EXTENDED REPORT

Angiogenic T cells are decreased in rheumatoid arthritis patients

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ABSTRACT

Objective The mechanisms underlying the increased cardiovascular risk (CVR) of rheumatoid arthritis (RA) patients remain unclear. Since the recently discovered angiogenic T cells (Tang) could have a role in endothelial repair through cooperating with endothelial progenitor cells (EPC), the main aim of this study was to analyse the Tang and EPC populations in relation to disease-specific features and traditional CVR factors.

Methods Tang ($CD3^+CD31^+CXCR4^+$) and EPC ($CD34^+VEGFR2^+CD133^+$) populations were quantified by flow cytometry in peripheral blood samples from 103 RA patients and 18 matched healthy controls (HC). Clinical features and traditional CVR factors were obtained from clinical records, and 28-joint Disease Activity Score was used for measuring disease activity. Interferon (IFN) α serum levels were measured by immunoassays.

Results Tang and EPC were strongly decreased in RA patients. In HC, but not in patients, both populations were positively correlated and inversely related to low density lipoprotein- and total-cholesterol levels. Sex, diabetes, dyslipidaemia, hypertension or obesity did not significantly influence Tang in patients, although detected in smokers. However, Tang were closely related to disease activity, autoantibody positivity and IFNα levels. Multiple regression analysis adjusted for traditional CVR factors confirmed that only disease activity, age at diagnosis, antinuclear antibody positivity and smoking habit could predict Tang frequency. Finally, patients who had suffered a CV event since their RA diagnosis presented higher Tang decrease and IFNα levels than those who were CV event-free.

Conclusions Disease-specific parameters, including disease activity, autoantibody profiles and IFNα levels, are associated with Tang decrease in RA, thus probably accounting for CVR.

INTRODUCTION

An increased prevalence of cardiovascular (CV) events, not fully explained by traditional risk factors,[1] has been widely reported in rheumatoid arthritis (RA) patients.[2 3] Therefore, other causes, such as disease activity, chronic inflammation, glucocorticoid treatment and genetic background, have been proposed as disease-related independent risk factors.[3–9] Such factors could increase CV risk by promoting the development of early atherosclerotic lesions and impairing the endothelial repair mechanisms. Moreover, it has been reported that endothelial repair mechanisms were impaired in RA and other autoimmune diseases, partly due to the altered number or function of endothelial progenitor cells (EPC), a haematopoietic-derived population involved in vasculogenesis and vascular repair.[10]

Although EPC levels have been considered as a surrogate marker of CV status in healthy subjects,[11 12] different and even contradictory data about their role in RA patients have been reported. Recently, a novel T cell subset, the so-called angiogenic T cells (Tang),[13] has been described that seems to cooperate with EPCs and enhance endothelial repair function, possibly through the secretion of proangiogenic cytokines. In fact, in vitro experiments showed that Tang depletion could abrogate EPC functionality.[13] Animal models of ischaemia also highlighted the relevance of the Tang population in capillary formation.[13] Tang cells are characterised by the coexpression of CD3, CD31 (platelet endothelial cell adhesion molecule) and CXCR4 (receptor for stromal-cell-derived factor-1) and may express CD4 or CD8. This subset is characterised by the coexpression of naive and memory markers, thus revealing its heterogeneous nature. A recent study in human patients revealed, for the first time, that lower Tang numbers are associated with vascular disease.[14] Thus, Tang may be used as a novel putative biological marker for CV disease.

On the other hand, among other factors that could impair endothelial repair, type I interferon (IFN) deserve to be noted. IFNα and related cytokines are a family of pleiotropic molecules with potent antiviral effects and an established relevance in systemic autoimmunity.[15] However, increasing evidence points out their roles in endothelial injury and repair failure. In fact, several mechanisms by which IFNα could damage the endothelium have been described.[16–19] In addition, this cytokine has been associated with the occurrence of CV disease in systemic lupus erythematosus independent of traditional CV risk factors.[20] Similar conclusions have been reached by our group when studying RA patients.[21]

Given the very recent description of the Tang population, no such studies in RA patients have yet been reported. Thus, we hypothesised that the increased CV risk of RA patients could be related to altered numbers of Tang cells. Therefore, the main aims of the present study were: (i) to quantify Tang population in RA patients, (ii) to evaluate clinical parameters that could be associated with these cells and (iii) whether IFNα serum levels could influence Tang numbers in these patients.

