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Expansion of T-Cell Receptor ζ^{dim} effector T Cells in Acute Coronary Syndromes

Ammirati, Expansion of TCR ζ^{dim} T Cells in ACS

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Abstract

Objective- The T-cell receptor zeta (TCR ζ)-chain is a master sensor and regulator of lymphocyte responses. Loss of TCR ζ -chain expression has been documented during infectious and inflammatory diseases and defines a population of effector T cells (TCR ζ^{dim} T cells) that migrate to inflamed tissues. We assessed the expression and functional correlates of circulating TCR ζ^{dim} T cells in coronary artery disease.

Methods and Results- We examined the expression of TCR ζ -chain by flow cytometry in 140 subjects. Increased peripheral blood CD4⁺TCR ζ^{dim} T cells were found in patients with acute coronary syndromes (ACS, n=66; median 5.3%, inter-quartile 2.6-9.1% of total CD4⁺T cells; p<0.0001) compared to chronic stable angina (CSA, n=32; 1.6%; 1.0-4.1%) and controls (n=42; 1.5%; 0.5-2.9%). Such increase was significantly greater in ACS patients with elevated levels of C-reactive protein, and it persisted after the acute event. Moreover, TCR ζ^{dim} cells were also more represented within CD8⁺T cell, NK and CD4⁺CD28^{null}T cell subsets in ACS compared to CSA and controls. Finally, CD4⁺ and CD8⁺ TCR ζ^{dim} T cells isolated from ACS displayed an enhanced transendothelial migratory capacity.

Conclusions- TCR ζ^{dim} T cells, an effector T-cell subset with trans-endothelial migratory ability, are increased in ACS, and may be implicated in coronary instability.

Condensed abstract

Reduction of TCR ζ -chain expression defines antigen-experienced effector T cells (TCR ζ^{dim} T-cells) described in inflammatory and infectious diseases. We found increased numbers of TCR ζ^{dim} T cells in patients with acute coronary syndromes compared to stable angina and controls, with enhanced trans-endothelial migratory ability, that may be implicated in the pathogenesis of coronary instability.

Key words

Acute coronary syndrome ■ lymphocytes ■ flow cytometry ■ immune system ■ receptors

Introduction

Inflammation plays a key role in the pathogenesis of atherosclerosis with participation of both innate and adaptive immunity.¹⁻³ Upregulation of proinflammatory cytokines is common in acute coronary syndromes (ACS).⁴ Elevated C-reactive protein (CRP) levels are associated with an adverse prognosis in patients with coronary artery disease (CAD), and healthy subjects.⁴ The activation of inflammatory pathways in ACS is not confined to coronary lesions but involves the activation of neutrophils, monocytes and lymphocytes in peripheral blood.⁵⁻⁷

Most T cells found in human atherosclerotic lesions exhibit an effector or memory phenotype with a Th1 bias, and a predominance of CD4⁺ over CD8⁺ T cells.⁸ Different subsets of T cells may drive or regulate inflammation during evolution of the atherosclerotic process, as in other inflammatory diseases.⁹ An increased prevalence of specific circulating T cell subsets, such as CD4⁺CD28^{null} T cells, was reported in patients with ACS, as well as in patients with rheumatoid arthritis.¹⁰⁻¹³

The TCR ζ -chain (CD247 or CD3 ζ) is a transmembrane protein with a small extracellular domain and an intracellular domain containing three immunoreceptor tyrosine-based activation motifs. The TCR ζ -chain subunit associates with the TCR-CD3 complex as a homodimer, and couples antigen recognition by the TCR to downstream intracellular signal-transduction pathways through phosphorylation and recruitment of downstream proteins. The TCR ζ -chain is also expressed in NK cells, where it is associated with the FcR γ III low affinity IgG receptor (CD16) and other activating receptors.¹⁴ TCR ζ -chain is

therefore a master regulator and sensor of innate as well as adaptive immune responses, and so it follows from this that aberrations in its expression or function should be expected to have profound effects on immune function.¹⁵

