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Senescent profile of angiogenic T cells from systemic lupus erythematosus patients

Patricia López,[*,1] Javier Rodríguez-Carrio,[*] Aleida Martínez-Zapico,[†] Luis Caminal-Montero,[†] and Ana Suarez[*]*

[*]Department of Functional Biology, Immunology Area, Faculty of Medicine, University of Oviedo, Oviedo, Spain; and [†]Department of Internal Medicine, Hospital Universitario Central de Asturias, Oviedo, Spain

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ABSTRACT

The chronic inflammatory environment associated with systemic lupus erythematosus can lead to an accelerated immunosenescence responsible for the endothelial damage and increased cardiovascular risk observed in these patients. The present study analyzed two populations with opposite effects on vascular endothelium, angiogenic T cells and the senescent $CD4^+CD28^{null}$ subset, in 84 systemic lupus erythematosus patients and 46 healthy controls. Also, 48 rheumatoid arthritis patients and 72 individuals with traditional cardiovascular risk factors participated as disease controls. Phenotypic characterization of $CD28^+$ and $CD28^{null}$ cells was performed by analyzing markers of senescence (CCR7, CD27, CD57) and cytotoxicity (CD56, perforin, granzyme B, IFN-γ). IL-1β, IL-6, IL-8, IL-10, IL-12, IL-17A, IFN-α, IFN-γ, TNF-α, B lymphocyte stimulator, and GM-CSF serum levels were analyzed in systemic lupus erythematosus patients and healthy controls. $CD4^+CD28^{null}$ cells were notably increased in the systemic lupus erythematosus patients and disease controls compared with healthy controls. In contrast, angiogenic T cells were only reduced in the disease controls (those with rheumatoid arthritis or traditional cardiovascular risk factors). Nevertheless, an anomalous presence of $CD28^{null}$-angiogenic T cells, with cytotoxic and senescent characteristics, was noted in systemic lupus erythematosus patients in association with anti-dsDNA titer, anti-SSA/Ro antibodies and circulating TNF-α, IL-8, IFN-α, and B lymphocyte stimulator amounts. This subset was also detected in those with traditional cardiovascular risk factors but not in the rheumatoid arthritis patients. In contrast, $CD28^+$-angiogenic T cells were reduced in the systemic lupus erythematosus patients with cardiovascular disorders. In conclusion, CD28 expression must be used to redefine the angiogenic T cell population, because in pathologic conditions, a senescent $CD28^{null}$-angiogenic T cell subset with inflammatory, rather than protective, effects could be present. *J. Leukoc. Biol.* 99: 405–412; 2016.

Introduction

SLE is an autoimmune disease characterized by a dysregulation of the immune system involving lymphocyte overactivation and autoantibody production, which drive chronic inflammation and consequent tissue damage [1, 2]. Accumulating evidence has suggested the chronic proinflammatory environment and repeated cell stimulation in autoimmune patients can lead to accelerated aging of the immune system, known as premature immunosenescence [3–6]. The progressive age-related deterioration of the lymphocyte compartment is associated with a decline in the proportion of $CD4^+$ cells and an increase in terminally differentiated T cells that have lost CD28 expression [3, 7]. In addition to elderly subjects, expansion of $CD4^+CD28^{null}$ cells has been reported in a variety of conditions, including patients with autoimmune and CV diseases [7–13] and could be considered a marker of immunosenescence itself. Functionally, $CD4^+CD28^{null}$ T cells secrete inflammatory mediators and release cytolytic molecules from intracellular granules capable of inducing damage in endothelial cells [12, 14]. $CD4^+CD28^{null}$ cells can migrate into inflamed tissue and play an important role in destabilizing atherosclerotic plaques [15].

Endothelial damage, accelerated atherosclerosis, and increased CV disease are well-recognized complications of SLE that cannot be completely explained by traditional risk factors [16]. Thus, a number of disease-specific features, including chronic inflammation, a dysregulated cytokine profile, and altered T cell subsets, have been proposed as important triggers of the imbalance between endothelial damage and repair that underlie the CV disorders developing in those with SLE [17].

Given this scenario, special interest should be given to a recently identified T cell population, T_{ang} cells, which promote repair of damaged endothelium by cooperating with endothelial progenitor cells [18]. T_{ang} cells are characterized by the expression of the platelet endothelial cell adhesion molecule (CD31) and the receptor for stromal-derived factor 1 (CXCR4) and the secretion of angiogenic molecules [18]. A decrease in T_{ang} frequency has been associated with vascular disease [19], and we have recently described a reduction of this subpopulation

Abbreviations: ACR = American College of Rheumatology, BLyS = B lymphocyte stimulator, CV = cardiovascular, HC = healthy control, RA = rheumatoid arthritis, SLE = systemic lupus erythematosus, T_{ang} = angiogenic T cell, tCVR = traditional cardiovascular risk factor

1. Correspondence: Department of Functional Biology, Immunology Area, Faculty of Medicine, University of Oviedo, C/Julián Clavería s/n, Oviedo 33006, Spain. E-mail: lopezpatricia@uniovi.es

in the circulation of RA patients, which was especially stronger in those presenting with CV disease [20].