MATERIAL AND METHODS

Patients and controls

Our study involved 103 RA patients consecutively recruited from the outpatient clinic from the Rheumatology Department at the Hospital Universitario Central de Asturias, fulfilling the 1987 American College of Rheumatology criteria. Routine clinical examination, including 28-joint Disease Activity Score (DAS28) calculation, was performed during the patients' visit. Then, patients' clinical records were exhaustively revised so as to obtain previous therapies, traditional CV risk factors and histories of previous CV events. Definition and classification of CV events and traditional risk factors (hypertension, diabetes, dyslipidaemia, obesity and smoking) was performed as previously established.[22 23] A CV event was considered if the patient suffered from heart failure, ischaemic heart disease or cerebrovascular accident since their RA diagnosis to the time of sampling. Simultaneously, matched healthy volunteers (n=18; 15 women; age range 23–63 years) without any pathology or treatment were recruited from the Centro Comunitario de Sangre y Tejidos de Asturias. Automatised blood count and serum lipids analysis were carried out for all the participants. Approval for the study was obtained from the Regional Ethics Committee for Clinical Investigation, according to the Declaration of Helsinki and all the participants gave written informed consent.

Flow cytometry analyses

EPC and Tang frequencies were measured by flow cytometry. EPCs were quantified as previously described.[22] Tang were stained with anti-CD3 PerCP-Cy5,5, anti-CD31 FITC and CXCR4 PE-Cy7, and those CD31/CXCR4$^+$ were considered Tang (figure 1A) (see online supplementary text).

Cytokine serum level quantification

Serum aliquots were stored at −80°C until cytokine measurements. IFNα and tumour necrosis factor (TNF)α serum levels were analysed by immunoassays (see online supplementary text).

In vitro cultures

Peripheral blood mononuclear cell (PBMC) cultures were carried out to investigate the effect of IFNα, TNFα and patients' serum on Tang frequency in vitro (see online supplementary text).

Statistical analysis

All data are presented as median (IQR) unless otherwise stated. Mann–Whitney, Spearman's ranks, ANOVA, χ^2 tests and multivariate regression analysis were used as appropriate. A p value<0.05 was considered statistically significant (see online supplementary text).

RESULTS

Angiogenic–T cells were reduced in RA patients

To evaluate Tang cells in RA patients in relation to EPC-mediated endothelial repair ability and traditional CV risk factors, blood samples from 103 RA patients and 18 healthy controls (HC) were analysed by flow cytometry, quantifying Tang population by means of their CD3, CD31 and CXCR4 expression (figure 1A), whereas EPC populations were determined according to their CD34, CD133 and VEGFR2 expression, as previously described.[24] Demographic and clinical characteristics of patients were summarised in table 1. Results showed a strong decrease of Tang population in RA patients compared with HC, both in absolute numbers (figure 1B) and as a percentage of T cells (2.06 (1.89)% vs 5.52 (4.77)%, p=0.0002). Circulating EPCs, as previously reported, were also decreased in patients (figure 1C).

On the other hand, we observed interesting associations between Tang and CV risk factors in HC that were absent in RA patients (table 2). First, these cells exhibited a strong positive correlation with EPC levels. Of note, this association was found with CD34$^+$CD133$^+$VEGFR2$^+$ cells (the so-called 'true EPC'), but not with total CD34$^+$ or CD34$^+$CD133$^+$ progenitor cells or with the CD34$^+$VEGFR2$^+$ population. In addition, Tang from HC were negatively associated with total cholesterol and low density lipoprotein (LDL)-cholesterol, but not with high density lipoprotein. Furthermore, EPC levels from HC showed similar correlations (total cholesterol: r=−0.573, p=0.013; LDL-cholesterol: r=−0.562, p=0.015), thus supporting the association of both Tang and EPC populations with CV risk factors. Nevertheless, these correlations were completely absent in RA patients. Moreover, male sex and the presence of diabetes, hypertension, dyslipidaemia or obesity did not significantly influence Tang in RA patients, although even lower levels were detected in smokers (p=0.037).

