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Rapid Communication

CD45RA−Foxp3^{low} non-regulatory T cells in the CCR7−CD45RA−CD27+CD28+ effector memory subset are increased in synovial fluid from patients with rheumatoid arthritis

Fumichika Matsuki [a,b], Jun Saegusa [a,b,*], Keisuke Nishimura [a], Yasushi Miura [c], Masahiro Kurosaka [c], Shunichi Kumagai [b,d], Akio Morinobu [a]

[a] Department of Rheumatology and Clinical Immunology, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan
[b] Department of Evidence-Based Laboratory Medicine, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan
[c] Department of Orthopaedic Surgery, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan
[d] The Center for Rheumatic Diseases, Shinko Hospital, 1-4-47 Wakinohama-cho, Chuo-ku, Kobe 651-0072, Japan

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ABSTRACT

Increased numbers of regulatory T (Treg) cells are found in synovial fluid from patients with rheumatoid arthritis (RASF) compared with peripheral blood. However, Treg cells in RASF have been shown to have a decreased capacity to suppress T cells. Here we phenotypically classified CD4+ T cells in RASF into six subsets based on the expression of CD45RA, CCR7, CD27 and CD28, and demonstrated that the CCR7−CD45RA−CD27+CD28+ T_{EM} subset was significantly increased in synovial fluid compared with peripheral blood. In addition, the proportion of Foxp3+ Treg cells in the CCR7−CD45RA−CD27+CD28+ T_{EM} subset was significantly increased in RASF. Furthermore, most of the Foxp3+ Treg cells in RASF were non-suppressive CD45RA−Foxp3^{low} non-Treg cells, and the frequency of the non-Treg cells in the CCR7−CD45RA−CD27+CD28+ T_{EM} subset was significantly increased in RASF. Our findings suggest that the pro-inflammatory environment in RA joints may induce the increase of CD45RA−Foxp3^{low} non-Treg cells in synovial fluid.

1. Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by synovial inflammation and hyperplasia in which T cells and monocytes play central pathogenic roles. Activated T cells infiltrate into the pannus and the synovial fluid, contributing to a series of inflammatory processes, and leading to the cartilage and bone destruction.

Human T cells can be divided into functionally distinct subsets. Two primary categories are T cells that have not been exposed to antigen (naïve) and those that are antigen-experienced (memory). Memory subset can be further subdivided into CCR7+CD45RA− central memory T cells (T_{CM}), which resides mainly in the secondary lymphoid tissues, and CCR7−CD45RA− effector memory T cells (T_{EM}), which reside in peripheral non-lymphoid tissues. In addition to CD45RA and CCR7, CD27 and CD28 have been used as surface markers to characterize CD4+ T cells. Previous reports established a model of CD4+ T cell differentiation characterized by a sequential down-regulation of CCR7, CD27 and then CD28, accompanied by alteration of their functional characteristics [1,2]. CD27 and CD28 are the main costimulatory molecules required to induce T cell activation, although memory T cells seem to be less dependent on CD27 and CD28 for their reactivation than naïve T cells [3,4]. As CD4+ T cells lose CD27 expression, they gain cytotoxic potential with the acquisition of lytic granules with granzymes, and acquisition of perforin at the CD28 negative stage, so that highly differentiated CD4+ T cells become cytotoxic. With the down-regulation of CD27 and CD28, CD4+ T cells exhibit higher IFN-γ-producing ability and shorter telomere length [5]. It has been shown that CD4+CD45RO+CD27− T cell subset contained higher proportions of IFN-γ-producing cells in synovial fluid from patients with juvenile idiopathic arthritis (JIA) [6]. We recently classified human peripheral blood CD4+ T cells into six functionally distinct subsets

Abbreviations: DAS28, disease activity score for 28 joints; ECD, energy-coupled dye; Foxp3, forkhead box P3; PB, peripheral blood; PerCPCy5.5, peridin chlorophyll protein-cyanin 5.5; RA, rheumatoid arthritis; RAPB, peripheral blood from RA patients; RASF, synovial fluid from RA patients; SF, synovial fluid; T_{CM}, central memory T cells; T_{EM}, effector memory T cells; Treg, regulatory T cells.

* Corresponding author at: Department of Rheumatology and Clinical Immunology, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan.
E-mail address: jsaegusa@med.kobe-u.ac.jp (J. Saegusa).

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Table 1
Characteristics of the study population.

RA patients	Peripheral blood	Synovial fluid
*N (male/female)	23 (4/19)	10 (3/7)
Median age (range)	61 ± 9.8 (39–74)	60 ± 12.3 (39–79)
Years with disease (range)	8.2 ± 7.6 (1–24)	14 ± 13.3 (7–34)
DAS28 average score (range)	3.2 ± 1 (1.4–5.3)	3.0 ± 0.7 (1.9–4.2)
Medication; N (average ± SD/range)		
Prednisolone (mg/day)	7 (5.7 ± 2.2/1–7.5)	6 (3.7 ± 2.3/1–6)
Methotrexate (mg/week)	5 (8.4 ± 7.6/4–8)	7 (9.4 ± 4.6/4–16)
Anti-TNF drugs; N	3	6

* N; number.

using the four markers, and characterized the pro-inflammatory and regulatory characteristics of the each subset [7].