Premature aging of the immune system seems to play a role in the development of CV disorders in autoimmune patients. Thus, the aim of the present work was to analyze 2 populations with opposite effects on vascular endothelium, T_{ang}, and $CD4^+CD28^{null}$ cells, in relation to clinical features and soluble factors associated with inflammation or endothelial damage in SLE patients.

MATERIALS AND METHODS

Patients and controls

A total of 84 SLE patients fulfilling the ACR criteria for SLE [21] were recruited from the Autoimmune Disease Unit (Hospital Universitario Central de Asturias, Oviedo, Spain). Information on the clinical manifestations, traditional CV risk factors, and CV disorders during the disease course were obtained after a review of the clinical histories, and the parameters of disease activity and treatments were recorded at sampling (**Table 1**). Also, 46 volunteers without any pathologic findings or treatment were recruited as HCs, and 48 RA patients fulfilling the 2010 ACR criteria and 72 individuals with tCVR without any previous CV event, were enrolled as disease controls (**Table 2**). The Regional Ethics Committee for Clinical Research (Servicio de Salud del Principado de Asturias) provided ethics approval for the present study, in accordance with the Declaration of Helsinki. All the subjects provided written informed consent before participation in the study.

Cytometry

To quantify the $CD4^+CD28^{null}$ and T_{ang} populations, blood samples from all participants were stained for 30 min at 4°C with anti-CD3-PerCP-Cy5.5 (Tonbo Biosciences, San Diego, CA, USA), anti-CD4-Pacific Blue (Immunostep, Salamanca, Spain), anti-CD31-FITC, anti-CXCR4-PE-Cy7, and anti-CD28-APC-Cy7 mAbs or with the corresponding isotype conjugated irrelevant mAb as a negative control (BD Biosciences, Franklin Lakes, NJ, USA). $CD4^+CD28^{null}$ cells were calculated within the $CD3^+CD4^+$ population and T_{ang} ($CD3^+CD31^+CXCR4^+$) among the $CD3^+$ cells. Phenotypic characterization of $CD28^{null}$- and $CD28^+$-T_{ang} subsets was performed in 20 SLE patients by extracellular staining with anti-CCR7-PE (BD Biosciences), CD27-APC, CD56-APC, or CD57-PE or the corresponding isotype controls (Miltenyi Biotec, Bergisch Gladbach, Germany). Additionally, blood cells were intracellularly stained with perforin-Pacific Blue, granzyme B-PE (Miltenyi Biotec), and IFN-γ-PE (BD Biosciences). A minimum of 20,000 $CD3^+$ cells per tube was acquired using a FACSCanto II flow cytometer (BD Biosciences) and analyzed using FlowJo software (Tree Star, Ashland, OR, USA). The specific fluorescence intensity was quantified as the mean fluorescence intensity calculated by subtracting the background of isotype-matched control staining from the total fluorescence intensity.

Cytokine quantification

IFN-α, IL-10, IL-6, IL-12, IL-1β, IL-17A, and GM-CSF were analyzed by Cytometric Bead Arrays (BD Biosciences) in accordance with the manufacturer's instructions. ELISA kits were used for TNF-α (PeproTech, Rocky Hill, NJ, USA), BLyS (eBioscience, San Diego, CA, USA), and IFN-γ (BD Biosciences).

Statistical analysis

The Kolmogorov-Smirnov test was used to evaluate normality. Data are expressed as the median (interquartile range). Differences between the disease and control groups were assessed using the nonparametric Mann-Whitney *U* test. Because comparisons were performed between groups with different sample sizes, Hedge's *g* statistic was calculated for each reported significant difference between the groups (*g* values >0.5 and >0.8 were considered to indicate a medium and large effect, respectively) [22]. Statistically significant differences in cytotoxic and senescence marker expression among $CD28^{null}$ T cell and $CD28^{null/pos}$ T_{ang} cell subpopulations were evaluated using the Friedman test and Dunn's post test. Linear regression analyses adjusted for sex and age and multiple backward regression models were applied to determine the influence of demographic and disease features in different cellular populations or serum factors using logarithmic transformations of non-normal variables. Thus, age at diagnosis, disease duration, SLE disease activity index, clinical manifestations (e.g., malar rash, discoid lesions, photosensitivity, oral ulcers, arthritis, serositis, cytopenia, renal disorder, neurologic disorder) and autoantibodies presence (antinuclear antibodies, anti-dsDNA, anti-SSA, anti-SSB, anti-Sm, anti-RNP, rheumatoid factor, anti-cardiolipin) were entered into the initial model. GraphPad Prism, version 5 (GraphPad, La Jolla, CA, USA), and SPSS, version 22.0 (IBM, Armonk, NY, USA), software packages were used for all determinations, and $P < 0.05$ was considered significant.