Finally, a stronger Tang–blood decrease was found in the subgroup of RA patients who had suffered a CV event since their RA diagnosis (n=19, time between RA diagnosis and CV event: 70.56±61.11 months) when compared with those without this complication (2.61 (1.88) vs 3.56 (3.75)·10^3 cells/μL, p=0.014). Thus, we analysed the influence of traditional CV risk factors in RA patients with and without previous history of CV events using logistic regression modelling. We found that none of the variables included in the analysis were significantly associated with the occurrence of a CV event (age: p=0.704, male sex: p=0.074, obesity: p=0.958, hypertension: p=0.079, dyslipidaemia: p=0.569, diabetes: p=0.211 and smoking habit: p=0.840). Therefore, traditional CV risk factors did not appear to be the most relevant causes for Tang decrease in RA patients.

Disease activity and autoantibodies influenced Tang in RA patients

Therefore, we aimed to look for disease-specific features which may be involved in Tang reduction in peripheral blood. Among the analysed clinical parameters, the strongest association was detected with DAS28 score (figure 2A), indicating that disease activity plays an important role in Tang decrease. Moreover, Tang and EPC levels remained correlated, although at a lower degree than in HC, in patients with low disease activity (DAS28<2.6, n=27) (figure 2B). This association was completely lost in patients with active disease (DAS28≥2.6, n=76). Other clinical parameters such as tender joint counts (r=−0.260, p=0.009), erythrocyte sedimentation rate (r=−0.330, p=0.001) and age at diagnosis (r=−0.352, p<0.0001), but not disease duration (r=0.009, p=0.929), were negatively correlated with Tang population. In fact, the analysis of RA patients recruited at diagnosis and without treatment (n=7) indicated that Tang were strongly decreased even at early stage of the disease compared with HC (3.98 (4.13)·10^3 vs 8.93 (6.63)·10^3 cells/μL, p=0.006), showing similar levels as they established disease counterparts (3.30 (3.28)·10^3 cells/μL).

Likewise, the analysis of immunological features showed that presence of autoantibodies was also related with Tang–blood population, since patients presenting rheumatoid factor (RF), anticyclic citrullinated peptide antibody (anti-CCP) or antinuclear antibody (ANA) exhibited lower Tang levels than their negative counterparts (figure 2C). In fact, Tang were negatively

