Clinical Immunology 187 (2018) 50–57

Contents lists available at ScienceDirect

Clinical Immunology

journal homepage: www.elsevier.com/locate/yclim

CD4$^+$ CD52^{lo} T-cell expression contributes to the development of systemic lupus erythematosus☆,☆☆

Masataka Umeda [a], Tomohiro Koga [a,b,*], Kunihiro Ichinose [a,*], Takashi Igawa [a], Tomohito Sato [a], Ayuko Takatani [a], Toshimasa Shimizu [a], Shoichi Fukui [a], Ayako Nishino [a], Yoshiro Horai [a,c], Yasuko Hirai [a], Shin-ya Kawashiri [a], Naoki Iwamoto [a], Toshiyuki Aramaki [d], Mami Tamai [a], Hideki Nakamura [a], Kazuo Yamamoto [e], Norio Abiru [f], Tomoki Origuchi [g], Yukitaka Ueki [d], Atsushi Kawakami [a]

[a] Department of Immunology and Rheumatology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
[b] Center for Bioinformatics and Molecular Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
[c] Department of Rheumatology, Clinical Research Center, National Hospital Organization Nagasaki Medical Center
[d] Department of Rheumatology, Sasebo Chuo Hospital, Nagasaki, Japan
[e] Biomedical Research Support Center, Nagasaki University School of Medicine, Nagasaki, Japan
[f] Department of Endocrinology and Metabolism, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
[g] Department of Rehabilitation Sciences, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

ARTICLE INFO

Article history:
Received 14 August 2017
Received in revised form 11 October 2017
Accepted with revision 11 October 2017
Available online 13 October 2017

Keywords:
CD52
T cells
Systemic lupus erythematosus

ABSTRACT

The cell-surface glycoprotein CD52 is widely expressed in lymphocytes. CD4$^+$CD52^{hi} T cells are functioning suppressor CD4$^+$T cells. We investigated the role of the immune regulation of CD4$^+$CD52 T cells in systemic lupus erythematosus (SLE). CD4$^+$CD52^{lo} T cells were increased in SLE patients, in positive correlation with SLEDAI, anti-ds-DNA antibody, and IgG concentration. Circulating follicular helper-like T cells (Tfh-like cells) were also increased in SLE, in positive correlation with CD4$^+$CD52^{lo} T cells. Chemokine receptor 8 (CCR8) expression in CD4$^+$CD52^{lo} T cells was increased. *In vitro* experiments using CD4 T cells of SLE patients showed that thymus and activation-regulated chemokine (TARC), a ligand of CCR8, contributed to the development of CD4$^+$CD52^{hi} T cells into CD4$^+$CD52^{lo} T cells. Our findings suggest that CD4 + CD52lo T-cell upregulation is involved in the production of pathogens by autoantibodies, and TARC may contribute to the development of SLE through an aberrant induction of CD4$^+$CD52^{lo} T cells.

1. Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease caused by a reduction of tolerance to nuclear antigens [1]. In SLE, multiple organs such as the kidneys, brain, and skin are injured by inappropriate immune responses, including auto-antibody production, auto-reactive T cells and B cells, auto-antigen presenting cells, and a local activation of the complement system [1]. If the treatment of SLE is inadequate, fatal complications can eventually occur.

CD52, also known as Campath-1, is a glycosylphosphatidylinositol (GPI)-anchored cell-surface glycoprotein that is widely expressed in lymphocytes, monocytes and eosinophils [2]. Changes in the quantity and/or quality of CD4$^+$CD52^{lo} T cells are suggested to be involved in autoimmune disease [3]. CD4$^+$CD52^{hi} T cells play a suppressor role, whereas CD4$^+$CD52^{lo} T cells have an activator function. It has been reported that CD4$^+$CD52^{lo} T cells are induced by glutamic acid decarboxylase (GAD) 65 or tetanus toxoid from healthy human peripheral blood mononuclear cells (PBMCs), and that the activated CD4$^+$CD52^{lo} T cells can produce interferon-gamma (IFN-γ) [4].

Importantly, the production of inflammatory cytokines, including IFN-γ, was inhibited under a co-culture with CD4$^+$CD52^{lo} and CD4$^+$CD52^{hi} T cells through the soluble fusion protein of CD52 cleaved by cell-surface CD52 and the immunoglobulin crystallizable fragment (CD52-Fc) [4]. This soluble CD52 is cleaved by phospholipase C, which functions as a ligand of siglec-10 on CD4$^+$CD52^{lo} T cells [4]. The signal of siglec-10 inhibits the phosphorylation of the T cell receptor-associated kinases Lck and Zap70 and T-cell activation [4].

☆ This work was supported by grants from JSPS KAKENHI (to M.U.: no. 16K19604; to K.I.: no. 24790999), the Japan Intractable Diseases Research Foundation (to K.I.), the Leading Initiative for Excellent Young Researchers of the Ministry of Education, Culture, Sports, Science and Technology (to T.K.: no. 16810055) and the Uehara Memorial Foundation (to T.K.: no 201510047).

☆☆ The authors declare no conflict of interest.

* Corresponding authors at: Department of Immunology and Rheumatology, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan.
E-mail addresses: tkoga@nagasaki-u.ac.jp (T. Koga), kichinos@nagasaki-u.ac.jp (K. Ichinose).

https://doi.org/10.1016/j.clim.2017.10.004
1521-6616/

These findings indicate that an imbalance between $CD4^+$ $CD52^{hi}$ T cells and $CD4^+$ $CD52^{lo}$ T cells is involved in the pathogenesis of autoimmune diseases. However, the expression and function of these cells in SLE have not been clarified. In this study, we examined the expressions of $CD4^+$ $CD52^{hi}$ and $CD4^+$ $CD52^{lo}$ T cells in SLE patients, and we evaluated their underlying mechanism of action in the pathogenesis of SLE.

2. Materials and methods

2.1. Study design and patients

We analyzed blood samples from individuals treated at Nagasaki University Hospital (Nagasaki, Japan) and Sasebo Chuo Hospital (Sasebo, Japan) during a 4-year period from 2013 through 2016: 64 patients with SLE (51 female and 13 male; mean age 41.4 years, standard deviation [SD] 15.4 years), 23 patients with rheumatoid arthritis (RA) (18 female and 5 male; mean age 65.3 years, SD 10.1 years), and 33 healthy controls (HCs) (22 female and 11 male; mean age 29.2 years, SD 6.5 years). The demographic characteristics of the subjects are summarized in Table 1. The subjects included in the analysis between $CD4^+$ $CD52^{lo}$ T cells, circulating follicular helper-like T cells (Tfh-like cells) and plasmablasts are shown separately in Suppl. Table S1. These subjects were included in the group of all subjects in Table 1.

All of the SLE patients fulfilled at least 4 of the 11 revised criteria of the American College of Rheumatology (ACR) [5]. Disease activity was assessed with the Safety of Estrogens in Lupus Erythematosus National Assessment-SLE Disease Activity Index (SELENA-SLEDAI) [6]. All of the RA patients fulfilled the 2010 RA classification criteria issued by the ACR/European League Against Rheumatism (EULAR) [7]. All of the patients and HCs gave their informed consent to participate in the study and have their data published, and the study was approved by the Institutional Review Board of Nagasaki University (approval no. 12012397-5).

2.2. Mice

Female MRL/MpJ-*Tnfrsf6*^{lpr} (MRL/*lpr*) mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and sacrificed at the end of their 6th or 16th week of age. Prior to sacrifice the mice were maintained in an SPF animal facility (Nagasaki University), and the experiments were approved by the Institutional Animal Care Committee of Nagasaki University (approval no. 1412261190-6).