TABLE 1. Demographic and clinical features of SLE patients

Variable	Value
Total SLE patients	84
Sex (female/male)	79/5
Age (yr)	49.30 ± 12.05
Age at diagnosis (yr)	35.02 ± 13.82
Disease duration (yr)	14.01 ± 10.21
SLEDAI score (mean ± SD)	3.85 ± 4.11
Clinical manifestations	
Malar rash	44 (52.38)
Discoid lesions	21 (25.00)
Photosensitivity	46 (54.76)
Oral ulcers	42 (50.00)
Arthritis	57 (67.86)
Serositis	18 (21.43)
Cytopenia	59 (70.24)
Renal disorder	22 (26.19)
Neurologic disorder	8 (9.52)
Autoantibodies	
ANAs	84 (100.00)
Anti-dsDNA titer (U/ml)	68 (80.95)/38.57 ± 63.34
Anti-SSA	44 (34.11)
Anti-SSB	14 (16.67)
Anti-Sm	6 (7.14)
Anti-RNP	11 (13.09)
RF	13 (15.48)
Anti-cardiolipin	12 (14.29)
Treatment	
None or NSAIDs	4 (4.76)
Antimalarial drugs	73 (86.90)
Glucocorticoids	33 (39.29)
Immunosuppressive drugs[a]	2 (2.38)
Traditional CV risk factors	
Dyslipidemia	18 (21.43)
Hypertension	21 (25.00)
Diabetes (type 2)	2 (2.38)
Obesity (BMI >30 kg/m^2)	14 (16.67)
Smoking habit	17 (20.23)
CV disease[b]	11 (13.09)

Data presented as *n* (%) or mean ± SD. ANAs, antinuclear antibodies; BMI, body mass index; NSAIDs, nonsteroidal anti-inflammatory drugs; RF, rheumatoid factor; SLEDAI, SLE disease activity index. [a]Azathioprine, mycophenolate mofetil. [b]Coronary heart/artery disease, cerebrovascular accidents, congestive heart failure, peripheral vascular disease, angina, myocardial infarction.

TABLE 2. Demographic and clinical features of healthy and disease control groups

Variable	Value
HC group	46
Sex (female/male)	36/10
Age at sampling (yr)	45.8 4 ± 8.99
RA patients	48
Sex (female/male)	40/8
Age at sampling (yr)	52.56 ± 14.36
Age at diagnosis (yr)	51.48 ± 14.31
Disease duration (yr)	1.10 ± 1.89
DAS28 score	4.47 ± 1.61
Autoantibodies	
RF titer (U/ml)	25 (54.35)/172.07 ± 276.86
Anti-CCP	27 (58.70)
Traditional CV risk factors	
Dyslipidemia	16 (33.33)
Hypertension	14 (29.17)
Diabetes (type 2)	2 (4.17)
Obesity (BMI >30 kg/m^2)	12 (25.00)
Smoking habit	10 (20.83)
CV disease[a]	3 (6.25)
tCVR group	72
Sex (female/male)	38/34
Age at sampling (yr)	55.99 ± 8.99
Traditional CV risk factors	
Dyslipidemia	27 (37.50)
Hypertension	41 (56.94)
Diabetes (type 2)	24 (33.33)
Obesity (BMI >30 kg/m^2)	25 (34.72)
Smoking habit	20 (27.78)

Data presented as n (%) or mean ± SD. Anti-CCP, cyclic citrullinated peptide antibody; BMI, body mass index; DAS28, disease activity score; RF, rheumatoid factor. [a]Heart failure in 2 and cerebrovascular accident in 1.

RESULTS

Senescent phenotype of T_{ang} cells in SLE patients

$CD4^+CD28^{null}$ and T_{ang} ($CD3^+CD31^+CXCR4^+$) populations were quantified in the SLE, HC, and disease control groups with expected low T_{ang} levels (RA patients and tCVR individuals) by flow cytometry (**Fig. 1**). The results showed a significant increase of $CD4^+CD28^{null}$ cells in those with RA and tCVR and, more strikingly, in SLE patients compared with HCs (**Fig. 2A**), supporting a disease-associated immunosenescence. However, no significant differences were observed in the frequency of T_{ang} cells between the SLE patients and HCs, although this population was notably reduced in both disease control groups. However, the absolute T_{ang} counts were slightly reduced in SLE patients compared with HCs [median 6.59 (range 2.91–11.91) vs. median 8.89 (range 5.23–15.26) cells/μl; $P = 0.048$], because most patients with SLE tend to present with lymphopenia.

