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SP676 The role of age-related T cell differentiation in dialysis patients

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Introduction

Persisting uremic conditions are made responsible for the increased aging of the immune system in dialysis patients.

Methods

In this study, we analyzed whether age-related differences in the differentiation of both recent-thymic-emigrant-(RTE)-regulatory (Tregs) and RTE-responder T cells (Tresps) into CD31--memory Tregs/Tresps led to differences in the suppressive activity of naïve and memory Tregs on autologous Tresps between healthy volunteers (n=89) and dialysis patients (n=80).

Results

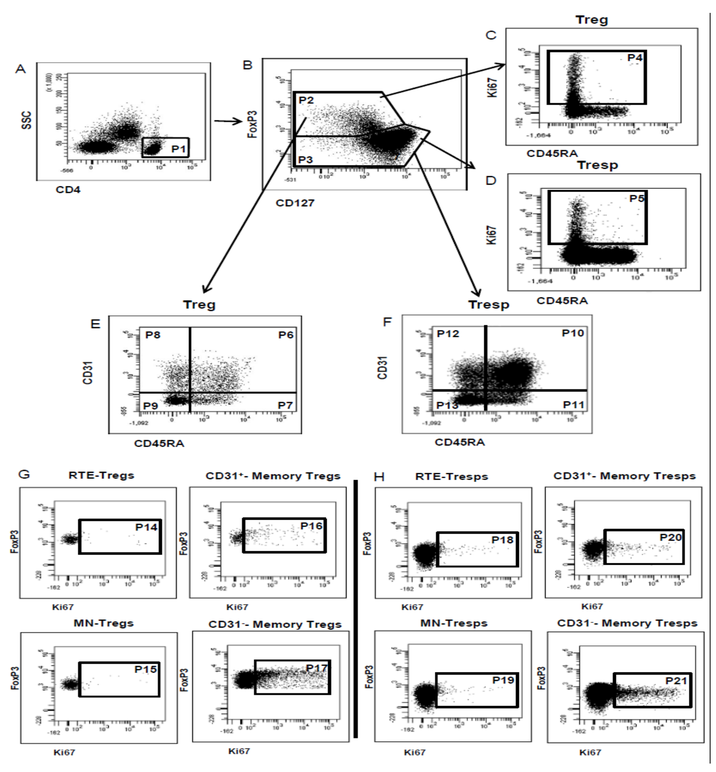
We found that regardless of the age, the differentiation of RTE-Treg/Tresps into CD31--memory-Treg/Tresps was significantly increased in dialysis patients. By analyzing the age-related differences in the differentiation of Tregs/Tresps, we saw that in healthy volunteers RTE-Tregs differentiate via CD31+-memory-Tregs, while RTE-Tresps differentiate via MN-Tresps into CD31--memory-Tresps. Functional analysis of Tregs showed an increasing suppressive activity with age in healthy individuals, suggesting that differential differentiation pathways of Tregs and Tresps may strengthen the reactivity of Tregs, but weaken the reactivity of Tresps. Our data reveal that RTE-Tresps of dialysis patients lose their capacity to differentiate via MN-Tresps into CD31--memory Tresps and their Tregs exhibit a decreasing suppressive activity with age.