Table 1
Demographics and characteristics of the subjects.

	SLE (n = 64)	RA (n = 23)	HC (n = 33)	p-value
Gender (female:male)	51:13	18:5	22:11	0.52
Age (yrs)	41.4 ± 15.4	65.3 ± 15.4	29.2 ± 15.4	<0.0001
SELENA-SLEDAI	8.3 ± 6.7			
Untreated	14 (21.9%)			
Prednisolone (mg/day)	11.0 ± 10.6			
Immunosuppressive drugs				
Tacrolimus	22 (34.4%)			
Cyclosporine	7 (10.9%)			
Mizoribine	6 (9.4%)			
Mycophenolate	1 (8.7%)			
WBC counts (/μL)	5812 ± 2550			
Lymphocyte counts (/mL)	1085 ± 555			
Anti-ds DNA antibody (U/ml)	61.2 ± 107.9			
C3 (mg/dl)	68.7 ± 27.4			
C4 (mg/dl)	13.2 ± 7.4			
CH50 (U/ml)	28.2 ± 12.5			
IgG (mg/dl)	1618 ± 730.6			

SLE: systemic lupus erythematosus; RA: rheumatoid arthritis; HC: healthy controls; SELENA-SLEDAI: Safety of Estrogens in Lupus Erythematosus National Assessment-SLE Disease Activity Index; WBC: white blood cells. Values are the number (%) of patients or the mean ± SD. Analyses were conducted by using Kruskal-Wallis test and Fisher's exact test.

2.3. Flow cytometry

We isolated peripheral blood mononuclear cells (PBMCs) from the blood samples of patients and HCs using density-gradient centrifugation with a RosetteSep™ system (Stemcell Technologies, Grenoble, France). Isolated PBMCs were stained for flow cytometry with antibodies (Abs) against CD3 (SK7; BD Biosciences, San Diego, CA), CD4 (SK3; BD Biosciences), CD8a (HIT8a; Biolegend, San Diego, CA), CD45 (HI30; BD Pharmingen, San Diego, CA), CD52 (097; Biolegend), CD25 (2A3; BD Biosciences), CD127 (HIL-7R-M21; BD Pharmingen), CD45RA (HI100; Biolegend), CXCR5 (J252D4; Biolegend), ICOS (C398.4A; Biolegend), PD-1 (EH12.2H7; Biolegend), CD19 (HIB19; Biolegend), CD27 (O323; Biolegend), CD38 (HIT2; Biolegend), or CCR7 (GO43H7; Biolegend) for 30 min at 4 °C.

Spleens were excised from the mice by teasing the organs through a nylon mesh. Isolated cells were stained for flow cytometry with Abs against CD3 (17A2; Biolegend), CD4 (GK1.5; Biolegend), CD8a (53–6.7; Biolegend) and CD52 (BTG-2G; MBL, Nagoya, Japan). Stained cells were analyzed by multiparameter flow cytometry using a FACS CANTO II (BD Biosciences) or FlowSight Imaging Flow Cytometer (Amnis Corp., Seattle, WA) and FlowJo ver. 10 software (Tree Star, Ashland, OR).

2.4. Cell sorting

Cell sorting was performed in a FACS Aria II cell sorter (BD Biosciences). PBMCs from SLE patients ($n = 5$) were isolated and then incubated with antibodies against CD3, CD4, CD8, CD45 and CD52 for 30 min at 4 °C. Cells were sorted into two different populations: (1) $CD3^+$ $CD45^+$ $CD4^+$ $CD8^-$ $CD52^{hi}$ cells and (2) $CD3^+$ $CD45^+$ $CD4^+$ $CD8^-$ $CD52^{lo}$ cells. The purity of the sorted cell populations ranged from 94% to 99%.

2.5. ELISA

Soluble CD52 in serum from the human blood samples was measured by a Human CAMPATH-1 Antigen (CD52) Enzyme-Linked Immunosorbent Assay (ELISA) Kit (Cusabio, College Park, MD).

2.6. Gene microarray analysis

To reveal the genetic characteristics of $CD4^+$ $CD52^{lo}$ T cells in SLE, we performed cDNA microarrays. $CD4^+$ $CD52^{lo}$ T cells and $CD4^+$ $CD52^{hi}$ T cells from five SLE patients were sorted by flow cytometry. The RNA was obtained from $CD3^+$ $CD45^+$ $CD4^+$ $CD8^-$ $CD52^{hi}$ cells and $CD3^+$ $CD45^+$ $CD4^+$ $CD8^-$ $CD52^{lo}$ T cells. Total cellular RNA was extracted using an RNeasy Plus Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's recommendations. RNA was quantified using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). We performed the cDNA microarray using a SurePrint G3 Human GE 8x60K Microarray (Agilent Technologies). The *in vitro* transcription, oligonucleotide array hybridization, and scanning were conducted with Takara Bio protocols (TaKaRa Bio, Shiga, Japan). We conducted a scatter plot analysis by using GeneSpring GX ver. 7.3.1 software (Agilent Technologies). The data have been deposited in the Gene Expression Omnibus repository (GSE94815).

2.7. Analysis of the effects of ligands

CD4 T cells were isolated using CD4 RosetteSep™. The isolated CD4 T cells (1×10^6) in RPMI 1640 medium with 10% fetal bovine serum (HyClone, Logan, UT) plus penicillin/streptomycin were incubated for up to 24 h at 37 °C (in 5% CO_2) in 96-well round-bottomed plates with or without the following ligands: growth-related oncogene (GRO) (50 ng/ml; Peprotech, Rocky Hill, NJ), monocyte chemoattractant protein-1 (MCP-1) (20 ng/ml; Prospec, East Brunswick, NJ), regulated

upon activation normal T-cell expressed and secreted (RANTES) (10 ng/ml; Peprotech), thymus and activation-regulated chemokine (TARC) (10 ng/ml; Novus Biologicals, Littleton, CO), and interleukin-8 (IL-8) (100 ng/ml; Prospec).

2.8. Statistical analyses

One-way analysis of variance (ANOVA), unpaired *t*-test, Wilcoxson signed rank test, paired *t*-test and Fisher's exact test were used. Spearman's correlation coefficient with two-tailed *p*-values was determined in the analysis of correlations. Statistical analyses were performed with GraphPad Prism 7.0 software (GraphPad Software, La Jolla, CA) or JMP® 13 (SAS Institute Inc., Cary, NC). A p-value <0.05 was considered significant.

3. Results

3.1. The proportion of $CD4^+$ $CD52^{lo}$ T cells was increased in the SLE patients

We analyzed single $CD3^+$ $CD45^+$ $CD4^+$ cells, dividing them into $CD52^{hi}$ and $CD52^{lo}$ cell subsets, which have been reported as the suppresser phenotype and effector-like phenotype, respectively [4]. We found that the percentage of $CD4^+$ $CD52^{lo}$ T cells in the SLE patients was significantly higher than those in the HC ($p < 0.01$) and RA ($p < 0.05$) groups (Fig. 1A, B). The expansion of $CD8^+$ $CD52^{lo}$ T cells was not seen in SLE patients (Suppl. Fig. S1A).

No significant difference was found in the percentage of conventional Tregs (identified as $CD3^+$ $CD4^+$ $CD25^+$ $CD127^-$ cells) among the three groups (Suppl. Fig. S1B, C). There was no correlation between the percentage of Tregs and $CD4^+$ $CD52^{hi}$ T cells in SLE patients (Suppl. Fig. S1D). The serum soluble CD52 was also analyzed by ELISA. The titer of serum soluble CD52 was decreased in the SLE group compared to the RA and HC groups (Fig. 1C).