Additional analysis of the T_{ang} cell population showed that >20% of total T_{ang} cells in SLE patients did not express CD28. This anomalous subset, named $CD28^{null}$-T_{ang}, was also observed in tCVR individuals but were nearly absent in the HCs and RA patients (Fig. 2B), pointing out relevant differences in the T_{ang} profile of RA and SLE. Multiple linear regression analyses adjusted for sex and age showed that $CD28^{null}$-T_{ang} cells in SLE patients was associated inversely with age at diagnosis ($\beta = -0.584$, $P < 0.001$) and directly with disease duration ($\beta = 0.341$, $P < 0.001$). Analyses performed with $CD4^+CD28^{null}$ cells provided similar results ($\beta = 0.384$, $P < 0.001$ for disease duration and $\beta = -0.643$, $P < 0.001$ for age at diagnosis), supporting the presence of premature immunosenescence in SLE that progresses with the disease course. Remarkably, multiple backward regression analysis, including demographic, clinical, and immunologic manifestations, indicated that anti-dsDNA titer ($\beta = 0.424$, $P = 0.022$) and the presence of anti-SSA/Ro antibodies ($\beta = 0.618$, $P = 0.001$) were significant predictors of high $CD28^{null}$-T_{ang} cell levels but not of a higher $CD4^+CD28^{null}$ cell amount. The increased $CD28^{null}$-T_{ang} subset in SLE patients compared with the HCs was also detected by analyzing absolute numbers [median 1.85 (range 1.02–3.73) vs. median 0.49 (range 0.24–0.99) cells/μl; $P < 0.001$]. Finally, no significant differences were found in patients receiving different therapies.

In contrast, the frequency of $CD28^+$-T_{ang} cells in the SLE patients was similar to that of the HCs, but the RA and tCVR

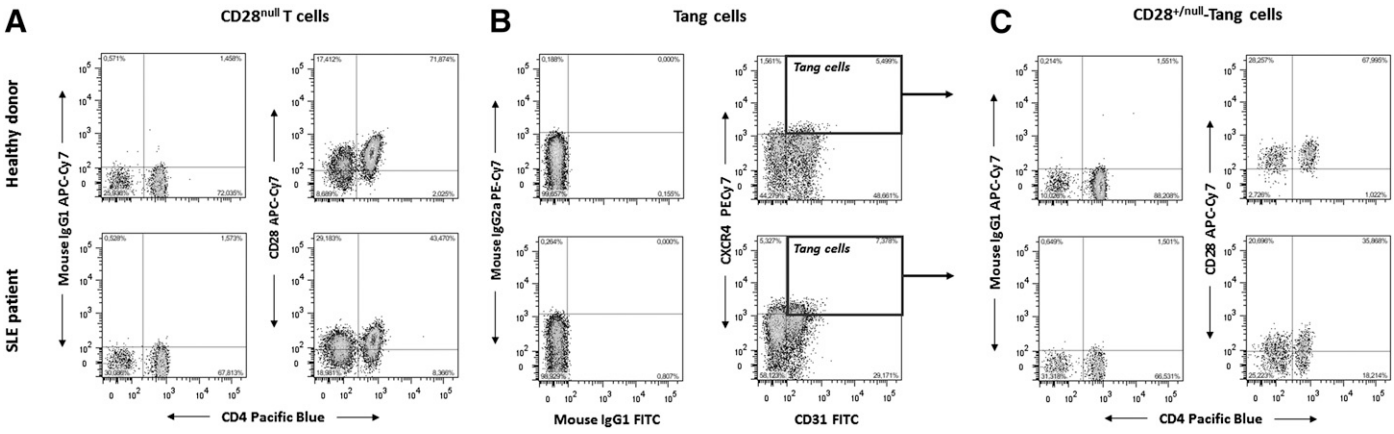

Figure 1. Gating strategy used to identify $CD4^+CD28^{null}$ and T_{ang} subsets by flow cytometry. Representative dot plots of a healthy donor and an SLE patient are shown. Quadrants were set according to the fluorescence signal provided by the isotype controls. (A) The $CD4^+CD28^{null}$ population was determined within the $CD3^+CD4^+$ population according to their CD4 and CD28 expression. (B) The T_{ang} population was identified as triple-positive CD3/CD31/CXCR4 cells in the lymphocyte gate. (C) CD4 and CD28 expression in gated T_{ang} cells from a representative healthy donor and an SLE patient.

