Negative Immune Checkpoint Molecules on T Regulatory Cells Distinguish RA, SLE and Healthy Controls

0092

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BACKGROUND

T regulatory cells (Tregs) play a crucial role in the regulation of the immune response and are of utmost interest when studying autoimmune diseases, such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Therefore, targeting specific Treg markers in therapy is now widely discussed. Expression of T cell immunoglobulin 3 (TIM-3) is associated with an enhanced immune suppression and are currently discussed as target in cancer therapy [1]. Fc receptor-like protein 3 (FCLR-3), which regulates Treg proliferation, was shown to be associated with the susceptibility in juvenile RA [2]. Other surface markers, like CD161 enable Tregs to produce IL-17A, IFNg and IL-2, hence promoting inflammation [3]. The aim of this study was to distinguish between RA and SLE using anti-and pro-inflammatory cell markers on Treg subsets.

MATERIAL & METHODS

Peripheral blood samples from 66 RA patients (mean ± SD; age 60 ± 10 years, female ratio: 0.68, disease duration 18 ± 14 years), 40 SLE patients (age 42 ± 13 years, female ratio 0.85, disease duration 11 ± 13 years) and 72 age-matched healthy participants (age 46 ± 17 years, female ratio 0.68) were drawn over a sampling period of 2 years. Freshly isolated PBMCs were stained and Treg subsets were identified by the expression of CD25, CD127, FoxP3, CD45RA and CD15 on the surface of CD3 and CD4 positive T cells. CD25+CD127+CD45- Tregs were further subclassified by the expression of TIM-3 (CD366) and FCLR-3 (CD307c). CD161 was used to identify Th17 type Tregs (CD15S-CD161+) and transitional Tregs (CD15S-CD161-). All cytometric measurements were performed using a standardized BD LSRFortessa platform. All statistical analysis was conducted using Rstudio version 1.1.442 [4].

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RESULS

Transitional Tregs (CD15S-CD161-) were significantly higher ($p < 0.001$) in RA patients compared to the SLE and healthy cohort (40.5 ± 13.4% vs. 28.7 ± 9.6% and 29.7 ± 9.4% respectively) (Fig. 1B). Regulation of the immune response via Tregs could hence play a more important role in RA than in SLE. Differences in the CD161+Th17 type Treg population could not be detected (Fig. 1A), however the number of CD161+ like Th17 Tregs was positively associated with the age of SLE patients (Fig. 2A). Differences in disease phenotype between SLE age groups are described [5] and could be explained by a shift in the abundance of different T cell subsets. The number of Tregs expressing TIM-3 was higher in both RA and SLE patients compared to healthy controls (2.8 ± 2.3%, $p = 0.0105$ and 2.6 ± 1.6%, $p = 0.0031$ vs. 0.8 ± 0.7% respectively), but did not differ between the rheumatic diseases (Fig. 1C). FCLR-3+Tregs distinguished RA and SLE patients (17.8 ± 13.3 vs. 25.3 ± 13.1%, $p = 0.0036$), as well as SLE patients and healthy controls (16.8 ± 12.9%, $p = 0.0112$) (Fig. 1D). Patients with RA displayed a lower number of FCLR-3+ and TIM-3+ Tregs than SLE patients and a positive association between those cell populations in RA patients was found (Fig. 2B). No findings were correlated with the disease activity of RA or SLE patients.

Table 1 Patient Characteristics

Patients	RA	SLE	HCs
sample size	68	40	72
Gender			
Female, n (%)	49 (68 %)	35 (85 %)	49 (68 %)
Age, years ± SD	60 ± 10	42 ± 13	46 ± 17
disease duration, years ± SD	18 ± 14	11 ± 13	-
Clinical parameters, mean ± SD			
cDAI	6.9 ± 5.5	-	-
SLEDAI	-	2.0 ± 1.7	-
Treatment, n (%)			
glucocorticides	26 (39 %)	31 (78 %)	-
methotrexate (MTX)	26 (39 %)	1 (3 %)	-
non-MTX DMARDs	8 (12 %)	0	-
TNF-blockers	17 (26 %)	7 (18 %)	-
NSAR	34 (52 %)	28 (70 %)	-