3.2. The expansion of $CD4^+$ $CD52^{lo}$ T cells was correlated with disease activity and autoantibody production in the SLE patients

To clarify whether the proportion of $CD4^+CD52^{lo}$ T cells can be a biomarker of SLE, we next analyzed the correlations between the percentage of $CD4^+$ $CD52^{lo}$ T cells in the PBMCs and clinical parameters including the SLEDAI value, the serum IgG concentration, serum anti-double strand-DNA antibody titer, and serum complement titer in the SLE patients. We found that the percentage of $CD4^+CD52^{lo}$ T cells in the SLE patients showed a positive correlation with the SLEDAI, the anti-double strand-DNA antibody titer, and the serum IgG concentration (Fig. 2).

Though the age differed among the three groups, there was no correlation between the percentage of $CD4^+$ $CD52^{lo}$ T cells and age in either SLE patients or all subjects (Suppl. Fig. S2A, B). On the basis of these results, we think that the age of subjects does not affect the proportion of $CD4^+$ $CD52^{lo}$ T cells. There was no difference in gender among the three groups, while the proportion of $CD4^+CD52^{lo}$ T cells was higher in females than males in both SLE patients and all subjects (Suppl. Fig. S2C, D).

There was no correlation between the titer of serum soluble CD52 and the SLEDAI score in SLE patients (Suppl. Fig. S3).

3.3. The $CD4^+$ $CD52^{lo}$ T cells increased with the disease development in MRL/lpr mice

To clarify the relationship between SLE progression and the expression of $CD4^+CD52^{lo}$ T cells, we used MRL/*lpr* mice, which develop immune complex-mediated nephritis and exhibit progressive lymphadenopathy by age 8–10 weeks [8]. We compared the percentage of $CD4^+CD52^{lo}$ T cells from splenocytes between pre-onset (6 weeks old) and established disease (16 weeks old). As shown in Fig. 3, the percentage of $CD4^+$ $CD52^{lo}$ T cells was significantly increased in the 16-week-old mice compared to the 6-week-old mice (Fig. 3).

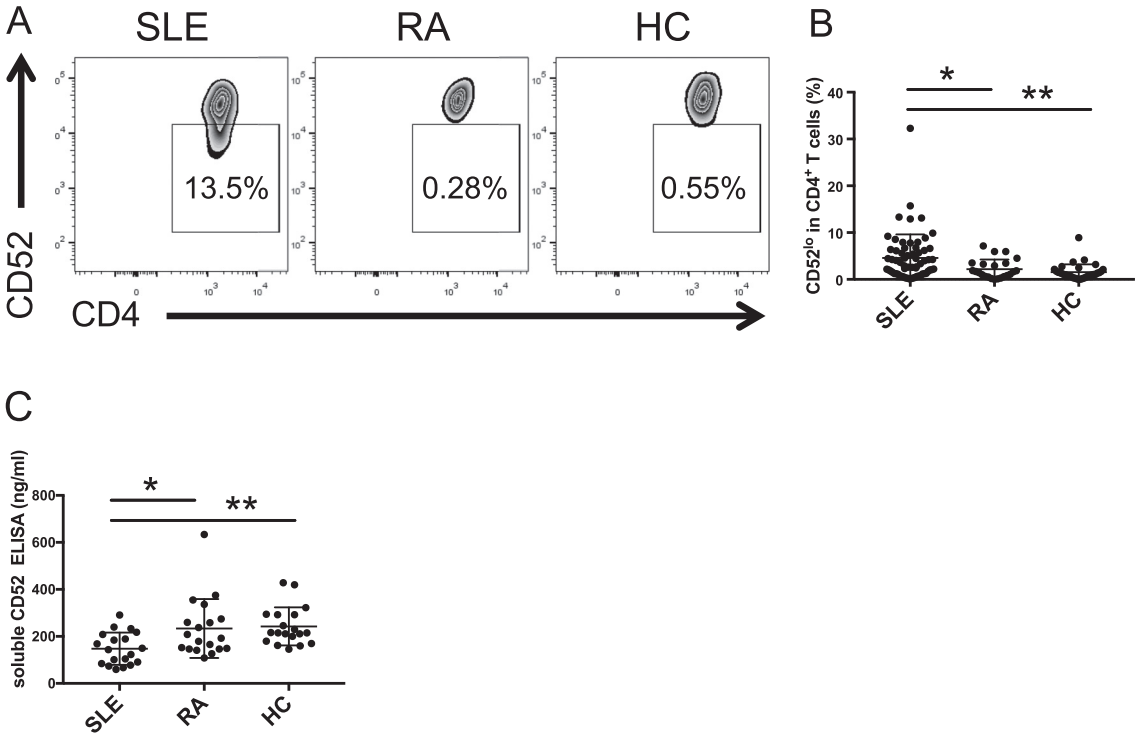

Fig. 1. Analysis of $CD4^+$ $CD52^+$ T cells. A, B: The percentage of $CD4^+CD52^{lo}$ T cells in the SLE patients (n = 64) was significantly higher than those of the HC (n = 33) and RA (n = 23) groups. **C:** Soluble CD52 was decreased in the SLE group compared to the other groups. Bars: mean ± SD. $^*p < 0.05$, $^{**}p < 0.01$ by one-way ANOVA. SLE: systemic lupus erythematosus; HC: healthy control; RA: rheumatoid arthritis.

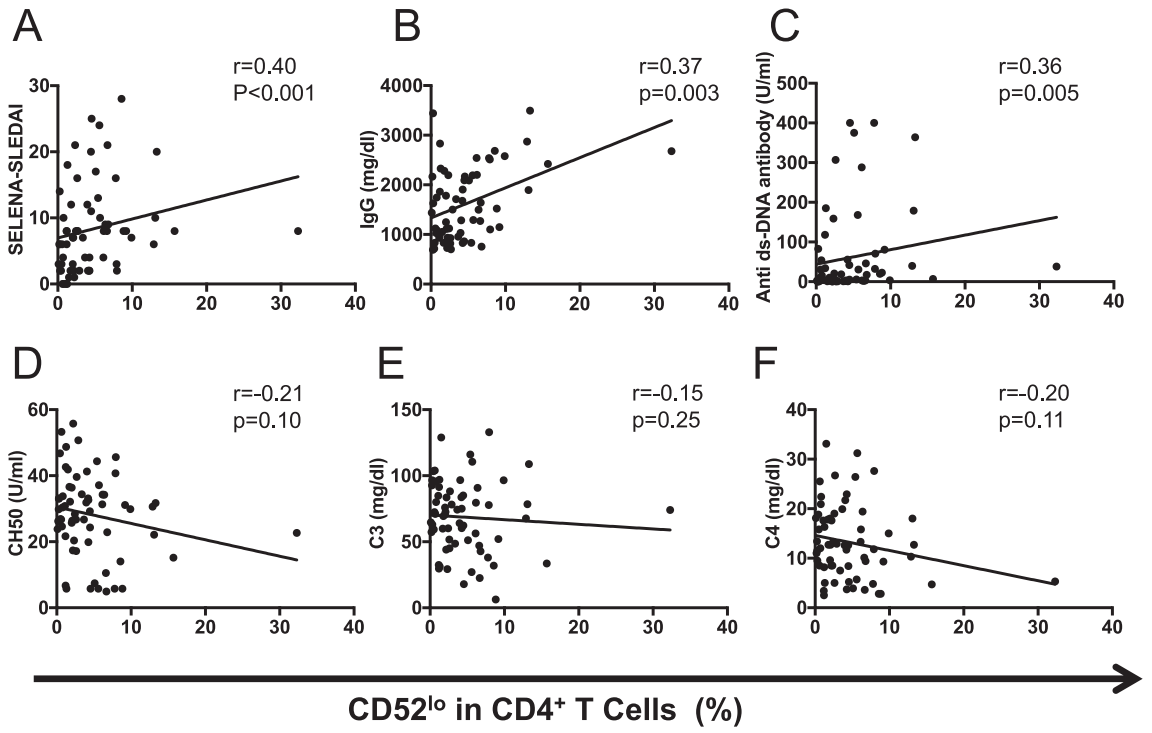

Fig. 2. A–C: The expression of $CD4^+$ $CD52^{lo}$ T cells of the 64 SLE patients was positively correlated with the SLEDAI, anti-ds-DNA antibodies and IgG. D–F: There was no correlation between $CD4^+$ $CD52^{lo}$ T cells and CH50, C3, or C4. All correlations were determined using Spearman's correlation coefficient.