A subset of T cells expressing low levels of TCR ζ -chain (hereafter TCR ζ^{dim} T cells) has been described in association with infectious, malignant and inflammatory diseases.¹⁴⁻¹⁸

Downregulation of the TCR ζ -chain, known to occur following antigen engagement or in response to inflammatory stimuli, may represent an attempt to modulate the immune response.¹⁴ However, we previously reported that the TCR ζ^{dim} T-cell subset, although refractory to TCR-induced proliferation,¹⁹ paradoxically displays features of antigen-experienced effector T cells.¹⁵ Cell surface markers and tetramer analysis revealed that TCR ζ^{dim} T-cells are enriched for memory CD45RO⁺ cells, and for cells that had previously engaged antigen. Moreover, TCR ζ^{dim} T cells produce high levels of interferon- γ (IFN γ) and tumor necrosis factor α (TNF α), and low levels of IL-10.¹⁵ Finally, TCR ζ^{dim} T-cells display effector functions, e.g. they can activate monocytes via cell contact-dependent pathways, and preferentially accumulate in inflamed tissues such as the rheumatoid joint.¹⁵

In the present study we report that the frequencies of subsets of T cells and NK cells expressing low levels of TCR ζ -chain are increased in patients with ACS when compared to patients with stable atherosclerotic lesions or to controls, particularly in patients with elevated levels of CRP, and remain elevated after the acute coronary event. TCR ζ^{dim} T

cells display an enhanced ability to migrate through activated endothelium *in vitro*. Our findings suggest the possibility that antigen experienced T cells, defined by the TCR ζ^{dim} phenotype, may contribute to the inflammatory process that triggers coronary instability by accumulating in coronary plaques.

Methods

Study Population

Institutional Ethics Committees approved the study and informed consent was obtained from all participating subjects. Venous peripheral blood samples were obtained from all patients on admission to San Raffaele Scientific Institute. Three groups participated in the study. *Control group* comprised 42 individuals with negative history, clinical and electrocardiographic signs of CAD. *CSA group* included 32 patients with effort angina (lasting more than 3 months and without previous history of unstable angina or myocardial infarction) with angiographic evidence of coronary artery stenosis (stenosis >50% diameter). *ACS group* comprised 66 patients with chest pain accompanied by ischemic electrocardiographic changes (ST-segment changes and/or T-wave inversions) or ST-segment elevation >2 mm in at least two consecutive leads associated to increase of troponin I. All samples were obtained on admission. Evidence of coronary artery stenosis/occlusion was documented by coronary angiography. *Exclusion criteria*: recent surgery, documented immune, infectious or neoplastic disease, immunosuppressive therapy, ACS due to intrastent thrombosis or occlusion of arterious and venous by-pass grafts (confirmed after angiography). In 17 patients with ACS the levels of TCR ζ -chain were also assessed after a 50-day median time (interquartile 39-83 days) from admission. Further analysis of TCR ζ -chain expression was performed in CD8⁺ T and NK cells (14 control, 9 CSA and 22 ACS participating in the study) and in CD4⁺CD28^{null} T cells (19 controls, 18 CSA and 23 ACS).

Cell isolation

Peripheral blood mononuclear cells (PBMCs) were purified by Ficoll-Hypaque (Becton Dickinson) density gradient centrifugation from anti-coagulated venous blood samples, before flow cytometry. Alternatively, flow cytometry was performed on fresh whole blood samples. For confocal microscopy and cell culture studies, CD3⁺ or CD3⁺CD4⁺ cells were further enriched by Robosep using EasySep Negative selection Human CD3⁺ or CD3⁺CD4⁺ T cell enrichment kit and procedure (Voden).