Clinical characteristics

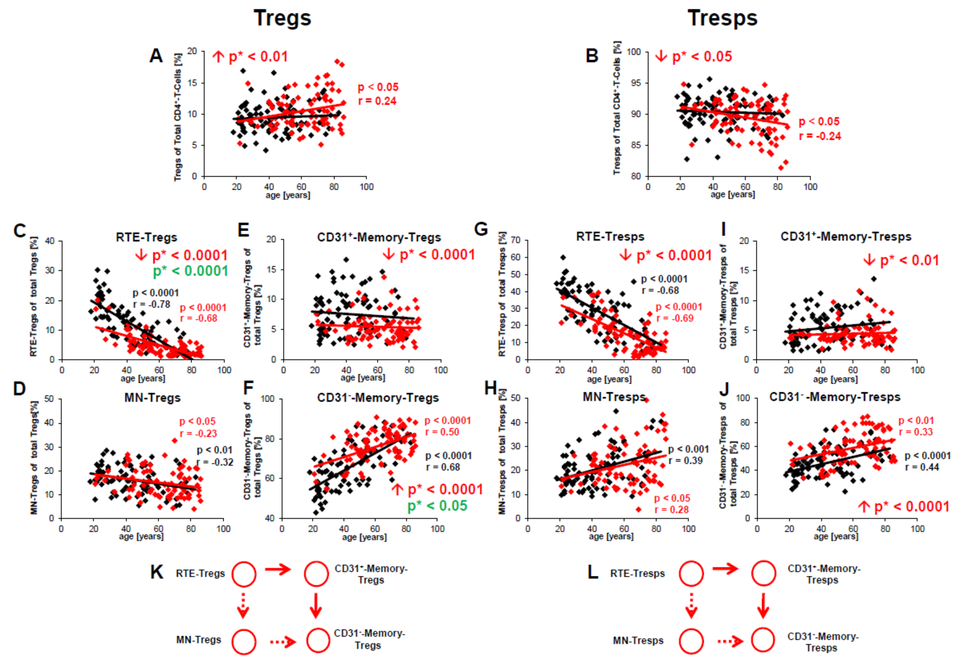
	Healthy Controls n = 89	Dialysis patients n = 80
Female Sex, n (%)	60 (67%)	23 (29%)
Age (years)	41 (18-83)	66 (21-86)
Primary disease, n (%)	-	
Diabetes		13 (16%)
Hypertension		3 (4%)
GN/vasculitis		19 (24%)
Interstitial nephritis		3 (4%)
Polycystic kidney disease		11 (14%)
Renal malformations		7 (9%)
Nephrectomy after renal carcinoma		3 (4%)
Cardio renal syndrome		3 (3%)
Obstructive uropathy		2 (2%)
Others		9 (11%)
Unknown		7 (9%)
Dialysis method	-	
HD, n (%)		63 (79%)
PD, n (%)		17 (21%)
Time since start of dialysis (years)	-	2,44 (0,003-26,95)
Creatinine (mg/dl)	0,83 (0,53-1,47)	7,92 (3,17-18,77)
CKD-EPI GFR (ml/min)	87,6 (48,3-130,7)	6,45 (3,0-18,7)
MDRD GFR (ml/min*1,73qm)	87,69 (53,62-119,21)	7,66 (3,0-19,0)

The data are presented as their median values together with their range (minimum-maximum); HD: Hemodialysis; PD: Peritoneal dialysis; n: number; GN: Glomerulonephritis; CKD-EPI GFR: Chronic Kidney Disease Epidemiology Collaboration estimated Glomerular Filtration Rate; MDRD GFR: Modification of Diet in Renal Disease Study estimated Glomerular Filtration Rate

Gating strategy

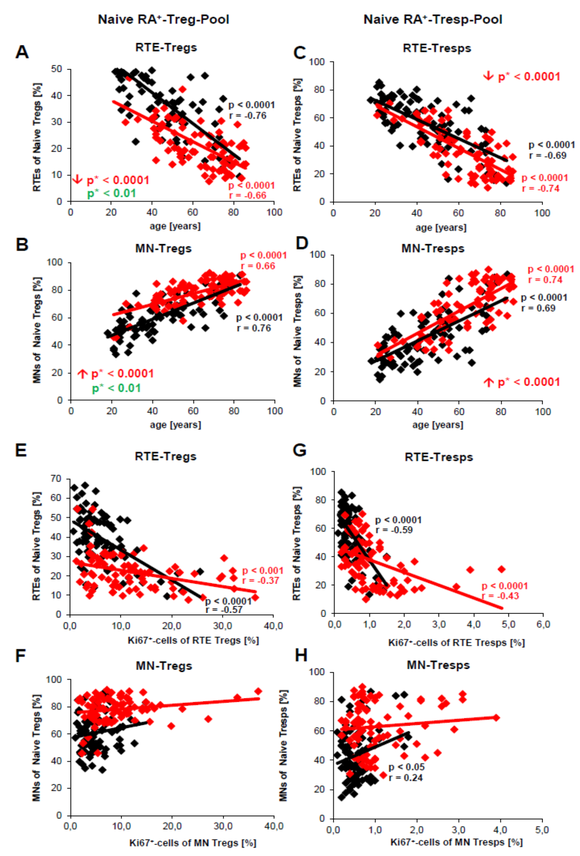


Changes in the composition of the total Treg/Tresp pool during the course of life in healthy volunteers and dialysis patients