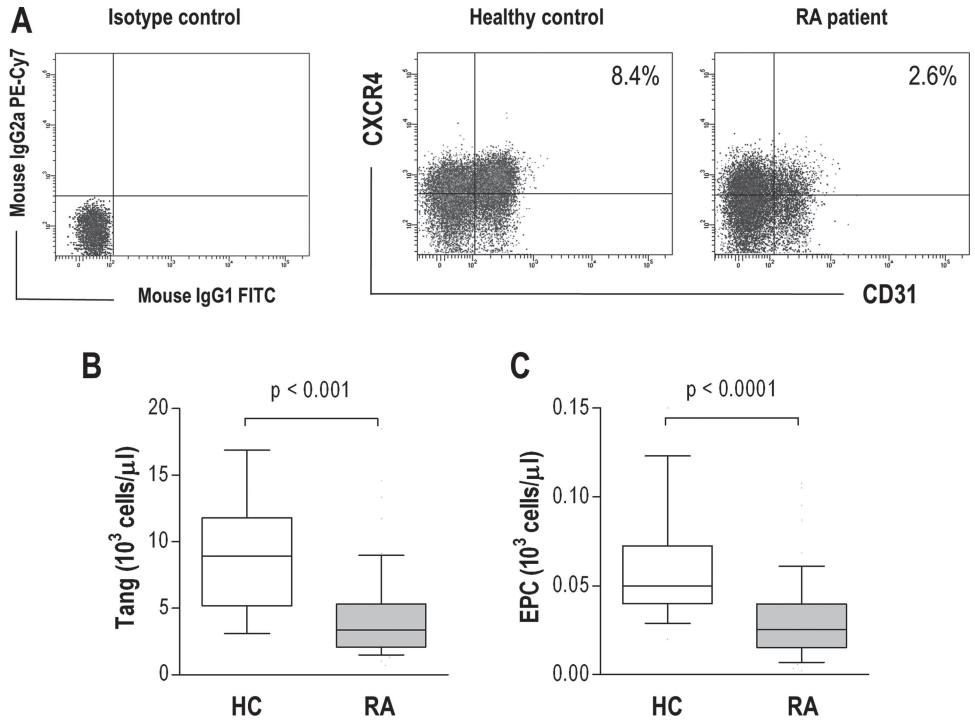

Figure 1 Tang and endothelial progenitor cell (EPC) are decreased in peripheral blood of rheumatoid arthritis (RA) patients. (A) Representative CD31 versus CXCR4 dot-plots of a healthy controls (HC) and a RA patient. Gated CD3 lymphocytes were analysed for CD31 and CXCR4 expression by flow cytometry. Tang population was identified as the triple-positive CD3/CD31/CXCR4 cells in the lymphocyte gate. Quadrants were set according to the fluorescence signal provided by the isotype controls. Box plots represent Tang (B) and EPC (C) peripheral blood reduction in RA patients compared with HC. Differences were evaluated by Mann–Whitney U test.

associated with RF and ANA titres (r=−0.473, p<0.0001 and r=−0.252, p=0.011, respectively). Additionally, patients with previous CV events presented higher frequency of anti-CCP (88.9% vs 62.5%, p=0.031) and a trend for RF positivity (83.3% vs 62.5%, p=0.091).

Therefore, in order to evaluate the relevance of clinical and immunological features in determining Tang frequencies, a multivariate linear regression analysis were performed. After adjusting for traditional CV risk factors (age, sex, dyslipidaemia, diabetes, hypertension, obesity and smoking), only disease activity (B (95% CI) −0.546 (−1.008 to −0.339), p=0.0001), age at diagnosis (−0.041 (−0.088 to −0.018), p=0.003), ANA positivity (−1.046 (−1.959 to −0.184), p=0.019) and smoking (−0.732 (−2.189 to −0.374), p=0.006) showed a significant effect in predicting Tang levels, thus supporting that, except for smoking, disease-specific parameters rather than traditional CV risk factors, are implicated in Tang numbers in RA patients.

IFNα levels were associated with Tang decrease in peripheral blood

In addition to clinical parameters, other factors involved in RA pathogenesis and inflammation burden could have a role in Tang frequency reduction. Thus, to evaluate possible serum markers associated with endothelial damage, we quantified circulating IFNα and TNFα, two cytokines involved in the pathogenesis of several autoimmune diseases. Results showed that serum levels of IFNα were significantly increased in patients (p=0.004) (see online supplementary table S1) and correlated inversely with Tang (figure 3A). Although IFNα was undetectable in the 49.3% of patients, this association remained significant in the IFNα-detectable subgroup (r=−0.233, p=0.048), which displayed lower Tang levels (2.64 (3.03)·10^3 vs 4.51 (4.48)·10^3 cells/µL, p=0.004). This relationship was completely absent in HC (r=−0.028, p=0.918), probably because IFNα was undetectable in most of them (88%). No associations with total CD3 or CD31 cells were found, suggesting that detrimental effects were specific for Tang population rather than a generalised effect on T cells. Moreover, RA patients with previous CV events exhibited higher IFNα serum levels compared with those without them (p=0.019, 78% of IFNα positive). On the other hand, TNFα levels, also increased in patients (p=0.014), failed to exhibit a significant association with Tang numbers (see online supplementary figure S2A), although patients with the highest levels (>80th percentile, 39.49 pg/mL) showed a trend to lower Tang counts (2.49 (1.74)·10^3 vs 3.92 (3.93)·10^3 cells/µL, p=0.081). No significant differences were detected in patients with previous CV events (p=0.573).