Human regulatory T (Treg) cells play an indispensable role for the maintenance of self tolerance and immune homeostasis [8]. Quantitative and/or qualitative deficiencies in Treg cells could lead to the development of autoimmune diseases. Foxp3 is a key transcription factor for the development of CD4+ Treg cells. A recent report showed that human Foxp3+CD4+ T cells can be separated into three functionally and phenotypically distinct subpopulations, based on the expression of CD45RA and Foxp3: (fraction (Fr.) I) CD45RA+Foxp3^{low} naïve Treg cells; (Fr. II) CD45RA−Foxp3^{high} activated/effector Treg cells, both of which are suppressive *in vitro*; and (Fr. III) non-suppressive cytokine-secreting CD45RA−Foxp3^{low} non-Treg cells. Terminally differentiated CD45RA−Foxp3^{high} activated/effector Treg cells rapidly died whereas CD45RA+Foxp3^{low} naïve Treg cells proliferated and converted into activated/effector Treg cells *in vitro* and *in vivo* [9]. Recent studies report the proportion of the three subpopulations differed in peripheral blood from patients with immunological diseases such as Systemic Lupus Erythematosus, Behcet's disease, sarcoidosis and Stevens-Johnson syndrome [9–11].

In this study, in order to determine the characteristics of CD4+ T cells and Foxp3+ Treg cells in RASF, we phenotypically classified synovial fluid CD4+ T cells and Foxp3+ T cells into several distinct subsets, and analyzed the distribution and characteristics of each subset. We demonstrated that most of the Foxp3+CD4+ cells are CD45RA−Foxp3^{low} non-Treg cells, and that non-Treg cells in the CCR7−CD45RA−CD27+CD28+ T_{EM} subset are increased in RASF.

2. Materials and methods

2.1. Sample collection

This study was approved by the ethics committee at the Kobe University Graduate School of Medicine. The human samples were

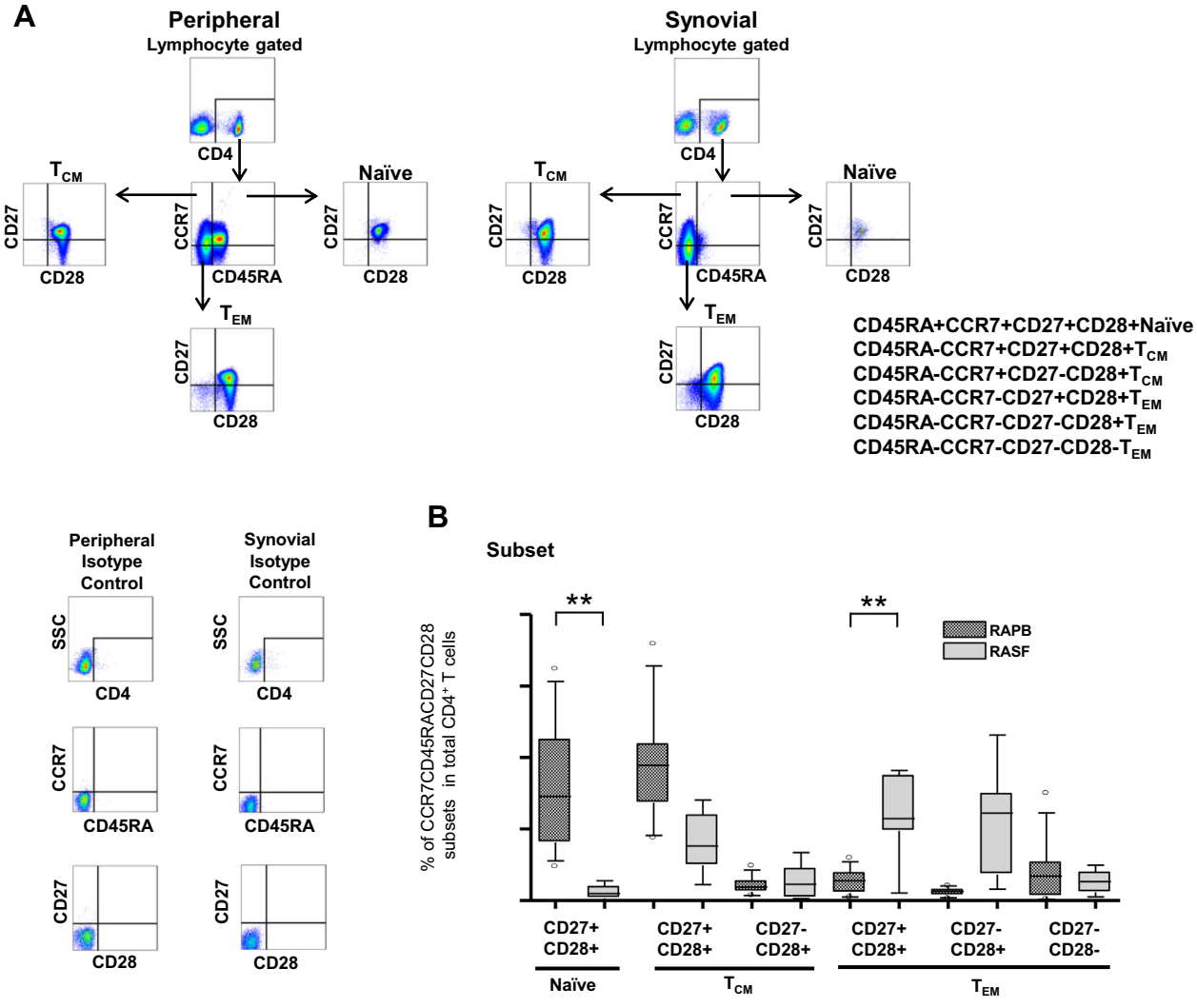

Fig. 1. The CD45RA+CCR7+CD27+CD28+ naïve subset is decreased, while CCR7−CD45RA−CD27+CD28+ T_{EM} subset is increased in RASF. (A) CD4+ T cells from RAPB (left panel) and RASF (right panel) were classified into six major populations using CD45RA, CCR7, CD27, and CD28. Representative flow cytometry results are shown. (B) The distribution of the six CD4+ T cell subsets was studied in RAPB and RASF (n = 12 in RAPB, n = 7 in RASF). **P < 0.01.