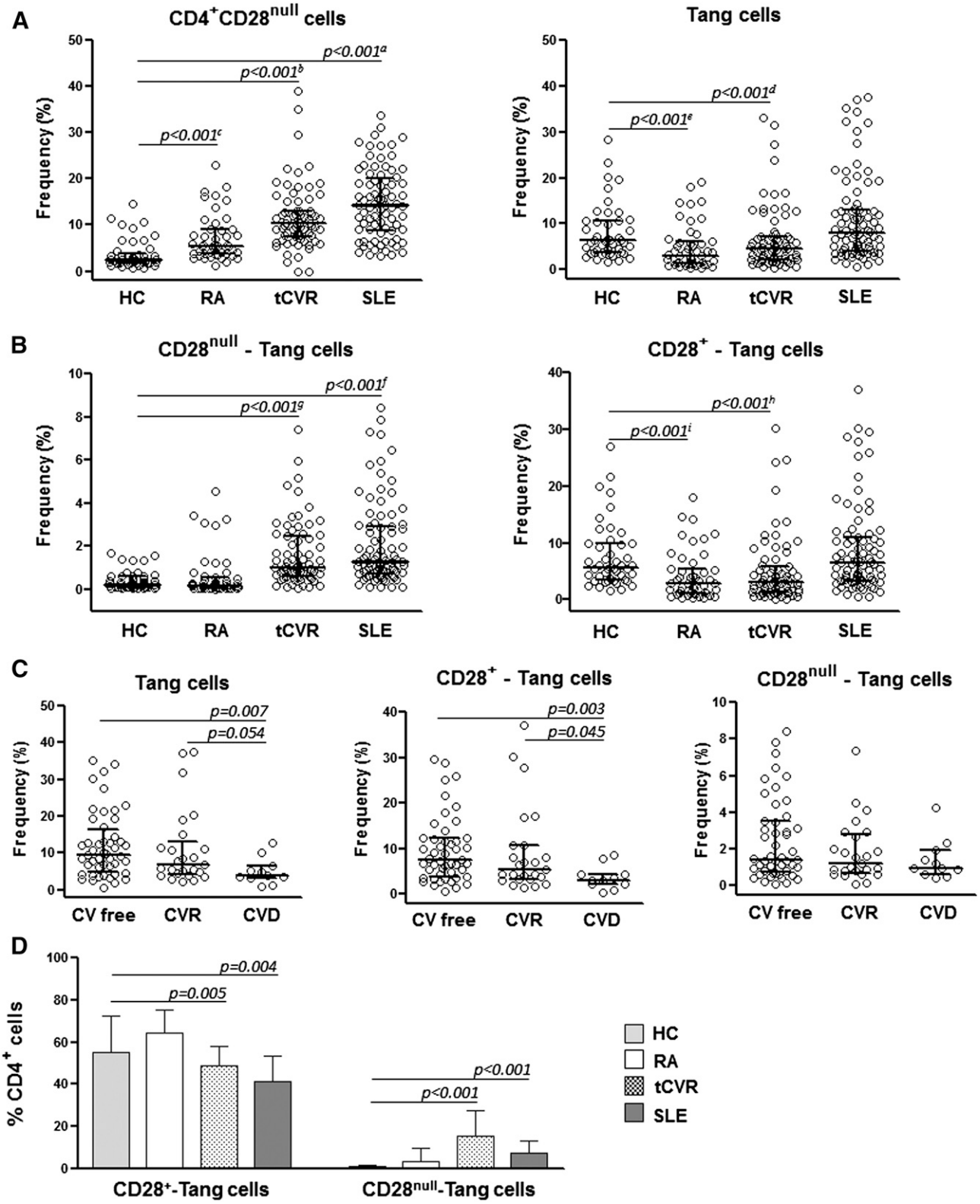

Figure 2. $CD4^+CD28^{null}$ and T_{ang} cells in SLE patients, HCs, and disease control groups. CD3, CD4, CD28, CD31, and CXCR4 expression was analyzed by flow cytometry in 84 SLE patients, 46 HCs, 48 RA patients, and 72 individuals with tCVR. The frequency of $CD4^+CD28^{null}$ and T_{ang} ($CD3^+CD31^+CXCR4^+$) cells (A) and T_{ang}-subsets ($CD28^+/CD28^{null}$-T_{ang}) (B) in SLE patients, HCs, and disease control groups (RA and tCVR). Hedge's *g* values: [a]1.73, [b]1.36, [c]0.83, [d]0.21, [e]0.58, [f]1.06, [g]1.01, [h]0.45, and [i]1.76. (C) T_{ang} populations in SLE patients with cardiovascular disorders (CVD) or tCVR compared with the rest of the patients (CV free). (D) Percentage of $CD4^+$ cells among $CD28^+$ and $CD28^{null}$ -T_{ang} subpopulations in patients and controls. Horizontal lines represent median and interquartile range. Statistical significance was assessed using the Mann-Whitney *U* test.