TCR ζ expression analysis

Surface staining of T cell subsets was performed by standard methods. For intracellular staining, cells were fixed with 2% formaldehyde and permeabilised in buffer containing 10 $\mu\text{g/ml}$ saponin. The efficiency of permeabilisation was determined by uptake of trypan blue (>99% in all experiments). Isotype-matched controls Abs were used to confirm expression specificity. The following Abs were purchased from Becton Dickinson: mouse IgG1 isotype control conjugated with PE (clone MOPC-21) or with FITC (clone A85-1), anti-CD3-FITC (clone UCHT1), anti-CD4-PE-Cychrome 5 (clone RPA-T4), anti-CD8-Cychrome 5 (clone HIT8a), anti-CD3-PE-Cychrome 5 (clone HIT3a), anti-CD16-FITC (clone 3G8), anti-CD3-Pacific Blue (PB) (clone UCHT1), anti-FoxP3-PB (clone 206D). Two different antibodies were utilized for TCR ζ expression studies: anti-TCR ζ -PE (clone 2H2D9, Immunotech, Coulter), anti-TCR ζ -FITC (clone G3, Dako). The 2 different clones used of anti-TCR ζ individuate the same percentage of TCR ζ^{dim} cells. The CD3⁻

CD56⁺ NK cell subset was found to be uniformly TCR ζ negative, and was not therefore studied further.

We confirmed data also with fresh whole blood analysis (cell viability >99%). Cell viability was assessed using the Molecular Probes Patented LIVE/DEAD Viability (Invitrogen) according to the manufacturer instructions. Moreover, frequencies of TCR ζ^{dim} within the CD3⁺CD4⁺CD28^{null} subset were determined by a four-colour flow cytometry on fresh whole blood performed using anti-CD3-Cascade Yellow (clone UCHT1, Dako), anti-CD4-APC-Cychrome 7 (clone SK3 Becton Dickinson), anti-CD28-FITC (clone CD28.2, Becton Dickinson) and anti-TCR ζ -PE (clone 2H2D9, Immunotech, Coulter).

Cells were analyzed on a Cyan ADP (Dako) flow cytometer. FCS Express version 3 (De Novo Software) was used for analysis. The percentage of TCR ζ^{dim} was determined within each lymphocyte subset of interest. In addition, we assessed the Median Fluorescent Intensity (MFI) of TCR ζ -chain expression as MFI index (MFI TCR ζ^{bright} /MFI TCR ζ^{negative}) as described.¹⁸

Confocal microscopy

Cells from 10 subjects randomly chosen among ACS patients (n=3), CSA patients (n=2) and controls (n=5) were fixed and stained for confocal microscopy with anti-CD3-PB (clone UCHT1, Becton Dickinson) or anti-CD3-FITC (clone UCHT1, Becton Dickinson) and, upon permeabilisation, anti-TCR ζ -FITC (clone G3, Dako) or anti-TCR ζ -PE (clone 2H2D9, Immunotech, Coulter). Cells were subsequently plated onto glass slides and

examined under a Leica TCS SP2 AOBS confocal microscope (Leica Microsystems). Z-series were collected from singles channels, processed to 2D free projection max images, and merged. Single stains either for CD3 and TCR ζ served as controls.

Measurement of high sensitivity CRP (hsCRP)

Peripheral blood samples were centrifuged and serum aliquots were stored at -80°C until assayed in single batch. hsCRP was assessed via nephelometry (BN II – Behring instrument).

Transendothelial Migration Assay

We performed transendothelial migration assays of T cells were performed in 17 study subjects (5 controls, 6 CSA and 6 ACS), by applying 10^6 lymphocytes to gelatin-coated transwell upper chambers containing a monolayer of human umbilical vein endothelial cells (HUVEC) previously stimulated with 10 ng/ml TNF α for 48 hours.¹⁵ After 24 hours at 37°C , T cells in the upper and lower chamber were recovered, and the numbers of migrating CD4⁺ and CD8⁺ TCR ζ^{bright} or TCR ζ^{dim} T cells were determined in each chamber by flow cytometry. Results are expressed as the percentage of cells migrating relative to the total number of each cell subset added to the transwell (TCR ζ^{bright} in the lower chamber at time=24hr/ TCR ζ^{bright} at time=0 vs TCR ζ^{dim} in the lower chamber time=24hr / TCR ζ^{dim} at time=0) .

