL is a subtype of

DLBCL according to our two-color (CD25/CD19) staining analysis (Fig. 4). Characterization of CD25⁺ DLBCL could be interesting because we found that recombinant IL-2 enhances the proliferation of CD25⁺/CD19⁺ B cells, which were purified from the lymph nodes of patients with RLH (not published data). Therefore, IL-2 might play a critical role in CD25⁺ DLBCL.

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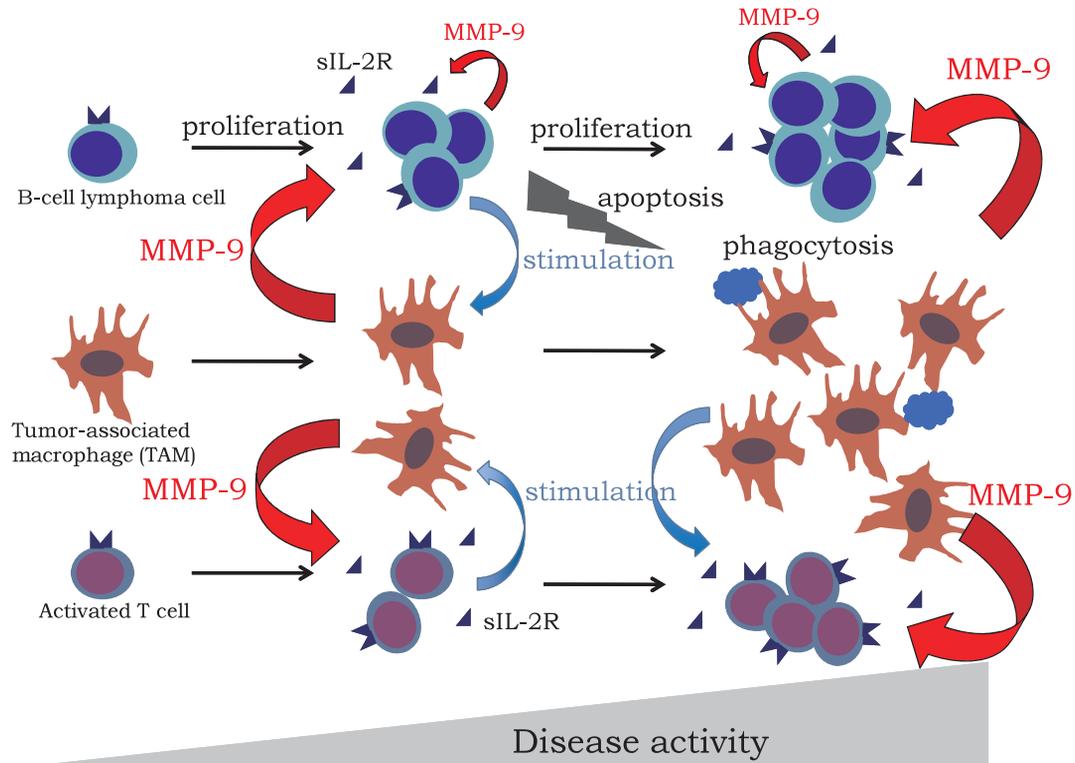


Fig. 6. Schematic model of soluble interleukin-2 receptor (sIL-2R) elevation in B-cell lymphomas. As lymphoma cells are proliferating, tumor-associated macrophages (TAMs) and activated T cells are accumulating around lymphoma cells to form tumor microenvironment (cancer related inflammation as mentioned by Mantovani *et al.*⁵⁴). MMP-9 is primarily released from TAMs (especially CD68⁺) and cleaves the IL-2R α chain on bystander T cells and B-cell lymphoma cells expressing CD25 (IL-2R α). Furthermore, cytokines released from TAMs might increase activated T cells including regulatory T cells. This mechanism might be involved in the elevation of sIL-2R levels in patients with B-cell lymphomas.