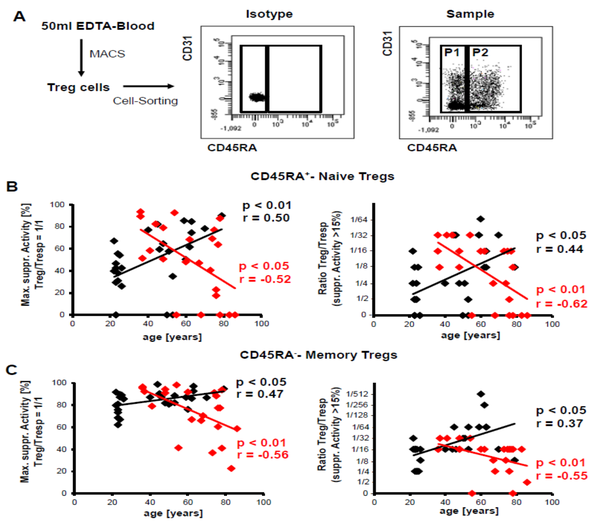
The percentages of total CD4⁺CD127$^{low+/-}$FoxP3⁺-Tregs (A) and total CD4⁺CD127⁺FoxP3⁻-Tresps (B) within total CD4⁺-T-cells was estimated in healthy volunteers (◆) and dialysis patients (◆) of different ages. In addition, the percentages of RTE-Tregs/Tresps (C and G), MN-Tregs/Tresps (D and H), CD31⁺-memory Tregs/Tresps (E and I) and CD31⁻-memory Tregs/Tresps (F and J) were estimated within the total Treg/Tresp pool in both patient groups. The figures present the regression lines concerning the changes in the percentages of Tregs/Tresps and their individual Treg/Tresp subsets with increasing age. Significantly decreased percentages (↓) of RTE-Tregs/Tresps and CD31⁺-memory Tregs/Tresps, but increased percentages (↑) of CD31⁻-memory-Tregs/Tresps within total Tregs/Tresps, independently of age (marked by red p*-values) suggest an enhanced differentiation of RTE-Tregs/Tresps via CD31⁺-memory-Tregs/Tresps into CD31⁻-memory-Tregs/Tresps in dialysis patients compared to healthy volunteers (K and L). Significant differences in the slopes of the regression lines between healthy volunteers and dialysis patients (marked by green p*-values) were detected for RTE-Tregs and CD31⁻-memory-Tregs (C and F), demonstrating that increased RTE-Treg differentiation occurs especially during early life in dialysis patients.