Statistical Analysis

The datasets did not conform to a normal distribution. Mann-Whitney *U* test and Kruskal-Wallis test with Dunn's multiple comparison test were used as appropriate. Wilcoxon matched paired test was used for repeated measures in the time. Spearman's rank test was used to test correlations between variables. GraphPad Prism 4 and GraphPad InStat 3 softwares were used for analysis. A probability value <0.05 was considered significant.

Results

Characteristics of Patients

No significant differences in demographic and risk factors were observed between CSA group and ACS group (Table 1). However, differences were found in therapeutic regimens at the time of the sampling. Such differences were likely due to the fact that for 80% of patients in the ACS group, this hospital admission represented their first clinical manifestation of coronary disease versus only 56% in the CSA group ($p=0.02$). However, at 50-day follow-up there were no statistical differences regarding therapy between the two groups ($p=NS$; data not shown). ACS samples were obtained very early after the onset of symptoms when the elevation of troponin I were still minimal (0.4 ± 0.0 - 5.9 ng/ml), thus ruling out the possible confounding effect of myocardial necrosis. The extent of coronary atherosclerosis was similar in patients in CSA and ACS group as documented by the number of diseased vessels in CSA, 2.0 ± 0.8 (expressed as mean \pm SD), compared to ACS, 1.9 ± 0.9 ($p=0.79$).

Substantial down-regulation of the TCR ζ -chain in CD4⁺ in ACS

Flow cytometric analysis (see representative dot-plots and histograms in Figure 1A-G) revealed a 3-fold increase in the percentage of CD3⁺TCR ζ ^{dim} T cells in ACS patients (median; inter-quartile range: 8.2%; 3.8-14.5%, $p<0.0001$; Supplemental Table I, please see <http://atvb.ahajournals.org>) compared to controls (2.6; 1.1-5.3%) and CSA (3.1; 1.7-7.0%). Confocal microscopy images confirmed the presence of CD3⁺ cells with downregulation of the expression of TCR ζ -chain in patients with ACS (Figure 1H-J and Supplemental Figure I and II, please see <http://atvb.ahajournals.org>). Circulating

CD4⁺TCR ζ^{dim} T cells showed a 3-fold increase in patients with ACS (5.3%; 2.6-9.1%; $p < 0.0001$, Figure 2A) when compared to controls (1.5; 0.5-2.9%) and CSA (1.6; 1.0-4.1%). The MFI index of TCR ζ -chain was significantly reduced in patients with ACS in comparison to controls and patient with CSA (Supplemental Figure III, please see <http://atvb.ahajournals.org>). To exclude that the increased levels of TCR ζ^{dim} T cells reported in ACS were merely due to cell death, we showed that cell viability in CD3⁺ T cells were >99% out of total CD3⁺ in fresh blood, and that TCR ζ^{dim} T cells were not confined to the non viable cells (Supplemental Figure IV, please see <http://atvb.ahajournals.org>).

Differences in statin therapy are unlikely to explain the differences in TCR ζ expression, as a subgroup analysis in patients without statin therapy (ACS n=54; CSA n=14) confirmed a significant increase of CD4⁺TCR ζ^{dim} T cells in ACS ($p=0.002$). Moreover, statin treatment itself was unlikely to provoke such changes as by comparing CSA patients with (n=18) or without (n=14) statin therapy (respectively 1.3; 0.9-3.7% vs 1.8; 0.9-5.1%; $p=0.46$) no significant differences were found.