3.4. The correlation of circulating follicular helper-like T cells and $CD4^+$ $CD52^{lo}$ T cells

Since our data showed that the percentage of $CD4^+$ $CD52^{lo}$ T cells was correlated with both the anti-ds-DNA antibody titer and the serum concentration of IgG, we determined the correlation between the percentage of $CD4^+$ $CD52^{lo}$ T cells, Tfh-like cells, and plasmablasts ($CD3^-$ $CD19^+$ $CD38^+$ $CD27^+$) from the SLE patients ($n = 27$), the HC ($n = 9$) and the RA patients (n = 9). The demographic characteristics of these subjects are summarized in Suppl. Table S1. The percentages of Tfh-like cells ($ICOS^{high}$ $PD\text{-}1^{high}$ in $CD4^+$ $CD45RA^-$ $CXCR5^{high}$) and plasmablasts ($CD38^+$ $CD27^+$ in $CD3^-$ $CD19^+$) in the SLE patients were significantly higher than those of the other groups (Fig. 4A, B, D, E), which is consistent with a previous report [9]. We found that the percentage of Tfh-like cells in the SLE group was positively correlated with that of $CD4^+$ $CD52^{lo}$ T cells (Fig. 4C), but there was no correlation between the plasmablasts and $CD4^+$ $CD52^{lo}$ T cells (Fig. 4F). To clarify that Tfh-like cells and $CD4^+$ $CD52^{lo}$ cells are not overlapped populations, we next analyzed $CD45RA^-$ $CXCR5^+$ in both $CD4^+$ $CD52^{hi}$ cells and $CD4^+$ $CD52^{lo}$ T cells in patients with SLE. We found that the percentage of $CD45RA^-$ $CXCR5^+$ cells was lower in the $CD4^+$ $CD52^{lo}$ T cells than the $CD4^+$ $CD52^{lo}$ T cells (Suppl. Fig. S4). From this result, we concluded that there was little overlap between the Tfh-like cells and $CD4^+$ $CD52^{lo}$ cells.

3.5. TARC induced the development of $CD4^+$ $CD52^{hi}$ T cells into $CD4^+$ $CD52^{lo}$ T cells in the SLE patients

The expression of messenger RNA from $CD4^+$ $CD52^{lo}$ T cells and $CD4^+$ $CD52^{hi}$ T cells was analyzed by microarray analysis. To identify

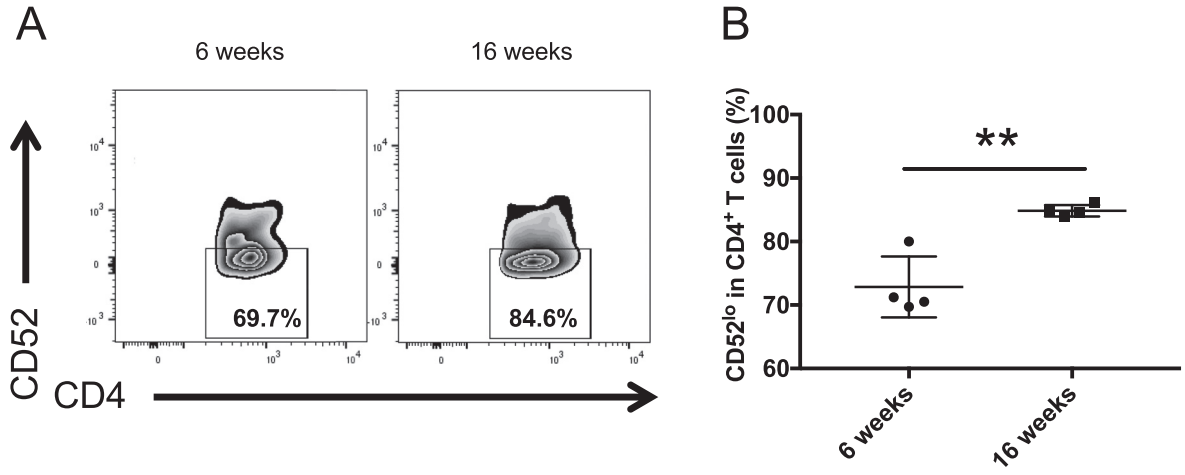

Fig. 3. A, B: Splenocytes from MRL/*lpr* mice were analyzed. $CD4^+$ $CD52^{lo}$ T cells were significantly increased in the 16-wk.-old mice ($n = 4$) compared to the 6-wk.-old mice ($n = 4$). Bars: mean ± SD. $^{**}p < 0.01$ by unpaired *t*-test.