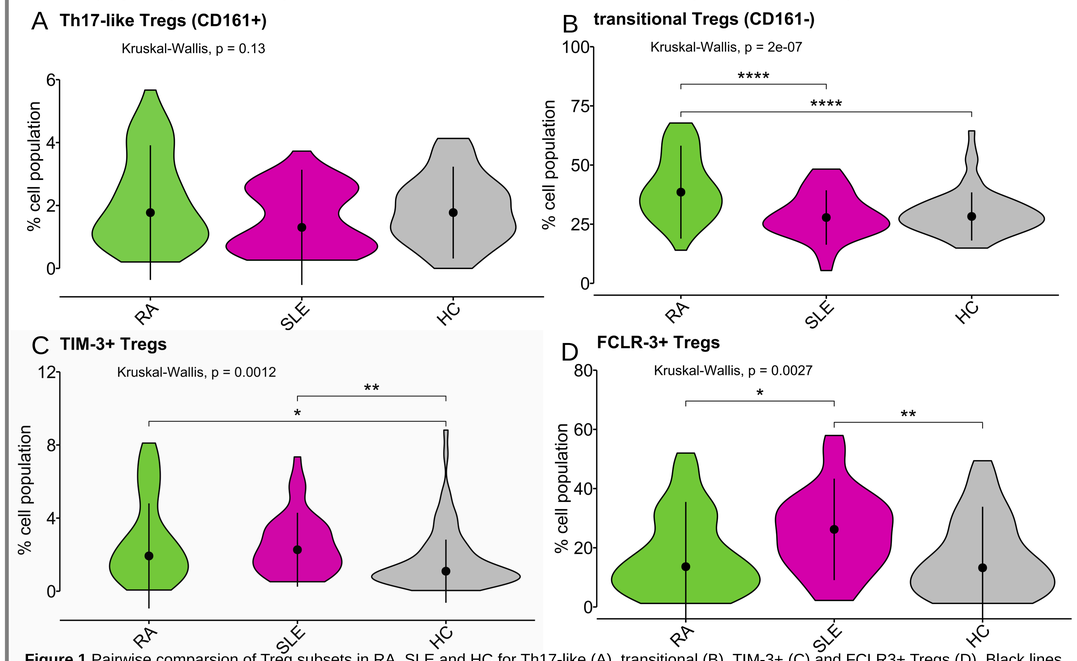

Figure 1 Pairwise comparsion of Treg subsets in RA, SLE and HC for Th17-like (A), transitional (B), TIM-3+ (C) and FCLR3+ Tregs (D). Black lines indicate the interquartile range (IQR). Pairwise comparsions were calculated using Bonferroni-corrected Wilcoxon Ranked-Sum tests.

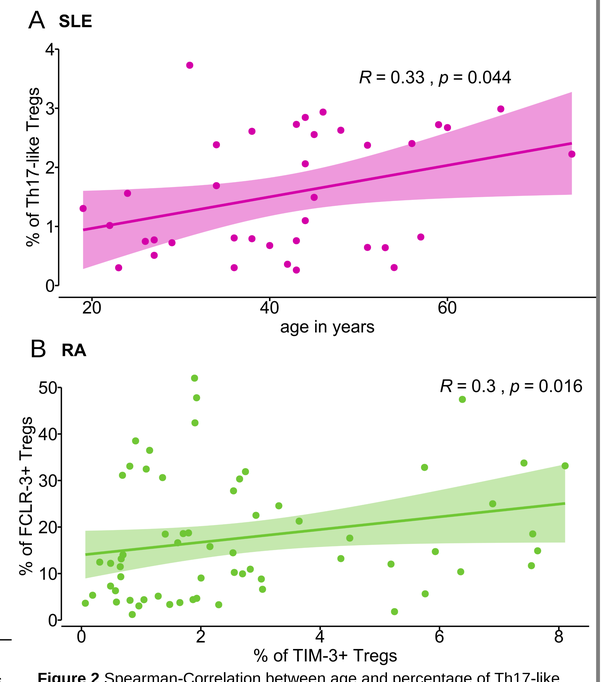

Figure 2 Spearman-Correlation between age and percentage of Th17-like Tregs in SLE (A) and percentage of TIM-3+ and FCLR3+ Tregs in RA (B).

CONCLUSION

Expression of negative immune checkpoints TIM-3 and FCRL-3 on Tregs not only distinguish healthy controls from RA and SLE patients but can be used to differentiate between different rheumatic diseases. These findings indicate that Tregs trigger the regulation of the immune response in RA and SLE, yet the activation of different Treg subsets is disease-specific.