Downregulation of TCR ζ -chain in CD8⁺ and NK cells in ACS

We further assessed that down-regulation of TCR ζ -chain was not limited to CD4⁺ T cells in ACS. Indeed TCR ζ^{dim} T cells represented 4.0% (2-5.7%) of peripheral circulating CD8⁺ T cells in patients with ACS in comparison to 0.6% (0.2-1.9%) in controls, and 1.2% (0.8-2.1%) in patients with CSA ($p < 0.0001$, Supplemental Figure VA, please see <http://atvb.ahajournals.org>). Also a 2-fold increase was observed in the levels of TCR ζ^{dim} within the circulating CD3⁺CD16⁺ NK cell subset (5.1%; 2.4-7.5%) in patients with

ACS, whereas they accounted for 2.5% (1.3-3.8%) and 1.6% (0.9-2.7%) in controls and in patients with CSA respectively ($p=0.001$, Supplemental Figure VB, please see <http://atvb.ahajournals.org>). No significant differences were observed between control individuals and CSA patients for either T-cell or NK-cell subsets.

Persistence of circulating CD4⁺TCR ζ ^{dim}T cells in ACS patients at follow-up

Frequencies of circulating CD4⁺TCR ζ ^{dim}T cells were unchanged at a 50-day follow-up after admission (6.4; 3.7-10.5% vs 5.5; 3.2-7.7%; $p=0.95$; Figure 2D), despite anti-ischemic therapy and risk factor management.

Increased frequencies of TCR ζ ^{dim}T cells are associated with higher CRP levels

We observed that the frequency of CD4⁺TCR ζ ^{dim}T cells, CD8⁺TCR ζ ^{dim}T cells and TCR ζ ^{dim}NK cells correlated with CRP levels (respectively: $r=0.30$; $p=0.0007$, $r=0.37$; $p=0.02$ and $r=0.48$; $p=0.002$) (Supplemental Figure VIA, B and C, please see <http://atvb.ahajournals.org>). Considering a cut-off of CRP ≥ 2 mg/l used in previous studies,²⁰ patients with ACS and CRP levels ≥ 2 mg/l had significantly increased percentages of CD4⁺TCR ζ ^{dim}T cells (7.7; 3.8-11.3%, $p=0.003$, $n=41$) compared to patients with ACS and low CRP levels < 2 mg/l (3.3; 1.7-7.7%, $n=25$; Figure 3).

Reduction of TCR ζ -chain in CD4⁺CD28^{null}T cells in patients with ACS

In 60 subjects, blood samples were simultaneously stained for CD3, CD4, CD28 and TCR ζ and analyzed by four-colour flow cytometry. We observed a significant correlation between frequencies of CD4⁺CD28^{null}T cells and CD4⁺TCR ζ ^{dim}T cells ($r=0.43$;

p=0.0006; figure 4A), especially within ACS patients ($r=0.62$; p=0.002; Supplemental Figure VIIA, please see <http://atvb.ahajournals.org>). Interestingly, a reduction of TCR ζ -chain expression was observed within the CD4⁺CD28^{null} subset in ACS (67.2; 39.4-82.9%; p<0.0001), as compared to controls (20.9; 11.1-33.1%; p<0.001) and CSA (29.2; 23.0-46.9%; p<0.05) (Figure 4B, 4C and Supplemental Figure VIIB, please see <http://atvb.ahajournals.org>).

Transendothelial migration of TCR ζ^{dim} T cells, both in CD4⁺ and CD8⁺T cells

We investigated the potential role of TCR ζ^{dim} T cells in the immunopathological pathways that drive ACS, by comparing the capacity of this subset to migrate across activated endothelium with their TCR ζ^{bright} counterparts. An *in vitro* transendothelial migration assays was performed on 17 individuals enrolled in the study. Increased migration of circulating TCR ζ^{dim} T cells (CD4⁺: 62.2; 48.3-75.9 % and CD8⁺: 69.0; 50.9-84.5%) were observed when compared to the TCR ζ^{bright} subset (CD4⁺: 47.8; 46.5-50.8%; p=0.005; and CD8⁺: 49.5; 42.3-52.4%; p=0.004; Figure 5 and Supplemental Figure VIII). Similar migratory behaviour of TCR ζ^{dim} T cells in comparison with their TCR ζ^{bright} counterparts were observed in ACS (n=5), CSA (n=6) and control (n=6) group (Supplemental Figure IX, please see <http://atvb.ahajournals.org>). Downregulation was not due to the transmigration process *per se*, since the total number of TCR ζ^{bright} and TCR ζ^{dim} cells in top and bottom chamber does not change during the 24hr transmigration assay.¹⁵