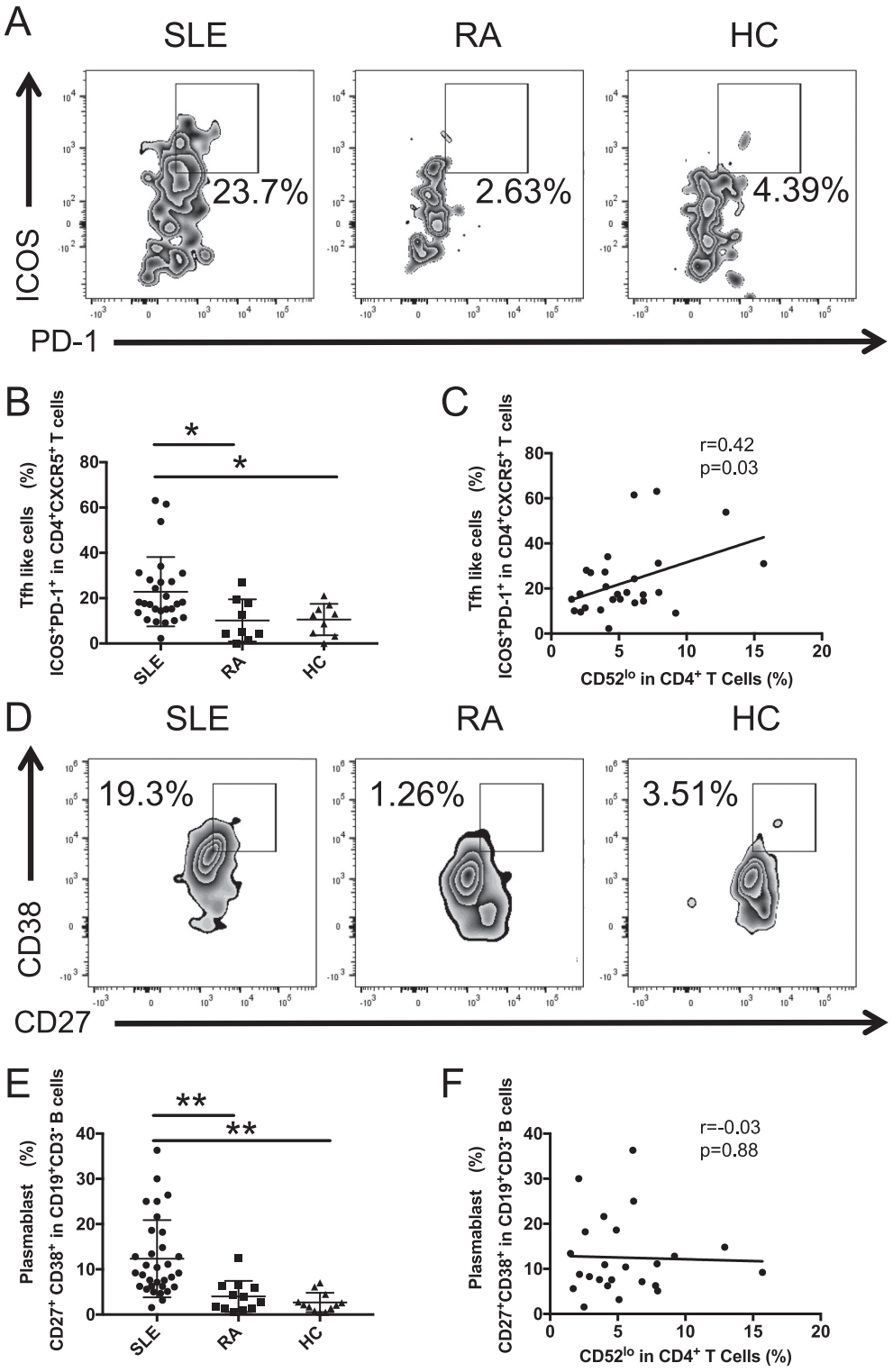

Fig. 4. A, B: The percentage of circulating follicular helper-like T cells (Tfh-like cells), identified as $ICOS^{high}$ $PD\text{-}1^{high}$ in $CD4^+$ $CXCR5^{high}$ T cells from the SLE patients (n = 27) was significantly higher than those in the HC (n = 9) and RA (n = 9) groups. C: The expansion of Tfh-like cells in the SLE group was positively correlated with $CD4^+CD52^{lo}$ T cells. D, E: The percentage of plasmablasts, identified as $CD38^+$ $CD27^+$ in $CD3^-$ $CD19^+$ B cells in the SLE patients ($n = 27$), was significantly higher than those in the HC ($n = 9$) and RA (n = 9) groups. F: There was no correlation between plasmablasts and $CD4^+CD52^{lo}$ T cells. Bars: mean ± SD. $^*p < 0.05$, $^{**}p < 0.01$ by one-way ANOVA. All correlations were determined using Spearman's correlation coefficient.

factors that affect the induction of $CD4^+$ $CD52^{lo}$ T cells, we compared the genes expressing the chemokine receptors between $CD4^+$ $CD52^{lo}$ T cells and $CD4^+$ $CD52^{hi}$ T cells. Of 35,324 genes, the expression of 3158 genes was >1.5-fold higher in $CD4^+CD52^{lo}$ T cells (Fig. 5A). Increased expression levels of several chemokine ligands and their cognate receptors have been found in lupus-prone mice and SLE patients [10]. We focused on the chemokine receptors that were upregulated by >1.5-fold in CD4 + $CD52^{lo}$ T cells, *i.e.*, chemokine receptor 2 (CCR2), CCR5, CCR8 and C-X-C motif chemokine receptor 2 (CXCR2).

