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Letter to the Editor

The potential role of $CD4^+CD52^{lo}$ T-cell populations in systemic lupus erythematosus

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To the editor:

Systemic lupus erythematosus (SLE), a chronic inflammatory disease of unknown cause, affects virtually all of the human body's organs [1]. Immunological abnormalities (in particular the production of numerous antinuclear antibodies) are another prominent feature of SLE. T-cell regulation is necessary to limit the inappropriate proliferation of lymphocytes and to prevent autoimmune diseases.

CD52, also known as campath-1, is a cell surface glycoprotein which is widely expressed in lymphocytes, monocytes, and eosinophils. Alterations in the amount and quality of $CD4^+CD52^{lo}$ T cells have been suggested to be involved in autoimmune diseases. A prior study suggested that $CD4^+CD52^{hi}$ T cells play the role of suppressor and that $CD4^+CD52^{lo}$ T cells are inflammatory in patients with type 1 diabetes mellitus (T1DM) [2].

Those findings indicated that the imbalance between $CD4^+CD52^{hi}$ T cells and $CD4^+CD52^{lo}$ T cells is involved in the pathogenesis of autoimmune diseases. We previously focused on this point and observed the expression and function of T cells in SLE [3] as follows. (*1*) The proportion of $CD4^+CD52^{lo}$ T cells is increased in diseased MRL/*lpr* mice and human lupus patients. (*2*) The proliferation of $CD4^+CD52^{lo}$ T cells showed a positive correlation with the Safety of Estrogens in Lupus Erythematosus National Assessment-SLE Disease Activity Index (SELENA-SLEDAI) and the titer of anti-ds-DNA antibodies. (*3*) We found that the proportion of follicular helper-like T cells (Tfh-like cells: $CD4^+CD45RA^-$ $CXCR5^{hi}$ $ICOS^{hi}$ $PD\text{-}1^{hi}$ T cells) in SLE patients was positively correlated with the proportion of $CD4^+CD52^{lo}$ T cells. (*4*) The expression of chemokine (C−C motif) receptor 8 (CCR8) was increased in $CD4^+CD52^{lo}$ T cells. (*5*) Thymus activation-regulated cytokine (TARC), known as one of the ligands for CCR8, induced the transition from $CD4^+CD52^{hi}$ T cells to $CD4^+CD52^{lo}$ T cells. (*6*) The percentage of $CD4^+CD52^{lo}$ T cells was reduced after the induction of intravenous cyclophosphamide (IVCY) therapy. These findings suggested that an increased number of $CD4^+CD52^{lo}$ T cells is involved in the production of autoantibodies and enhances the disease activity in SLE as well as T1DM.

In the present study, we compared the gene expression and gene ontology in $CD4^+CD52^{lo}$ T cells compared to $CD4^+CD52^{hi}$ T cells. $CD4^+CD52^{lo}$ T cells and $CD4^+CD52^{hi}$ T cells from five individual SLE patients were obtained by sorting with a FACSAria II cell sorter (BD Bioscience, San Jose, CA) and were > 95% pure. The characteristics of the studied SLE patients are summarized in Supplementary Table S1. The patients' data are from their initial onset of SLE, and the patients had not been treated with any immunosuppressive agents. The RNA was obtained from $CD3^+CD45^+CD4^+CD8^-$ $CD52^{hi}$ cells and $CD3^+CD45^+CD4^+CD8^-$ $CD52^{lo}$ T cells.

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 quantification of fluorescence intensity in each spot was calculated as the net intensity, i.e., the difference between the background and the raw intensity.

This dataset has been made publicly available at the Gene Expression Omnibus GEO database [4] under submission no. GSE94815.

The patterns of gene expression in $CD4^+CD52^{lo}$ T cells and $CD4^+CD52^{hi}$ T cells are completely different (Suppl. Fig. S1). The results of the microarray analysis demonstrated that *FOXO3*, *FASLG*, *DIAPH3*, *SLC15A4*, *MAPK1*, *NOS2*, *SCUBE1*, *JAK2*, *HLA-A*, *TNFSF12* and *Fli1*, which are also known as disease susceptibility genes of murine and/or human lupus, were significantly elevated in $CD4^+CD52^{lo}$ T cells compared to $CD4^+CD52^{hi}$ T cells (Table 1).

To reveal the putative role of $CD4^+CD52^{lo}$ T cells, we performed multiplex cytokine/chemokine bead assays using diluted serum supernatants from 30 SLE patients and Milliplex MAP Human Cytokine/Chemokine Panel 1 Pre-mixed 41Plex (Merck Millipore, Darmstadt, Germany), analyzed with a Bio-Plex® MAGPIX™ Multiplex Reader (Bio-Rad, Hercules, CA) according to the manufacturers' instructions. The cytokines/chemokines measured by the Milliplex MAP Human Cytokine/Chemokine Panel 1 Pre-mixed 41Plex included.