Discussion

We report for the first time expansion of circulating T cells expressing reduced levels of the invariant TCR ζ -chain in patients with ACS compared to patients with CSA and controls. CD4⁺TCR ζ^{dim} T cells remained elevated for at least 50 days after the acute event. In addition we also documented that TCR ζ -chain down-regulation was not limited to T cell subpopulations, but was also present in NK cells. Enrichment of TCR ζ^{dim} cells correlated with the magnitude of the systemic inflammatory response, raising the possibility that TCR ζ^{dim} cells might participate to the ongoing inflammatory and immune response in ACS.

Functional aspects of TCR ζ^{dim} T cells in coronary artery disease

Growing similarities are emerging between atherosclerosis and chronic inflammatory diseases, including the expansion of circulating and lesional effector cells such as T-lymphocyte subsets.¹⁰ Despite T cells with low levels of TCR ζ -chain expression being refractory to TCR signalling,¹⁹ we have recently reported that TCR ζ^{dim} T cells express the hallmarks of memory antigen experienced effector T cells,¹⁵ produce abundant inflammatory cytokines such as TNF α and IFN γ , and promote monocyte activation through cell contact dependent pathways.¹⁵ Moreover, TCR ζ^{dim} T cells do not produce anti-inflammatory cytokine such as IL-10,¹⁵ and do not express the T regulatory cell marker Forkhead Box Protein P3 (FoxP3; Supplemental Figure X, please see <http://atvb.ahajournals.org>). Collectively these observations point towards the requirement for intact TCR signalling to maintain immune homeostasis through the generation or function of regulatory T cell subsets.²¹

Furthermore, in this study we also observed that also in patients with CAD the ability of circulating TCR ζ^{dim} T cells to migrate through endothelium is enhanced compared to their TCR ζ^{bright} counterparts. In previous study we showed also enhanced chemotactic migratory ability of TCR ζ^{dim} T cells in response to chemokines, such as CCL5 and CXCL10.¹⁵ Consistent with these *in vitro* observations, we previously documented that TCR ζ^{dim} T cells are enriched in synovial fluid and tissue from patients with rheumatoid arthritis¹⁵. Moreover, in 3 patients with ACS enrolled in the study we documented a decrease in the number of CD3⁺CD4⁺TCR ζ^{dim} T cells in blood acquired from the great cardiac vein before angioplasty compared to levels of CD3⁺CD4⁺TCR ζ^{dim} T cells sampled from the aorta⁵ (Supplemental Figure XI, please see <http://atvb.ahajournals.org>). These results could suggest an accumulation of this cell population in the coronary circulation during the acute phase of the disease. Thus, antigen experienced effector TCR ζ^{dim} T cells with enhanced migratory competence have the potential to extravasate and exert local pro-inflammatory actions in atherosclerotic plaques, thus contributing to plaque destabilization.

Possible mechanisms of TCR ζ -chain down-regulation

In our study the enrichment of circulating CD4⁺TCR ζ^{dim} T cell in patients with ACS, was associated with higher levels of hsCRP, suggesting that the inflammatory milieu in patients with ACS may contribute to TCR ζ -chain down-regulation. Antigen-dependent and antigen-independent mechanisms have been advocated to explain downregulation of the TCR ζ -chain in human and experimental models.¹⁴ Both mechanisms may have a role in TCR ζ -chain down-regulation in ACS. For instance, prolonged exposure to bacterial