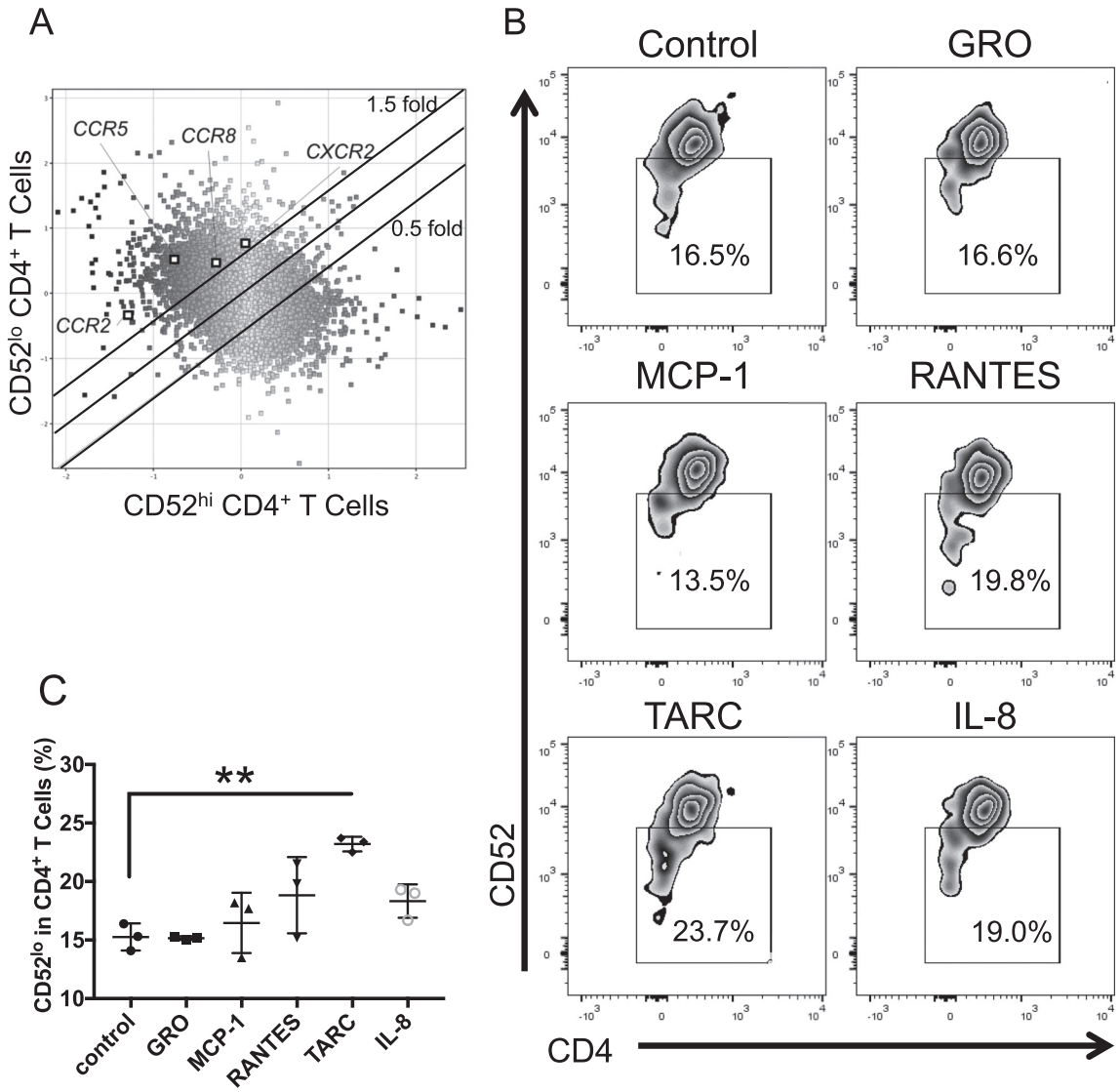

Fig. 5. A: Scatter plot of the entire gene set considered to be expressed in CD4 + CD52^{lo} and CD4 + CD52^{hi} T cells by microarray analysis (SrePrint G3 Human GE 8x60K). The position of each dot on the scatter plot corresponds to the normalized average signal intensity (log scale) of a single gene. The lines represent CD4 + CD52^{lo} T cells/CD4 + CD52^{hi} T cells ratios of 1.5 and of 0.5. The microarray analysis revealed that the expressions of chemokine receptor 2 (CCR2), CCR5, CCR8 and C-X-C Motif Chemokine Receptor 2 (CXCR2) were increased in CD4^{+}CD52^{lo} T cells. B: *In-vitro* experiments using CD4 T cells from patients with SLE. The isolated CD4^{+} T cells were incubated with and without ligands for 24 h: growth related oncogene (GRO) as a ligand of CXCR2, monocyte chemoattractant protein-1 (MCP-1) as a ligand of CCR2 and CCR8, regulated upon activation normal T-cell expressed and secreted (RANTES) as a ligand of CCR5, thymus and activation-regulated chemokine (TARC) as a ligand of CCR8, and interleukin-8 (IL-8) as a ligand of CXCR2. Then the CD4^{+} CD52^{+} T cells were analyzed by flow cytometry. C: TARC induced the conversion of CD4 + CD52^{hi} T cells into CD4 + CD52^{lo} T cells. Bars: mean ± SD. $^{**}p < 0.01$ by one-way ANOVA.

To examine the function of these chemokine receptors in CD4^{+} CD52^{lo} T cells, we conducted *in vitro* experiments using CD4^{+} T cells from SLE patients. The isolated CD4^{+} T cells were incubated with or without ligands related to these chemokine receptors for 24 h: GRO as a ligand of CXCR2 [11], MCP-1 as a ligand of CCR2 [11], RANTES as a ligand of CCR5 [11], TARC as a ligand of CCR8 [12], and IL-8 as a ligand of CXCR2 [11]. We used flow cytometry to analyze the percentage of CD4^{+} CD52^{+} T cells after the stimulation of these ligands, and we found that only TARC induced the development of CD4^{+} CD52^{hi} T cells into CD4^{+} CD52^{lo} T cells (Fig. 5B, C).

4. Discussion

Our findings revealed that the percentage of CD4^{+} CD52^{lo} T cells in the peripheral blood of the SLE patients was increased, and the expansion of CD4^{+} CD52^{lo} T cells was positively correlated with the SLEDAI score, the anti-ds-DNA antibody titer, and the serum IgG concentration. In addition, the expansion of circulating Tfh-like cells, which is responsible for B-cell differentiation in the germinal center, was positively correlated with the percentage of CD4^{+}CD52^{lo} T cells. These results suggested that increased CD4^{+}CD52^{lo} T cells along with decreased serum soluble CD52 are involved in the pathogenic autoantibody production.

We also found that the gene expression of *CCR8* was increased in CD4^{+}CD52^{lo} T cells. The *in vitro* experiments showed that TARC, which is known as one of the ligands for CCR8, induced the development of CD4^{+}CD52^{hi} T cells into CD4^{+}CD52^{lo} T cells.

Humans with type 1 diabetes have been reported to have a lower frequency and diminished function of CD4^{+}CD52^{hi} T cells responsive to the autoantigen GAD65 [4]. CD4^{+}CD52^{hi} T cells exert a suppressor function by releasing soluble CD52 [4]. Soluble CD52 binds to siglec-10 on activated T cells, including CD4^{+}CD52^{lo} T cells, which leads to the inhibition of these cells by impairing the phosphorylation of the T-cell receptor-associated kinases Lck and Zap70 [4]. An anti-CD52 antibody (alemtuzumab) has been used to treat multiple diseases: RA [13–15], non-Hodgkin's lymphoma [16,17], T-cell lymphoma [18,19], and relapsing-remitting multiple sclerosis [20,21].

The efficacy of alemtuzumab is brought about by anti-CD52 antibody-mediated lymphocytic depletion as immune ablation. However, multiple sclerosis patients treated with alemtuzumab have been reported to develop autoimmune diseases with autoantibodies, such as Grave's disease, immune thrombocytopenic purpura, and anti-glomerular basement membrane disease [22,23]. It has been suggested that anti-CD52 antibody treatment may induce autoimmune diseases by a preferential removal of $CD4^+$ $CD52^{hi}$ regulatory T cells and soluble CD52 [4,24]. In light of our present findings, a decreased percentage of $CD4^+$ $CD52^{hi}$ T cells along with decreased soluble CD52 in the serum of patients with SLE may contribute to the impairment of the inhibitory signal of activated T cells.

Our present investigation revealed that the expansion of $CD4^+$ $CD52^{lo}$ T cells correlates with disease activity and autoantibody production in both SLE patients and a murine lupus-prone model. It was proposed that follicular helper T cells are necessary for B-cell differentiation in the germinal center [25] and that circulating Tfh-like cells, identified as $CD4^+$ $CD45RA^-$ $CXCR5^{hi}$ $ICOS^{hi}$ $PD\text{-}1^{hi}$, were increased in the PBMCs from individuals with SLE [9]. In the present study, the expansion of Tfh-like cells was positively correlated with $CD4^+$ $CD52^{lo}$ T cells in the SLE patients, suggesting that $CD4^+$ $CD52^{lo}$ T cells are involved in the abnormal production of antibodies through the differentiation of Tfh-like cell-mediated plasma cells. However, our investigation could not clarify the mechanisms by which the $CD4^+$ $CD52^{lo}$ T cells induce Tfh-like cells. We speculate that $CD4^+$ $CD52^{lo}$ T cells from SLE patients may produce IL-21, which skews toward to Tfh-like cells. Further research is required to elucidate the precise mechanisms underlying the induction of Tfh-like cells by $CD4^+$ $CD52^{lo}$ T cells.

To determine whether the proportion of $CD4^+$ $CD52^{lo}$ T cells could be a candidate marker for the clinical response of SLE patients, we evaluated the percentage of $CD4^+$ $CD52^{lo}$ T cells before and after intravenous cyclophosphamide (IVCY) treatment, and as shown in Suppl. Fig. S5, we observed that the percentage of $CD4^+$ $CD52^{lo}$ T cells was reduced after the induction of IVCY therapy. Previous studies showed that CD52 expression is downregulated as lymphocytes divide and upregulated after the induction of cell quiescence through the expression of the transcription factor LKLF (lung Krüppel-like factor) [26,27].

Cyclophosphamide is an alkylating agent that interferes with the duplication of DNA, resulting in an impairment of cell division [28]. Cell quiescence induced by IVCY may contribute to the decrease of $CD4^+$ $CD52^{lo}$ T cells in SLE patients. In addition, our present experiments demonstrated that in the murine lupus-prone model, the percentage of $CD4^+$ $CD52^{lo}$ T cells was greater in the 16-week-old mice compared to the 6-week-old mice (Fig. 3). In light of these results, we propose that $CD4^+$ $CD52^{lo}$ T cells could serve as a useful biomarker for evaluating the disease activity and therapeutic responses of individuals with SLE.

Interestingly, even though the expansion of $CD8^+$ $CD52^{lo}$ T cells was not seen in SLE patients, the $CD8^+$ $CD52^{lo}$ T cells were expanded in the patients with RA (Suppl. Fig. S1A). Abundant $CD8^+$ T cells have been shown to infiltrate the rheumatoid synovium [29] and are also detected in preclinical stages [30]. $CD8^+$ $CD52^{lo}$ T cells may play some role in the pathogenesis in RA. Further research is needed on this point.