Finally, culture assays were performed in order to evaluate the effects of these cytokines on Tang population. Thus, PBMCs were cultured for 4 days in medium alone or in the presence of IFNα (1000 U/mL). Tang frequency was significantly reduced (up to a 26.6%) in IFNα-treated cells (figure 3B), although the total amount of viable T lymphocytes was similar in both cultures (p=0.516). Therefore, to determine the possible effect of the IFNα present in RA serum, PBMCs were cultured in medium supplemented with 10% of pooled sera from either HC or RA patients and with increasing concentrations of anti-IFNα or control rabbit IgG antibodies added to RA serum. At day 4, RA serum-treated cells displayed a strong Tang decrease compared with those HC-treated that was partially restored, dose-dependently, by anti-IFNα blockade. No differences in total CD3 counts were observed after IFNα or RA serum treatment. Moreover, the Annexin V/7-AAD staining showed that both early and late Tang apoptosis was not different

Table 1 Demographic and clinical parameters of RA patients

	RA patients (n=103)
Gender (female : male)	83:20
Age at sampling, years (mean±SD)	54.81±14.37
Disease features	
Disease duration, years (mean (range))	5.58 (0–30.00)
Age at diagnosis, years	47.83 (13.92)
Disease activity (DAS28)	3.49 (1.90)
Tender joint count	2.00 (4.00)
Swollen joint count	1.00 (3.00)
Patient global assessment (0–100)	37 (36.50)
ESR, mm/h	13.00 (22.50)
CRP, mg/L	2.00 (3.90)
HAQ (0–3)	0.87 (1.21)
RF (+), n (%)	65 (63.1)
αCCP (+), n (%)	66 (64.0)
ANA (+), n (%)	51 (49.1)
Shared epitope, n (%)	41 (39.8)
Erosive disease, n (%)	48 (46.6)
Traditional CV risk factors, n (%)	
Dyslipidaemia	36 (34.9)
Hypertension	35 (33.9)
Diabetes	9 (8.7)
Obesity (BMI>30)	20 (19.4)
Smoking habit	34 (33.0)
CV events, n (%)	
Previous CV events	18 (17.4)
Ischaemic heart disease	8 (7.7)
Heart failure	8 (7.7)
Cerebrovascular accidents	2 (1.9)
Treatments, n (%)	
None or NSAIDs	7 (6.7)
Glucocorticoids	56 (54.3)
Methotrexate	77 (74.7)
TNFα blockers	44 (42.7)
Tocilizumab	12 (11.6)
Statins	20 (19.4)

Categorical variables are summarised as n (%), and continuous one as median (IQR) unless otherwise was stated.
αCCP, cyclic citrullinated peptide antibody; ANA, antinuclear antibody; BMI, Body Mass Index; CRP, C reactive protein; CV, cardiovascular; DAS28, 28-joing Disease Activity Score; ESR, Erythrocyte Sedimentation Rate; HAQ, Health Assessment Questionnaire; NSAIDs, non-steroidal anti-inflammatory drugs; RA, rheumatoid arthritis; RF, rheumatoid factor; TNFα, tumour necrosis factor α.

Table 2 Associations of angiogenic T cells with EPC populations and CV risk factors

	HC	RA
EPC populations		
$CD34^+VEGFR2^+CD133^+$ (EPC)	r=0.886 p<0.0001	r=−0.052 p=0.679
CD34	r=0.252 p=0.313	r=0.126 p=0.263
$CD34^+CD133^+$	r=0.318 p=0.198	r=0.142 p=0.205
$CD34^+VEGFR2^+$	r=0.362 p=0.139	r=−0.010 p=0.933
Traditional CV risk factors		
Total cholesterol (mg/dL)	r=−0.688 p=0.002	r=0.040 p=0.709
HDL-cholesterol (mg/dL)	r=−0.099 p=0.695	r=0.112 p=0.300
LDL-cholesterol (mg/mL)	r=−0.670 p=0.002	r=0.107 p=0.325
Male sex	p=0.360	p=0.282
Diabetes		p=0.712
Hypertension		p=0.570
Obesity (BMI >30)		p=0.119
Smoking habit		p=0.037

Associations between Tang and continuous variables were assessed by Spearman rank correlations (r coefficient and p value are shown), and Mann–Whitney U test was used with categorical variables (p value is shown).
BMI, Body Mass Index; CV, cardiovascular; EPC, endothelial progenitor cell; HC, healthy controls; HDL, high density lipoprotein; LDL, low density lipoprotein; RA, rheumatoid arthritis.

in IFN-treated cultures than in the negative control (early: 0.53 ±0.21 vs 0.82±0.28, p=0.114; late: 0.16±0.20 vs 0.08±0.07, p=0.771). However, IFNα seemed to be able to downregulate CXCR4 expression (MFI in Tang: 7630.50±1575.82 vs 5318.50±3253.80; in CD3 cells: 4359±799.23 vs 3606.25 ±454.77), thus being one potential mechanism to explain lower Tang numbers.