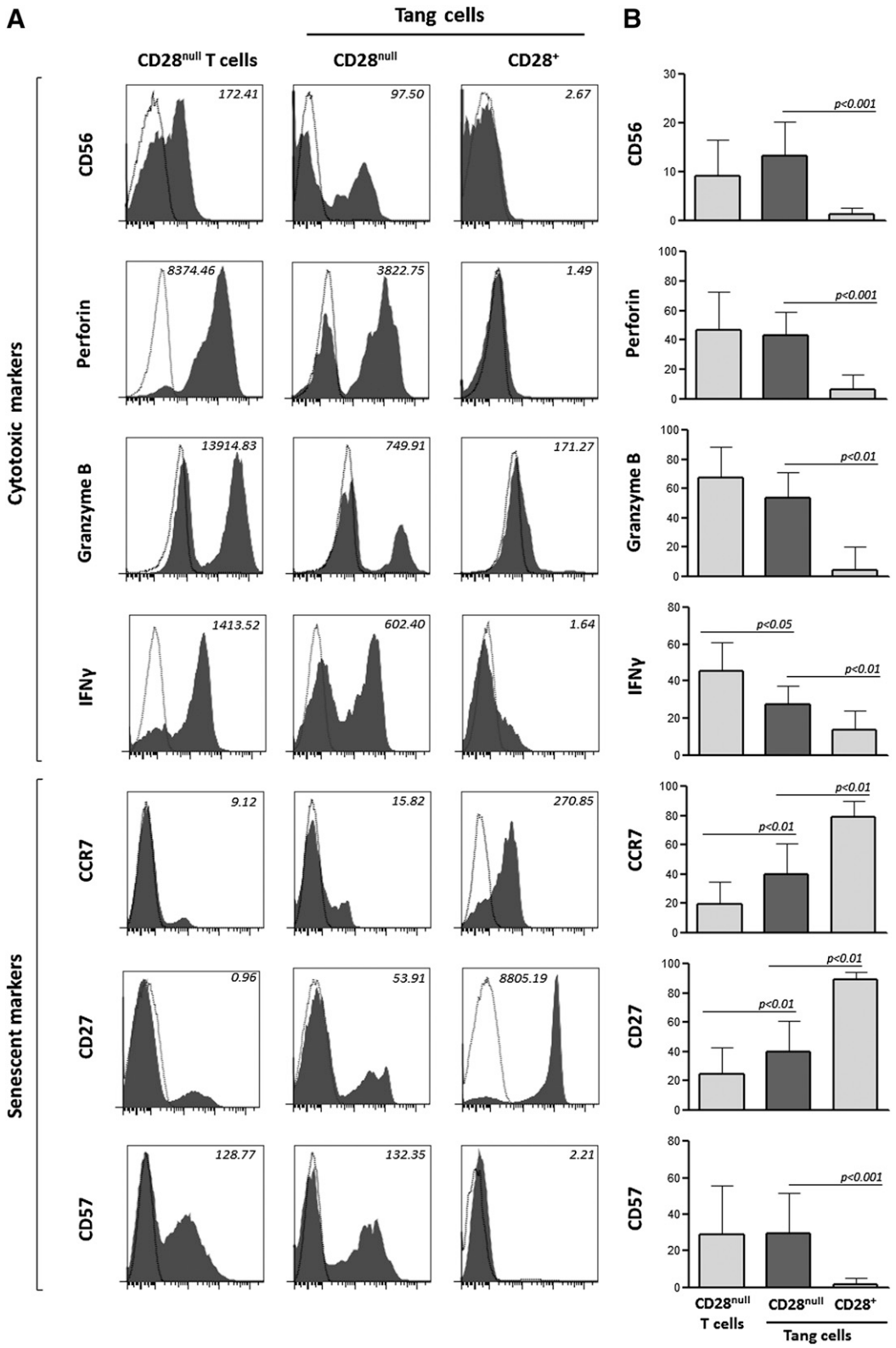

Figure 3. Phenotypic characterization of $CD28^{null}$-T_{ang} cells in SLE patients. Expression of cytotoxic and senescence markers in total $CD28^{null}$ T cells and $CD28^{null}$-T_{ang} and $CD28^{+}$-T_{ang} subsets from 20 SLE patients. (A) Histograms represent molecule expression in a SLE patient as an example (shaded) with the respective isotype matched control antibody (black dotted line). Numbers in plots indicate the median fluorescence intensity of each marker staining, with the matched irrelevant control value subtracted. (B) Bars represent the median and interquartile range for each marker in the $CD28^{null}$ T cells and $CD28^{null}$-T_{ang} and $CD28^{+}$-T_{ang} subsets from 20 SLE patients. Statistical differences among the 3 populations were evaluated by the Friedman test ($P < 0.001$, for all analyzed molecules), and Dunn's posttest was conducted to determine which groups' pairs had different medians.