In a present study, there was no correlation between the percentage of $CD4^+$ $CD25^+$ $CD127^-$ regulatory T cells and $CD4^+$ $CD52^{hi}$ T cells in the SLE patients. In addition, the microarray analysis in the present study showed that the mRNA expression level of *FOXP3*, a master transcription gene in Tregs, was nearly identical between $CD4^+$ $CD52^{lo}$ T cells and $CD4^+$ $CD52^{hi}$ T cells (1.16-fold difference). In the previous studies, the generation of human $CD4^+$ regulatory T-cell clones against the pancreatic islet autoantigen GAD65 was indicated to be a function of regulatory T cells [4,31]. These regulatory T-cell clones did not show CD25 expression or inhibitory mechanisms with typical Tregs. The clones also showed that a depletion of $CD25^+$ T cells from PBMCs did not affect the generation of $CD4^+$ $CD52^{hi}$ suppressive T cells [4]. These results support our present findings and suggest that $CD4^+$ $CD52^{hi}$ T cells with suppressor functions are not derived from $CD4^+$ $CD25^+$ conventional regulatory T cells.

We also analyzed the differentiation of T cells such as effector memory, central memory and naïve cells in both $CD4^+$ $CD52^{lo}$ T cells and $CD4^+$ $CD52^{hi}$ T cells (shown in Suppl. Fig. S6). We found that $CD4^+$ $CD52^{lo}$ T cells were polarized into effector T cells. The microarray analysis revealed that the mRNA expression of *IFNG*, the gene encoding IFN-γ, was upregulated in $CD4^+$ $CD52^{lo}$ T cells compared to $CD4^+$ $CD52^{hi}$ T cells (2.66-fold difference). These results suggest that $CD4^+$ $CD52^{lo}$ T cells in SLE patients have the activated effector functions.

TARC, also known as chemokine ligand 17 (CCR17), is expressed in the thymus and is produced by dendritic cells, endothelial cells, keratinocytes and fibroblasts [32]. TARC has affinity as a ligand for CCR4 and CCR8, which are predominantly expressed by Th2 cells [12,33]. In the present study, we observed that (1) the expression of *CCR8* but not *CCR4* mRNA was increased in $CD4^+$ $CD52^{lo}$ T cells, and (2) a recombinant TARC protein induced the development of CD4+ $CD52^{hi}$ T cells into CD4+ $CD52^{lo}$ T cells in SLE patients. A previous study implicated TARC in T-cell activations and the development of CCR8-transfected Jurkat cells [12]. It was also reported that the concentration of TARC is elevated in serum from SLE patients [34] and from New Zealand mice, a lupus-prone murine model [35]. Collectively, these findings suggest that TARC, by interacting with CCR8, may induce the development of $CD4^+$ $CD52^{hi}$ T cells into $CD4^+$ $CD52^{lo}$ T cells *via* direct or indirect mechanisms.

In conclusion, our data suggest that an increased percentage of $CD4^+$ $CD52^{lo}$ T cells, in conjunction with an increased percentage of Tfh-like cells, is involved in the pathogenic autoantibody production and that TARC may contribute to the development of SLE *via* an aberrant induction of $CD4^+$ $CD52^{lo}$ cells.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2017.10.004.

Acknowledgments

We thank Kaori Furukawa, Yuko Mizuno and Rie Yamashita for their excellent technical assistance.

Author contributions

Drs. Ichinose and Koga had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study design: K. Ichinose, T. Koga.

Acquisition of data: M. Umeda, T. Koga, K. Ichinose, T. Sato, T. Shimizu, S. Fukui, A. Nishino, A. Kawakami.

Analysis and interpretation of data: M. Umeda, K. Ichinose, T. Koga, T. Igawa, Y. Hirai, S. Kawashiri, N. Iwamoto, T. Aramaki, M. Tamai, H. Nakamura, K. Yamamoto, T. Origuchi, Y. Ueki, A. Kawakami.

Manuscript preparation: M. Umeda, K. Ichinose, T. Koga.

Statistical analysis: M. Umeda.

References

[1] G.C. Tsokos, Systemic lupus erythematosus, N. Engl. J. Med. 365 (2011) 2110–2121.
[2] S.P. Rao, J. Sancho, J. Campos-Rivera, P.M. Boutin, P.B. Severy, T. Weeden, S. Shankara, B.L. Roberts, J.M. Kaplan, Human peripheral blood mononuclear cells exhibit heterogeneous CD52 expression levels and show differential sensitivity to alemtuzumab mediated cytolysis, PLoS One 7 (2012), e39416.
[3] B. Samten, CD52 as both a marker and an effector molecule of T cells with regulatory action: identification of novel regulatory T cells, Cell. Mol. Immunol. 10 (2013) 456–458.
[4] E. Bandala-Sanchez, Y. Zhang, S. Reinwald, J.A. Dromey, B.H. Lee, J. Qian, R.M. Bohmer, L.C. Harrison, T cell regulation mediated by interaction of soluble CD52 with the inhibitory receptor Siglec-10, Nat. Immunol. 14 (2013) 741–748.
[5] E.M. Tan, A.S. Cohen, J.F. Fries, A.T. Masi, D.J. McShane, N.F. Rothfield, J.G. Schaller, N. Talal, R.J. Winchester, The 1982 revised criteria for the classification of systemic lupus erythematosus, Arthritis Rheum. 25 (1982) 1271–1277.