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REFERENCES

- Rubin LA, Galli F, Greene WC, Nelson DL, Jay G: The molecular basis for the generation of the human soluble interleukin 2 receptor. *Cytokine* 2:330-336, 1990
- Yasuda N, Lai PK, Ip SH, Kung PC, Hinuma Y, *et al.*: Soluble interleukin 2 receptors in sera of Japanese patients with adult T cell leukemia mark activity of disease. *Blood* 71:1021-1026, 1988
- Kamihira S, Atogami S, Sohda H, Momita S, Yamada Y, *et al.*: Significance of soluble interleukin-2 receptor levels for evaluation of the progression of adult T-cell leukemia. *Cancer* 73:2753-2758, 1944
- Ennishi D, Yokoyama M, Terui Y, Asai H, Sakajiri S, *et al.*: Soluble interleukin-2 receptor retains prognostic value in patients with diffuse large B-cell lymphoma receiving rituximab plus CHOP (RCHOP) therapy. *Ann Oncol* 20:526-533, 2009
- Goto N, Tsurumi H, Goto H, Shimomura YI, Kasahara S, *et al.*: Serum soluble interleukin-2 receptor (sIL-2R) level is associated with the outcome of patients with diffuse large B cell lymphoma treated with R-CHOP regimens. *Ann Hematol* 91:705-714, 2012
- Katsuya H, Yamanaka T, Ishitsuka K, Utsunomiya A, Sasaki H, *et al.*: Prognostic index for acute- and lymphoma-type adult T-cell leukemia/lymphoma. *J Clin Oncol* 30:1635-1640, 2012
- The International Non-Hodgkin's Lymphoma Prognostic Factors Project: A predictive model for aggressive non-Hodgkin's lymphoma. *N Engl J Med* 329:987-994, 1993
- Federico M, Bellei M, Marcheselli L, Luminari S, Lopez-Guillermo A, *et al.*: Follicular lymphoma international prognostic index 2: a new prognostic index for follicular lymphoma developed by the international follicular lymphoma prognostic factor project. *J Clin Oncol* 27:4555-4562, 2009
- Morgan DA, Ruscetti FW, Gallo R: Selective *in vitro* growth of T lymphocytes from normal human bone marrow. *Science* 193:1007-1008, 1976
- Bayer AL, Yu A, Adeegbe D, Malek TR: Essential role for interleukin-2 for CD4⁺ CD25⁺ T regulatory cell development during the neonatal period. *J Exp Med* 201:769-777, 2005
- Fontenot JD, Rasmussen JP, Gavin MA, Rudensky AY: A function for interleukin 2 in Foxp3-expressing regulatory T cells. *Nat*