Heat-maps of nearest-neighbor correlations of $CD4^+CD52^{lo}$ T cells (%), cytokines, chemokines, and growth factors in the serum of SLE patients are provided in Supplementary Fig. S2. For each $CD4^+CD52^{lo}$ T cell (%), cytokine, chemokine, and growth factor analyzed, the distance was determined based on Spearman's correlation coefficient. According to the heat-maps in the figure, it became clear that the correlations between $CD4^+CD52^{lo}$ T cells and the cytokines, chemokines and growth factors can be divided into two broad groups by the line of $CD52^{lo}$ (%) (surrounded by a red square in Suppl. Fig. S2).

The two groups are the positive correlations with a proinflammatory

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Table 1
Confirmation of changed genes that are known as disease susceptibility genes of murine and/or human lupus.

Gene symbol	Probe ID	GenBank accession no.	$CD52^{lo}$ average value	$CD52^{hi}$ average value	Average ratio	*p*-Value
FOXO3	A_33_P3240392*	NM_001455	6277.71	4099.73	1.5312	0.0020
FASLG	A_23_P369815	NM_000639	1837.76	109.89	16.7235	0.0117
DIAPH3	A_23_P162719	NM_030932	719.24	358.13	2.0083	0.0157
SLC15A4	A_33_P3406449	NM_145648	2502.53	1303.6	1.9197	0.0192
MAPK1	A_23_P257895	NM_138957	2798.47	1554.77	1.7999	0.0205
NOS2	A_23_P502464	NM_000625	139.29	40.8	3.4139	0.0248
SCUBE1	A_33_P3480395	AK055463	148.73	83.17	1.7883	0.0261
JAK2	A_33_P3878772	NM_004972	1855.78	834.13	2.2248	0.0324
HLA-A	A_33_P3379962	NM_002116	345,397.19	307,775.43	1.1222	0.0366
TNFSF12	A_33_P3406623	NM_003809	1697.76	1030.42	1.6476	0.0384
Fli1	A_24_P355649	NM_002017	6234.15	5339.08	1.1676	0.0474

The expression of selected genes shown to be upregulated in $CD4^+CD52^{lo}$ T cells compared to $CD4^+CD52^{hi}$ T cells of the same SLE patients' peripheral blood mononuclear cells by an Agilent 2100 Bioanalyzer.

* $p < .05$.

cytokine, chemokine, or growth factor, and the negative correlations with them. We found that the $CD4^+CD52^{lo}$ T cells (%) were significantly positively correlated with IL-2 ($p = .0085$), IL-6 ($p = .0218$), IL-12p40 ($p = .0446$), IL-12p70 ($p = .0059$), FGF-2/basic FGF ($p = .0001$), and MCP-3/CCL7 ($p = .0114$). The $CD4^+CD52^{lo}$ T cells (%) were significantly negatively correlated with eotaxin ($p = .0494$) and RANTES ($p = .0351$).

Multiple studies have identified higher IL-6 [5], IL-12 [6], FGF-2 [7] and MCP-3/CCL7 [8] levels in different serum samples from active SLE patients. In contrast, the level of IL-2 is known to be decreased in $CD4^+$ T cells of SLE patients and various murine lupus models, as are autoantibody production and proteinuria [9]. IL-2 is necessary for T-cell activation, and it is needed following clonal expansion to control the lymphoproliferation of a T-cell clone. The positive correlation between $CD4^+CD52^{lo}$ T cells (%) and IL-2 may result from a dichotomic function of $CD4^+CD52^{lo}$ T cells as a positive activator and a negative regulator of the signal transduction in $CD4^+CD52^{lo}$T cells.

Here, we clarified that the imbalance between $CD4^+CD52^{hi}$ T cells and $CD4^+CD52^{lo}$ T cells is involved in the pathogenesis of SLE. $CD4^+CD52^{lo}$ T cells may have a positive activator of the signal transduction involved in the pathology of SLE. Patterns of gene expression are considerably different between $CD4^+CD52^{lo}$ T cells and $CD4^+CD52^{hi}$ T cells, and $CD4^+CD52^{lo}$ T cells were linked to multiple genes that are reported as disease susceptibility genes in SLE. This suggested that $CD4^+CD52^{lo}$ T cells may be involved in the etiology of SLE.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2019.01.007.

Authors' contributions

K.I. and M.U. 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 conception and design: K.I., T.K., A.K.

Acquisition of data: K.I., M.U.

Analysis and interpretation of data: K.I., M.U., T.K.

All authors read and approved the final manuscript.

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Kunihiro Ichinose*, Masataka Umeda, Tomohiro Koga, Atsushi Kawakami
Department of Immunology and Rheumatology, Unit of Advanced Preventive Medical Sciences, Division of Advanced Preventive Medical Sciences, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
E-mail address: kichinos@nagasaki-u.ac.jp (K. Ichinose).

* Corresponding author.