These results point out the detrimental role of IFNα on Tang subset and the involvement of other serum factors in this effect. In fact, TNFα was also able to slightly decrease Tang frequencies (7.4%) in vitro (see online supplementary figure S2B), although no associations were observed in patients.

DISCUSSION

In recent years, several studies have been performed to explore the mechanisms underlying the increased CV risk and endothelial damage observed in RA patients. The results presented herein are the first reporting a new factor that seems to be implicated in this condition: the recently described subpopulation of immune cells, the so-called Tang.

It has been suggested that Tang cells may be used as a biomarker for CV risk and endothelial function.[13] Accordingly, the evaluation of Tang cells in healthy individuals performed in this work showed that this population was negatively correlated with total and LDL-cholesterol levels. In addition, Tang were positively associated with $CD34^+VEGFR2^+CD133^+$ cells, the true EPC population, but not with other phenotypes ($CD34^+VEGFR2^+$ or $CD34^+CD133^+$). This result highlights the special relevance of CD133 labelling for EPC measurements by flow cytometry.[25] Although a positive correlation between Tang and EPC colonies in vitro has been previously reported,[13] this is the first study showing a correlation between EPC and Tang in human peripheral blood. These findings suggest a connection between decreased Tang numbers and increased CV risk.

The most important finding of our work, however, was the striking circulating Tang decrease detected in RA patients, even at diagnosis, which, additionally, was unrelated to EPC levels and traditional CV risk factors, except for smoking. Instead, disease activity and presence of autoantibodies seemed to have detrimental effects on the Tang population. These cells were decreased in a disease activity-dependent manner in RA patients, thus suggesting that specific disease features were implicated in Tang decrease. In fact, the association between EPC and Tang was partially recovered in patients with low disease activity. These findings are in line with the idea that an accurate control of the disease will have a positive impact on CV risk management.[26] However, our data could support an alternative role of Tang in chronic