and viral antigens has been shown to induce TCR ζ -chain down-regulation *in vivo*.¹⁴ In the setting of ACS, a variety of infectious and endogenous antigen(s) have been implicated (i.e. modified low-density lipoprotein, endogenous and infectious agents-derived heat shock proteins) and could potentially lead to TCR ζ -chain downregulation.²²⁻²⁴ Antigen independent mechanisms, e.g. prolonged exposure inflammatory cytokines including IFN γ and TNF α , as well as reactive oxygen intermediates have also been advocated for TCR ζ -chain downregulation.¹⁹ In our study, the downregulation of the TCR ζ -chain was not limited to CD4⁺ and CD8⁺ T cells, but also present in the NK-cell population, in which it may arise through FcR engagement, perhaps via immune complexes or through cytokine stimulation.¹⁴

Interestingly, the loss of CD28, as the down-regulation of TCR ζ -chain, has been linked to both chronic antigen and/or cytokine exposure.^{19, 25, 26} CD4⁺CD28^{null}T cells, a terminally differentiated effector T cell population, was identified both in patients with rheumatoid arthritis with vascular complications and in patients with ACS.^{12, 13, 27} More than 65% of CD4⁺CD28^{null}T cells from patients with ACS had selectively reduced TCR ζ -chain expression. Hence, in the context of ACS, similar mechanisms might explain the reduced TCR ζ -chain expression and the loss of CD28, and the generation of T-cell subsets with perturbation of the classical immunological synapse.

Conclusion

We show that circulating TCR ζ^{dim} cells are increased in ACS, and such increase is associated with higher levels of hsCRP. TCR ζ^{dim} cells display an increased ability to cross activated endothelium, and might carry the potential to accumulate in

atherosclerotic plaques. Both antigen dependent and antigen independent mechanisms may contribute to the emergence of this unusual T-cell surface phenotype. The aberrant expression of TCR ζ -chain in circulating cell subsets further supports the role of dysregulation of the immune response in the pathogenesis of ACS. Moreover, our findings may contribute to explain the increased incidence of major cardiovascular events in systemic lupus erythematosus and rheumatoid arthritis²⁸.

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Disclosures

None.

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Figure legend

Figure 1. Lymphocytes (A) and CD3⁺CD4⁺ cells (B). Dot-plots from CSA (C-D) and ACS (E-F). The percentage of CD3⁺CD4⁺TCR ζ^{dim} T are indicated in the low right quadrant. Histograms of TCR ζ -chain MFI within CD3⁺CD4⁺ population (G). Confocal microscopy images (H-J) showed TCR ζ^{dim} T cells (green arrows, J). For details, please see <http://atvb.ahajournals.org>

Figure 2. CD3⁺CD4⁺TCR ζ^{dim} T cells (as percentage of CD3⁺CD4⁺T) were increased in ACS compared with CSA and controls (Kruskall-Wallis and Dunn's test). Dashed lines show median, continuous lines show 25th and 75th percentiles (A). Circulating CD4⁺TCR ζ^{dim} T cells in ACS (n=17) persisted increased after a 50-day median time follow-up (Wilcoxon test) (B).

Figure 3. Patients with ACS and CRP \geq 2 mg/l displayed a significantly increased percentage of CD3⁺CD4⁺TCR ζ^{dim} T cells compared to patients with CRP<2 mg/l (Mann-Whitney *U* test was used). Dots represent individual patient data; dashed lines show median value and continuous lines show 25th and 75th percentiles.

Figure 4. CD4⁺TCR ζ^{dim} T correlate with CD4⁺CD28^{null}T cells (as percentage of CD3⁺CD4⁺T) (Spearman's test, A). In ACS CD4⁺CD28^{null}T display reduction of TCR ζ -chain, as compared to CSA and controls (Kruskall-Wallis and Dunn's test) Dashed and continuous lines show median, 25th and 75th percentiles (B). ACS representative dot plots (C)(circled in A, B).