- Immunol 6:1142-1151, 2005
- 12 Malek TR: The biology of interleukin-2. *Annu Rev Immunol* 26:453-479, 2008
 - 13 Furtado GC, Curotto de Lafaille MA, Kutchukhidze N, Lafaille JJ: Interleukin 2 signaling is required for CD4⁺ regulatory T cell function. *J Exp Med* 196:851-857, 2002
 - 14 Almeida AR, Légrand N, Papiernik M, Freitas AA: Homeostasis of peripheral CD4⁺ T cells: IL-2R α and IL-2 shape a population of regulatory cells that controls CD4⁺ T cell numbers. *J Immunol* 169:4850-4860, 2002
 - 15 Curotto de Lafaille MA, Lino AC, Kutchukhidze N, Lafaille JJ: CD25⁻ T cells generate CD25⁺ Foxp3⁺ regulatory T cells by peripheral expansion. *J Immunol* 173:7259-7268, 2004
 - 16 Setoguchi R, Hori S, Takahashi T, Sakaguchi S: Homeostatic maintenance of natural Foxp3⁺ CD25⁺ CD4⁺ regulatory T cells by interleukin (IL)-2 and induction of autoimmune disease by IL-2 neutralization. *J Exp Med* 201:723-735, 2005
 - 17 Kim HP, Imbert J, Leonard WJ: Both integrated and differential regulation of components of the IL-2/IL-2 receptor system. *Cytokine Growth Factor Rev* 17:349-366, 2006
 - 18 Leonard WJ: Cytokines and immunodeficiency disease. *Nat Rev Immunol* 1:200-208, 2001
 - 19 Liao W, Lin JX, Leonard W: IL-2 family cytokines: new insights into complex roles of IL-2 as a broad regulator of T helper cell differentiation. *Cur Opin Immunol* 23:598-604, 2011
 - 20 Rochman Y, Spolski R, Leonard WJ: New insights into the regulation of T cells by γ_c family cytokines. *Nat Rev Immunol* 9:480-490, 2009
 - 21 Blackman MA, Tigges MA, Minie ME, Koshland ME: A model system for peptide hormone action in differentiation: interleukin 2 induces a B lymphoma to transcribe the J chain gene. *Cell* 47:609-617, 1986
 - 22 Henney CS, Kuribayashi K, Kern DE, Gillis S: Interleukin-2 augments natural killer cell activity. *Nature* 291:335-338, 1981
 - 23 Friedmann MC, Migone TS, Russell SM, Leonard WJ: Different interleukin 2 receptor beta-chain tyrosines couple to at least two signal pathways and synergistically mediate interleukin 2-induced proliferation. *Proc Natl Acad Sci U S A* 93:2077-2082, 1996
 - 24 Lin JX, Migone TS, Tsang M, Friedmann M, Weatherbee JA, *et al.*: The role of shared receptor motifs and common Stat proteins in the generation of cytokine pleiotropy and redundancy by IL-2, IL-4, IL-7, IL-13, and IL-15. *Immunity* 2:331-339, 1995
 - 25 Murakami S: Soluble interleukin-2 receptor in cancer. *Front Biosci* 9:3085-3090, 2004
 - 26 Witkowska AM: On the role of sIL-2R measurements in rheumatoid arthritis and cancers. *Mediators Inflamm* 2005:121-130, 2005
 - 27 Yang ZZ, Grote DM, Ziesmer SC, Manske MK, Witzig TE, *et al.*: Soluble IL-2R α facilitates IL-2-mediated immune responses and predicts reduced survival in follicular B-cell non-Hodgkin lymphoma. *Blood* 118:2809-2820, 2011
 - 28 Yoshida N, Oda M, Kuroda Y, Katayama Y, Okikawa Y, *et al.*: Clinical significance of sIL-2R levels in B-cell lymphomas. *Plos One* 11: e78730, 2013
 - 29 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* 107:3639-3646, 2006
 - 30 Hilchey SP, De A, Rimsza LM, Bankert RB, Bernstein SH: Follicular lymphoma intratumoral CD4⁺ CD25⁺ GITR⁺ regulatory T cells potently suppress CD3/CD28-costimulated autologous and allogeneic CD8⁺ CD25⁻ and CD4⁺ CD25⁻ T cells. *J Immunol* 178:4051-4061, 2007
 - 31 Levine SJ: Mechanisms of soluble cytokine receptor generation. *J Immunol* 173:5343-5348, 2004
 - 32 Egeblad M, Werb Z: New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer* 2:161-174, 2002
 - 33 Kessenbrock K, Plaks V, Werb Z: Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell* 141:52-67, 2010
 - 34 Stetler-Stevenson M, Mansoor A, Lim M, Fukushima P, Kehrl J, *et al.*: Expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in reactive and neoplastic lymphoid cells. *Blood* 89:1708-1715, 1997
 - 35 Hayashibara T, Yamada Y, Onimaru Y, Tsutsumi C, Nakayama S, *et al.*: Matrix metalloproteinase-9 and vascular endothelial growth factor: a possible link in adult T-cell leukaemia cell invasion. *Br J Haematol* 116:94-102, 2002
 - 36 Mori N, Sato H, Hayashibara T, Senba M, Hayashi T, *et al.*: Human T-cell leukemia virus type I Tax transactivates the matrix metalloproteinase-9 gene: potential role in mediating adult T-cell leukemia invasiveness. *Blood* 99:1341-1349, 2002
 - 37 Redondo-Muñoz J, Escobar-Díaz E, Samaniego R, Terol MJ, García-Marco JA, *et al.*: MMP-9 in B-cell chronic lymphocytic leukemia is up-regulated by $\alpha_4\beta_1$ integrin or CXCR4 engagement via distinct signaling pathways, localizes to podosomes, and is involved in cell invasion and migration. *Blood* 108:3143-3151, 2006
 - 38 Pennanen H, Kuitinen O, Soini Y, Turpeenniemi-Hujanen T: Prognostic significance of p53 and matrix metalloproteinase-9 expression in follicular lymphoma. *Eur J Haematol* 81:289-297, 2008
 - 39 Redondo-Muñoz J, Ugarte-Berzal E, Terol MJ, Van den Steen PE, Hernández del Cerro M, *et al.*: Matrix metalloproteinase-9 promotes chronic lymphocytic leukemia B cell survival through its hemopexin domain. *Cancer Cell* 17:160-172, 2010
 - 40 Bingle L, Brown NJ, Lewis CE: The role of tumor-associated macrophages in tumor progression: implications for new anti-cancer therapies. *J Pathol* 196:254-265, 2002
 - 41 Mantovani A, Sozzani S, Locati M, Allavena P, Sica A: Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol* 23:549-555, 2002
 - 42 Gordon S: Alternative activation of macrophages. *Nat Rev Immunol* 3:23-35, 2003
 - 43 Martinez FO, Helming L, Gordon S: Alternative activation of macrophages: an immunologic functional perspective. *Annu Rev Immunol* 27:451-483, 2009