[6] M. Petri, M.Y. Kim, K.C. Kalunian, J. Grossman, B.H. Hahn, L.R. Sammaritano, M. Lockshin, J.T. Merrill, H.M. Belmont, A.D. Askanase, W.J. McCune, M. Hearth-Holmes, M.A. Dooley, J. Von Feldt, A. Friedman, M. Tan, J. Davis, M. Cronin, B. Diamond, M. Mackay, L. Sigler, M. Fillius, A. Rupel, F. Licciardi, J.P. Buyon, Combined oral contraceptives in women with systemic lupus erythematosus, N. Engl. J. Med. 353 (2005) 2550–2558.
[7] D. Aletaha, T. Neogi, A.J. Silman, J. Funovits, D.T. Felson, C.O. Bingham III, N.S. Birnbaum, G.R. Burmester, V.P. Bykerk, M.D. Cohen, B. Combe, K.H. Costenbader, M. Dougados, P. Emery, G. Ferraccioli, J.M. Hazes, K. Hobbs, T.W. Huizinga, A. Kavanaugh, J. Kay, T.K. Kvien, T. Laing, P. Mease, H.A. Menard, L.W. Moreland, R.L. Naden, T. Pincus, J.S. Smolen, E. Stanislawska-Biernat, D. Symmons, P.P. Tak, K.S. Upchurch, J. Vencovsky, F. Wolfe, G. Hawker, 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European league against rheumatism collaborative initiative, Ann. Rheum. Dis. 69 (2010) 1580–1588.
[8] W. Rodriguez, C. Mold, L.L. Marnell, J. Hutt, G.J. Silverman, D. Tran, T.W. Du Clos, Prevention and reversal of nephritis in MRL/lpr mice with a single injection of C-reactive protein, Arthritis Rheum. 54 (2006) 325–335.
[9] J.Y. Choi, J.H. Ho, S.G. Pasoto, V. Bunin, S.T. Kim, S. Carrasco, E.F. Borba, C.R. Goncalves, P.R. Costa, E.G. Kallas, E. Bonfa, J. Craft, Circulating follicular helper-like T cells in systemic lupus erythematosus: association with disease activity, Arthritis Rheumatol. 67 (2015) 988–999.
[10] X. Liao, T. Pirapakaran, X.M. Luo, Chemokines and chemokine receptors in the development of lupus nephritis, Mediat. Inflamm. 2016 (2016) 6012715.
[11] A. Zlotnik, O. Yoshie, H. Nomiyama, The chemokine and chemokine receptor superfamilies and their molecular evolution, Genome Biol. 7 (2006) 243.
[12] G. Bernardini, J. Hedrick, S. Sozzani, W. Luini, G. Spinetti, M. Weiss, S. Menon, A. Zlotnik, A. Mantovani, A. Santoni, M. Napolitano, Identification of the CC chemokines TARC and macrophage inflammatory protein-1 beta as novel functional ligands for the CCR8 receptor, Eur. J. Immunol. 28 (1998) 582–588.
[13] S.J. Brett, G. Baxter, H. Cooper, W. Rowan, T. Regan, J. Tite, N. Rapson, Emergence of CD52-, glycosylphosphatidylinositol-anchor-deficient lymphocytes in rheumatoid arthritis patients following Campath-1H treatment, Int. Immunol. 8 (1996) 325–334.
[14] J.D. Isaacs, R.A. Watts, B.L. Hazleman, G. Hale, M.T. Keogan, S.P. Cobbold, H. Waldmann, Humanised monoclonal antibody therapy for rheumatoid arthritis, Lancet 340 (1992) 748–752.
[15] E.L. Matteson, D.E. Yocum, E.W. St Clair, A.A. Achkar, M.S. Thakor, M.R. Jacobs, A.E. Hays, C.K. Heitman, J.M. Johnston, Treatment of active refractory rheumatoid arthritis with humanized monoclonal antibody CAMPATH-1H administered by daily subcutaneous injection, Arthritis Rheum. 38 (1995) 1187–1193.
[16] J. Lundin, A. Osterborg, G. Brittinger, D. Crowther, H. Dombret, A. Engert, A. Epenetos, C. Gisselbrecht, D. Huhn, U. Jaeger, J. Thomas, R. Marcus, N. Nissen, C. Poynton, E. Rankin, R. Stahel, M. Uppenkamp, R. Willemze, H. Mellstedt, CAMPATH-1H monoclonal antibody in therapy for previously treated low-grade non-Hodgkin's lymphomas: a phase II multicenter study. European Study Group of CAMPATH-1H Treatment in Low-Grade Non-Hodgkin's Lymphoma, J. Clin. Oncol. 16 (1998) 3257–3263.
[17] A. Osterborg, A. Werner, E. Halapi, J. Lundin, U. Harmenberg, H. Wigzell, H. Mellstedt, Clonal CD8+ and CD52− T cells are induced in responding B cell lymphoma patients treated with Campath-1H (anti-CD52), Eur. J. Haematol. 58 (1997) 5–13.
[18] L. Ginaldi, M. De Martinis, E. Matutes, N. Farahat, R. Morilla, M.J. Dyer, D. Catovsky, Levels of expression of CD52 in normal and leukemic B and T cells: correlation with in vivo therapeutic responses to Campath-1H, Leuk. Res. 22 (1998) 185–191.
[19] R. Pawson, M.J. Dyer, R. Barge, E. Matutes, P.D. Thornton, E. Emmett, J.C. Kluin-Nelemans, W.E. Fibbe, R. Willemze, D. Catovsky, Treatment of T-cell prolymphocytic leukemia with human CD52 antibody, J. Clin. Oncol. 15 (1997) 2667–2672.
[20] K.P. Garnock-Jones, Alemtuzumab: a review of its use in patients with relapsing multiple sclerosis, Drugs 74 (2014) 489–504.
[21] J.L. Jones, A.J. Coles, Mode of action and clinical studies with alemtuzumab, Exp. Neurol. 262 (Pt A) (2014) 37–43.
[22] M. Cossburn, A.A. Pace, J. Jones, R. Ali, G. Ingram, K. Baker, C. Hirst, J. Zajicek, N. Scolding, M. Boggild, T. Pickersgill, Y. Ben-Shlomo, A. Coles, N.P. Robertson, Autoimmune disease after alemtuzumab treatment for multiple sclerosis in a multicenter cohort, Neurology 77 (2011) 573–579.
[23] M. Gupta, K. Jafri, R. Sharim, S. Silverman, S.B. Sindher, A. Shahane, M. Kwan, Immune reconstitution inflammatory syndrome associated with biologic therapy, Curr Allergy Asthma Rep 15 (2015) 499.
[24] S. von Kutzleben, G. Pryce, G. Giovannoni, D. Baker, Depletion of CD52-positive cells inhibits the development of central nervous system autoimmune disease, but deletes an immune-tolerance promoting CD8 T-cell population. Implications for secondary autoimmunity of alemtuzumab in multiple sclerosis, Immunology 150 (2017) 444–455.
[25] N. Fazilleau, L. Mark, L.J. McHeyzer-Williams, M.G. McHeyzer-Williams, Follicular helper T cells: lineage and location, Immunity 30 (2009) 324–335.
[26] R.E. Haaland, W. Yu, A.P. Rice, Identification of LKLF-regulated genes in quiescent CD4+ T lymphocytes, Mol. Immunol. 42 (2005) 627–641.
[27] H. Kubota, H. Okazaki, M. Onuma, S. Kano, M. Hattori, N. Minato, Identification and gene cloning of a new phosphatidylinositol-linked antigen expressed on mature lymphocytes. Down-regulation by lymphocyte activation, J. Immunol. 145 (1990) 3924–3931.
[28] A.G. Hall, M.J. Tilby, Mechanisms of action of, and modes of resistance to, alkylating agents used in the treatment of haematological malignancies, Blood Rev. 6 (1992) 163–173.
[29] I.B. McInnes, Leukotrienes, mast cells, and T cells, Arthritis Res. Ther. 5 (2003) 288–289.
[30] M.J. de Hair, M.G. van de Sande, T.H. Ramwadhdoebe, M. Hansson, R. Landewe, C. van der Leij, M. Maas, G. Serre, D. van Schaardenburg, L. Klareskog, D.M. Gerlag, L.G. van Baarsen, P.P. Tak, Features of the synovium of individuals at risk of developing rheumatoid arthritis: implications for understanding preclinical rheumatoid arthritis, Arthritis Rheumatol. 66 (2014) 513–522.
[31] J.A. Dromey, B.H. Lee, H. Yu, H.E. Young, D.J. Thearle, K.P. Jensen, S.I. Mannering, L.C. Harrison, Generation and expansion of regulatory human CD4(+) T-cell clones specific for pancreatic islet autoantigens, J. Autoimmun. 36 (2011) 47–55.
[32] H. Saeki, K. Tamaki, Thymus and activation regulated chemokine (TARC)/CCL17 and skin diseases, J. Dermatol. Sci. 43 (2006) 75–84.
[33] A. Zingoni, H. Soto, J.A. Hedrick, A. Stoppacciaro, C.T. Storlazzi, F. Sinigaglia, D. D'Ambrosio, A. O'Garra, D. Robinson, M. Rocchi, A. Santoni, A. Zlotnik, M. Napolitano, The chemokine receptor CCR8 is preferentially expressed in Th2 but not Th1 cells, J. Immunol. 161 (1998) 547–551.
[34] H. Okamoto, K. Koizumi, H. Yamanaka, T. Saito, N. Kamatani, A role for TARC/CCL17, a CC chemokine, in systemic lupus erythematosus, J. Rheumatol. 30 (2003) 2369–2373.
[35] H. Okamoto, H. Nishimura, N. Kamatani, A role for TARC/CCL17, a CC chemokine, in New Zealand mice, Rheumatology (Oxford) 44 (2005) 819–820.