TABLE 3. Serum levels of cytokines in healthy donors and SLE patients

Cytokine	Healthy donors (n = 46)	SLE patients (n = 84)	P value
IL-1β (pg/ml)	0.74 (0.58–1.12)	1.13 (1.03–1.30)	<0.001
IL-6 (pg/ml)	2.02 (1.73–23.91)	2.83 (2.22–36.53)	<0.001
IL-8 (pg/ml)	13.64 (10.95–15.66)	28.02 (21.94–36.33)	<0.001
IL-10 (pg/ml)	0.06 (0.01–1.24)	1.44 (1.26–1.74)	<0.001
IL-12 (pg/ml)	1.54 (1.46–1.66)	1.78 (1.56–2.14)	0.006
IL-17A (pg/ml)	3.37 (3.02–3.66)	5.15 (0.30–12.89)	0.393
IFN-α (pg/ml)	1.69 (1.24–2.24)	8.24 (4.23–13.41)	<0.001
IFN-γ (pg/ml)	3.29 (1.90–6.44)	2.63 (1.87–4.33)	0.141
TNF-α (pg/ml)	42.74 (3.90–129.44)	161.86 (96.07–222.28)	<0.001
GM-CSF (pg/ml)	0.56 (0.30–1.22)	1.84 (0.30–4.44)	0.020
BLyS (pg/ml)	558.03 (443.29–937.10)	1898.53 (1454.59–2264.51)	<0.001

Data presented as median (interquartile range). Differences between groups were evaluated by the Mann-Whitney U test.

groups both showed reduced levels. However, analysis of the SLE patients with CV disorders and those presenting with tCVR showed a significant decrease in $CD28^+$- but not $CD28^{null}$-T_{ang} cells compared with the other patients (Fig. 2C). $CD28^+$-T_{ang} cells were significantly reduced in SLE patients with CV disease compared with the levels in HCs (P = 0.008). No significant differences in total T_{ang} cells or their subsets were detected in SLE, RA, or tCVR groups, according to their CV risk factor. The proportion of $CD4^+$ lymphocytes among the $CD28^+$-T_{ang} subset was reduced in tCVR individuals and, in particular, in SLE patients. In contrast, the opposite occurred among the $CD28^{null}$-T_{ang} cells (Fig. 2D). Therefore, T_{ang} cells from SLE patients, even those expressing CD28, seem to exhibit an altered phenotype.

Characterization of $CD28^{null}$-T_{ang} cells

Next, we wanted to determine whether the downregulation of CD28 expression in a subset of SLE T_{ang} cells was accompanied by other senescent features. Thus, we performed a phenotypic characterization of $CD28^{null}$- and $CD28^+$-T_{ang} subsets in SLE patients (n = 20) by analyzing the expression of a range of cytotoxic and senescent markers usually present in $CD28^{null}$ T cells (**Fig. 3**). The results showed that $CD28^{null}$-T_{ang} cells expressed perforin, granzyme B, and CD56, similar to the expression in the whole $CD3^+CD28^{null}$ population, as well as significant levels of IFN-γ, thus suggesting a cytotoxic and inflammatory ability of this subset. Likewise, the expression of CCR7, CD27, and CD57 markers supported the senescent profile of $CD28^{null}$-T_{ang} cells. As expected, $CD28^+$-T_{ang} cells did not exhibit these cytotoxic and senescent characteristics.

Serum cytokines associated with $CD28^{null}$ cells

The senescent profile of T_{ang} cells from SLE patients suggests they could exert a proinflammatory, rather than a protective, effect on endothelial cells. Therefore, to estimate the possible harmful role of $CD4^+CD28^{null}$ and $CD28^{null}$-T_{ang} cells in SLE, we analyzed these subpopulations in relation to several cytokines and chemokines quantified in the serum of SLE patients and HCs (**Table 3**). Most of the analyzed molecules were significantly altered in the SLE patients, according to the wide rang of SLE dysregulation. Linear regression analyses adjusted by age and sex revealed significant positive associations of both $CD4^+CD28^{null}$ and $CD28^{null}$-T_{ang} subsets with several proinflammatory mediators in HCs and SLE patients (**Table 4**).

Moreover, analyses of significantly increased cytokines using a multiple backward regression model that included the demographic and clinical features of SLE patients as covariates confirmed the associations of both $CD4^+CD28^{null}$ and $CD28^{null}$-T_{ang} subsets with higher circulating amounts of the inflammatory mediators TNF-α and IL-8 (**Table 5**). Additionally, $CD28^{null}$-T_{ang} cells increased with increasing levels of BLyS and IFN-α, 2 cytokines with particular relevance to SLE activity. Therefore, all $CD28^{null}$ cells were associated with an inflammatory environment, but the $CD28^{null}$-T_{ang} subset seems to be, in addition, closely related to SLE pathology.

DISCUSSION

The present work provides the first evidence for the presence, in pathologic conditions, of a proportion of T_{ang} cells with a senescent and cytotoxic profile ($CD28^{null}$-T_{ang}). Thus, the inclusion of CD28 positivity as a new marker for the true T_{ang} population is proposed.

TABLE 4. Significant association of $CD4^+CD28^{null}$ and $CD28^{null}$ -T_{ang} cells with serum cytokines

Variable	β Coefficient	P value
$CD4^+CD28^{null}$		
TNF-α	0.534	<0.001
IL-8	0.429	<0.001
BLyS	0.664	<0.001
$CD28^{null}$-T_{ang}		
TNF-α	0.498	<0.001
IL-8	0.292	0.003
BLyS	0.557	<0.001
IFN-α	0.242	0.016
IL-10	0.210	0.035

Linear regression analyses in SLE patients (n = 84) and HCs (n = 46) performed with each cytokine as the dependent variable and adjusted by age and sex.