Changes in the composition of the naïve CD45RA⁺-Treg/Tresp



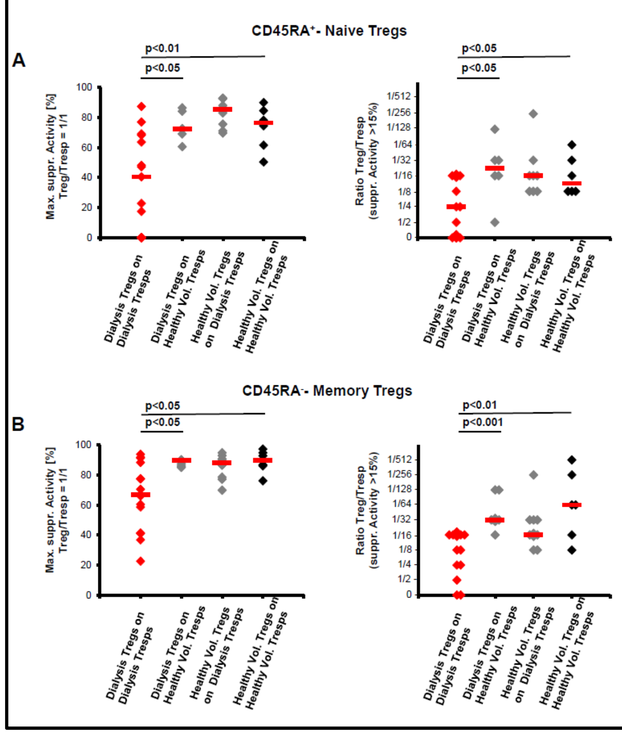
The percentages of RTE-Tregs/Tresps (A and C) and MN-Tregs/Tresps (B and D) within naïve CD45RA⁺-Tregs/Tresps were estimated in healthy volunteers (◆) and dialysis patients (◆) of different ages. The figures present the regression lines concerning the changes within the naïve CD45RA⁺-Treg/Tresp pool with increasing age. Significantly decreased (↓) percentages of RTE-Tregs/Tresps, but increased (↑) percentages of MN-Tregs/Tresps within total naïve CD45RA⁺-Tregs/Tresps, independently of age (marked by red p*-values) confirm an increased aging of the naïve CD45RA⁺-Treg/Tresp pool in dialysis patients compared to healthy volunteers. A significant negative correlation between the percentage of RTE-Tregs/Tresps and their Ki67 expression was detected for both healthy volunteers and dialysis patients (E and G). A significant correlation between the percentage of MN-Tregs and their Ki67 expression was neither found for healthy volunteers nor for dialysis patients (F). However, in healthy volunteers, a significant positive correlation was ascertained for MN-Tresps, which could not be found in dialysis patients (H).

Suppressive activity of naïve CD45RA⁺-Tregs and CD45RA⁻-memory Tregs



Total CD4⁺CD127$^{low+/-}$CD25⁺-Tregs were isolated by magnetic-activated-cell-sorting (MACS), stained with anti-CD45RA- and anti-CD31-monoclonal antibodies and sorted into CD45RA⁻-memory Tregs (P1) and naive CD45RA⁺-Tregs (P2), (A). The suppressive activity of the different Treg subsets was estimated by suppression assays. Blood samples were obtained from healthy volunteers (◆) and dialysis patients (◆) of different ages. The figure shows the individual and median values of the maximum suppressive activity (Treg/Tresp = 1/1) and of the ratio of Treg/Tresp up to which the purified naïve CD45RA⁺-Tregs (B) and CD45RA⁻-memory-Tregs (C) could be diluted to achieve a minimum suppressive activity of at least 15 %. The suppressive activity of both Treg subsets increased significantly with age in healthy volunteers, but decreased in dialysis patients (B and C).

Suppressive activity of naïve CD45RA⁺-Tregs, and CD45RA⁻-memory Tregs in old-aged healthy volunteers and dialysis patients on autologous and non-autologous Tresps



The suppressive activity of naïve CD45RA⁺- (A) and CD45RA⁻-memory Tregs (B) was tested in old-aged dialysis patients (> 55 years) on autologous Tresps (◆; n =15) and on non-autologous Tresps of age-matched healthy volunteers (◆; n= 6). Furthermore, the suppressive activity of both Treg subsets was tested in elderly healthy volunteers (> 55 years) on non-autologous Tresps of age-matched dialysis patients (◆; n = 8) and on autologous Tresps (◆; n = 6). The suppressive activity of Tregs of old-aged dialysis patients was significantly lower than that of old-aged healthy volunteers when tested on autologous Tresps. However, their suppressive activity was significantly increased when tested on non-autologous Tresps of aged-matched healthy volunteers. There were no significant differences in the suppressive activity of Tregs of old-aged healthy volunteers when tested on autologous or non-autologous Tresps.

Conclusion

These findings suggest an age-related increase of Tresp reactivity in these patients and may explain the increased incidence of chronic inflammation especially in elderly dialysis patients.

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