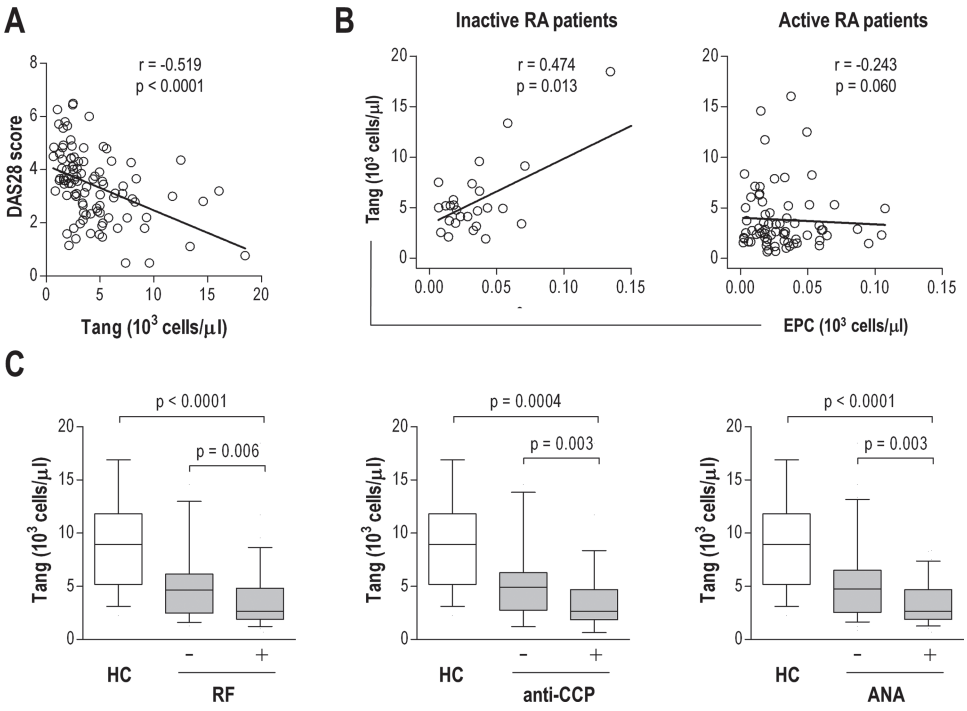

Figure 2 Disease activity and autoantibody positivity were associated with Tang decrease. (A) Tang cells were decreased in rheumatoid arthritis (RA) patients in a disease activity-dependent manner and (B) were positively correlated with endothelial progenitor cells (EPC) populations in inactive patients (Disease Activity Score (DAS)<2.6, n=27) but not in active ones (DAS≥2.6, n=66). (C) Autoantibodies positivity (rheumatoid factor (RF): n=65, α cyclic citrullinated peptide antibody (CCP): n=66 and antinuclear antibody (ANA): n=51) was associated with Tang reduction. Correlations were assessed by Spearman ranks test and differences were evaluated by Mann–Whitney U test.

inflammation, since Tang behaviour under this situation is yet unknown. In fact, it might be expected that Tang–blood cells would migrate to the inflamed tissues, thus explaining the low circulating counts and the inverse relationship with inflammatory markers. Therefore, we cannot exclude that Tang cells in patients with an active inflammatory disease could be involved in the chronic inflammation rather than in angiogenic repair.

On the other hand, Tang were also associated with late age at diagnosis and the presence of ANA, RF and anti-CCP antibodies, all of them previously associated with poor prognosis and CV risk in RA.[27 28] Moreover, autoantibody positivity has been previously related to CV events in RA[29] and other clinical conditions.[30 31] Therefore, our data suggest that the negative effect of autoantibodies on Tang could be one of the underlying mechanisms by which they influenced CV risk, at least in RA.