- 44 Qian BZ, Pollard JW: Macrophage diversity enhances tumor progression and metastasis. *Cell* 141:39-51, 2010
- 45 Kunisch E, Fuhrmann R, Roth A, Winter R, Lungershausen W, *et al.*: Macrophage specificity of three anti-CD68 monoclonal antibodies (KP1, EBM11, and PGM1) widely used for immunohistochemistry and flow cytometry. *Ann Rheum Dis* 63:774-784, 2004
- 46 Clear AJ, Lee AM, Calaminici M, Ramsay AG, Morris KJ, *et al.*: Increased angiogenic sprouting in poor prognosis FL is associated with elevated numbers of CD163⁺ macrophages within the immediate sprouting microenvironment. *Blood* 115:5053-5056, 2010
- 47 Niino D, Komohara Y, Murayama T, Aoki R, Kimura Y, *et al.*: Ratio of M2 macrophage expression is closely associated with poor prognosis for angioimmunoblastic T-cell lymphoma (AITL). *Pathol Int* 60:278-283, 2010
- 48 Steidl C, Lee T, Shah SP, Farinha P, Han G, *et al.*: Tumor-associated macrophages and survival in classic Hodgkin's lymphoma. *N Engl J Med* 362:875-885, 2010
- 49 Wada N, Zaki MA, Hori Y, Hashimoto K, Tsukaguchi M, *et al.*: Tumour-associated macrophages in diffuse large B-cell lymphoma: a study of the Osaka Lymphoma Study Group. *Histopathology* 60:313-319, 2012
- 50 Tan KL, Scott DW, Hong F, Kahl BS, Fisher RI, *et al.*: Tumor-associated macrophages predict inferior outcomes in classic Hodgkin lymphoma: a correlative study from the E2496 Intergroup trial. *Blood* 120:3280-3287, 2012
- 51 Komohara Y, Niino D, Saito Y, Ohnishi K, Horlad H, *et al.*: Clinical significance of CD163⁺ tumor-associated macrophages in patients with adult T-cell leukemia/lymphoma. *Cancer Sci* 104:945-951, 2013
- 52 Dave SS, Wright G, Tan B, Rosenwald A, Gascoyne RD, *et al.*: Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. *N Engl J Med* 351:2159-2169, 2004
- 53 Murai M, Turovskaya O, Kim G, Madan R, Karp CL, *et al.*: Interleukin 10 acts on regulatory T cells to maintain expression of the transcription factor Foxp3 and suppressive function in mice with colitis. *Nat Immunol* 10:1178-1184, 2009
- 54 Mantovanin A, Sica A: Macrophages, innate immunity and cancer: balance, tolerance, and diversity. *Curr Opin Immunol* 22:231-237, 2010