TABLE 5. Association of $CD4^+CD28^{null}$ and $CD28^{null}$-T_{ang} cells with cytokine serum levels in SLE patients

Variable	β Coefficient	*P* value
$CD4^+CD28^{null}$		
TNF-α	0.460	0.001
IL-8	0.389	0.002
$CD28^{null}$-T_{ang}		
TNF-α	0.561	<0.001
IL-8	0.283	0.042
BLyS	0.380	0.006
IFN-α	0.208	0.038

Multiple backward linear regression analyses performed with each cytokine as the dependent variable and including the demographic and disease features as covariates.

In agreement with the low levels of total and $CD28^+$-T_{ang} cells exhibited by our tCVR and RA groups, a reduced T_{ang} population has been previously described in CV conditions [8] and in RA patients, especially those who had experienced a CV event [20]. In line with this, SLE patients presenting with CV disorders showed a significant decrease in the true $CD28^+$-T_{ang} population, and the senescent $CD28^{null}$-T_{ang} subset increased even in the CV-free SLE group. The reduced proportion of $CD4^+$ lymphocytes within the $CD28^+$-T_{ang} subset in SLE patients is in accordance with a senescent profile and suggests impaired function. Moreover, this alteration was also observed in tCVR individuals, the other disease group with increased $CD28^{null}$-T_{ang} cell levels and usually characterized by premature immunosenescence [13, 23].

An expansion of $CD4^+CD28^{null}$ cells, most of them autoreactive, has been reported in several autoimmune diseases [3, 7, 9, 20]. It has been suggested that they could enhance autoimmunity through the production of proinflammatory cytokines that might activate autoreactive T cells [24]. Given the striking increase of circulating $CD4^+CD28^{null}$ cells found in SLE patients, and to a lesser extent in RA patients and tCVR individuals, the presence of $CD28^{null}$-T_{ang} cells should not be surprising. However, this subset was almost absent in RA patients, ruling out a general condition present in autoimmune diseases. Alternatively, this might be explained by the migration of T_{ang} blood cells (regardless of CD28 expression) to the inflamed joints, where they could be involved in the aberrant synovial angiogenesis present in these patients [25]. It has been suggested that this migration could underlie the low circulating T_{ang} counts and the chronic inflammation detected in RA patients with active disease [20]. In contrast, although both total and $CD28^+$-T_{ang} cells were decreased in the tCVR group, the $CD28^{null}$-T_{ang} subset was notably augmented. Therefore, the T_{ang} profile seems to be disease specific, highlighting the complexity of this poorly understood cell population.

It has been shown that $CD4^+CD28^{null}$ cells, which produce large amounts of inflammatory cytokines, can be isolated from ruptured atheromas [12, 15]. Thus, we hypothesized that the $CD4^+CD28^{null}$-T_{ang} subset reported in the present study could have a proatherogenic role. Accordingly, the proportion of $CD4^+$ T cells among the $CD28^{null}$-T_{ang} subset was significantly increased in both tCVR and SLE groups. However, $CD28^{null}$-T_{ang} cells, in view of their senescent and cytotoxic phenotype, might be expected to have a deleterious, rather than a protective, effect on endothelial cells. This population was related to higher levels of TNF-α and IL-8, similar to $CD4^+CD28^{null}$ cells, but also to several SLE features, including increased levels of IFN-α, BLyS, anti-dsDNA titer, and anti-SSA antibodies, which were associated with endothelial damage and a poor prognosis. Therefore, senescent $CD28^{null}$-T_{ang} cells could amplify the damage caused in SLE by the action of IFN-α, BLyS, and anti-dsDNA antibodies in a vicious circle [26].

In conclusion, our results have revealed the existence of an aberrant $CD28^{null}$-T_{ang} population in SLE patients. This aberrant population is characterized by a senescent and cytotoxic phenotype, which was associated with anti-dsDNA titer and an increased production of proinflammatory mediators. It is also associated with other SLE-related pathogenic factors involved in endothelial dysfunction and cardiovascular disease, such as IFN-α or BLyS. Therefore, blocking these molecules in patients with high $CD28^{null}$-T_{ang} levels could reduce the premature immunosenescence and accelerated atherosclerosis characteristic of most SLE patients.

AUTHORSHIP

P.L. performed most of the experimental procedures, data analysis and interpretation, and manuscript preparation. J.R.-C. performed some of the experimental procedures. A.M.-Z. and L.C.-M. contributed to sample collection and the review of the demographic data, clinical manifestations, disease activity, and patient therapy. A.S. contributed to the study design and conduct of the study, data interpretation, and manuscript preparation.

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DISCLOSURES

The authors declare no competing financial interests.

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KEY WORDS:
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