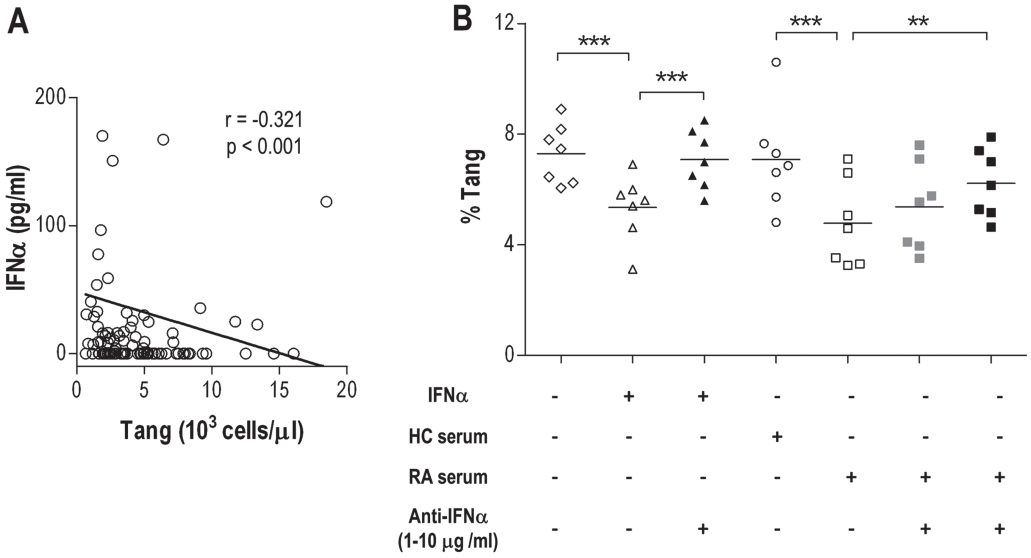

Figure 3 Interferon (IFN)α exhibited negative effects on Tang population. (A) IFNα serum levels were negatively correlated with Tang frequency in rheumatoid arthritis (RA) patients. (B) Peripheral blood mononuclear cell (PBMC) cultured in the presence or absence (medium) of 1000 U/mL of recombinant human IFNα prompted an in vitro Tang reduction, which was totally abrogated by IFNα blockade. A similar decrease was observed when culturing in the presence of RA pool serum (IFNα: 159.65 pg/mL; TNFα: 88.63 pg/mL) compared with those healthy controls sera-treated (IFNα: undetectable; TNFα: 5.09 pg/mL). Serum RA-treated reduction was partially recovered by IFNα blockade in a dose-dependent fashion: 1 μg/mL (■) and 10 μg/mL (■). Independent cultures were performed with freshly isolated PBMC from different blood donors (n=7). Correlations were analysed by Spearman ranks test and differences between among treatments were evaluated by a repeated measures ANOVA and Bonferroni post hoc test. Horizontal bars represent the mean value ***p<0.001, **p<0.01.

According to our results, disease-specific features rather than traditional CV risk factors, apart from smoking, appear to be associated with Tang reduction in RA, thus supporting the idea that new factors should be taken into account in CV risk assessment in RA. In line with this, an interesting result of this work was the suggested harmful role played by IFNα on Tang cells. Type I IFN signature has been widely associated with the pathogenesis of autoimmunity, first in SLE,[32] but currently also in a subset of RA and other disorders.[15 33 34] Moreover, type I IFNs have been associated with disease activity[35] and clinical features[36 37] as well as with atherosclerosis markers[19 38 39] and CV disease.[20] In fact, different ways by which IFNα could damage the endothelium have been described.[40] Additionally, IFNα treatment has been associated with increased CV events in non-RA subjects.[41–43] Thus, the role of IFNα in Tang decrease reported here may suggest a new way by which this cytokine could have a negative impact on endothelial repair and CV risk, in the subgroup of RA patients with the IFNα 'signature'.[15 34] Recently, systemic disease has been associated with the occurrence of CV events in RA patients.[44] Our results are in line with all these findings, since patients with higher IFNα levels are characterised by a higher rate of CV events and lower Tang frequencies. Thus, in addition to inflammatory and disease-specific markers, high IFNα levels might be helpful in the identification of RA patients with high CV risk.

Finally, the analysis of patients with a history of CV events may support the use of Tang as a putative marker of endothelial damage and CV disease in RA, as was suggested in other pathologies.[14] These patients exhibited lower Tang counts than those CV-free, highlighting the role of Tang cells in vascular repair. These patients also displayed increased levels of IFNα, previously associated with the development of premature atherosclerosis[20 39] and CV disease[19] in lupus. Accordingly, type I IFN signature has been found to be upregulated even several years after CV event occurrence.[17] Therefore, Tang cells could be an interesting target in RA and CV disease.

In conclusion, our data indicate that peripheral Tang decrease, in addition to an altered EPC function, is associated with the increased CV risk in RA patients, probably by impairing endothelial repair. These low Tang levels are closely related to disease-specific parameters. Specifically, high disease activity and autoantibody positivity are strong indicators of Tang reduction, whereas presence of high IFNα levels could be considered an additional factor in a subgroup of patients. We cannot exclude, however, that severe disease, chronic inflammation and IFNα can directly promote endothelial dysfunction, thus increasing CV risk independently of Tang population. In any case, these disease features could be interesting tools to account for CV risk in RA patients. Although further studies are needed to investigate the functionality of these cells in inflammatory conditions, increasing Tang number and/or function might be a promising intervention in RA patients, mainly in those with high risk or history of CV disease. In this sense, IFNα blockade[45] could be a valuable therapy for patients with high levels of this cytokine.

Contributors JR-C performed most of the flow cytometry analyses and data collection as well as wrote the manuscript. PL participated in immunoassays measurements and experimental procedures. MA-L, SA-C and JB-G were in charge of patients' recruitment and clinical data collection. AS conceived and coordinated the study, collected the data, performed the statistical analyses and corrected the manuscript. All the authors read and approved the final version of the manuscript.

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Patient consent Obtained.

Ethics approval Regional Ethics Committee for Clinical Investigation (Hospital Universitario Central de Asturias).

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