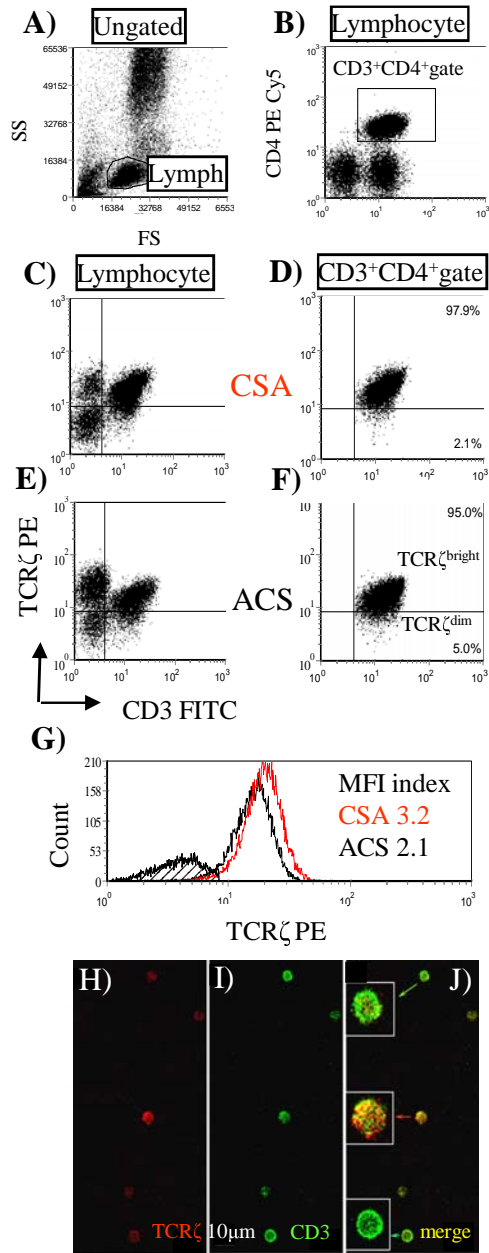
Figure 5. Results comparing $\text{TCR}\zeta^{\text{bright}}$ with $\text{TCR}\zeta^{\text{dim}}$ T-cell migratory ability are expressed as the percentage of cells migrating relative to the total number of each cell subset added to the transwell ($\text{TCR}\zeta^{\text{bright}}$ in the lower chamber at T=24hr/ $\text{TCR}\zeta^{\text{bright}}$ at T=0 vs $\text{TCR}\zeta^{\text{dim}}$ in the lower chamber T=24hr / $\text{TCR}\zeta^{\text{dim}}$ at T=0).

	Controls	CSA	ACS
n	42	32	66
Age - means (\pmSD)	57 (\pm 16)	65 (\pm 11)	63 (\pm 10)
Male sex – n (%)	33 (79)	27 (84)	55 (83)
Risk factors – n (%)			
Hypertension	20 (48) [†]	23 (72)	49 (74)
Smoking	3 (7)*	15 (47)	24 (36)
Diabetes	0 (0)	4 (13)	4 (6)
Hypercholesterolemia	14 (44)	14 (64)	27 (51)
Laboratory parameters (admission)			
- medians (interquartile ranges)			
White blood cell count ($\times 10^9/l$)	6.3 (5.6-7.9)*	7.2 (6.4-9.3)	9.2 (6.9-11.9)
Troponin I (ng/ml)	0	0	0.4 (0.0-5.9)
Angiographic characteristics			
N° of diseased vessels (means (\pm SD))	0 [†]	2 (\pm 0.8)	1.9 (\pm 0.9)
Medication on admission – n (%)			
β -Blockers	2 (5)*	19 (59) [†]	19 (29)
Aspirin	3 (7)*	29 (93)*	32 (48)
ACE inhibitors	5 (12) [‡]	13 (41)	17 (26)
Statins	2 (5)*	18 (56)*	12 (18)

* <0.001; [†]<0.01; [‡]<0.05 vs ACS

Table 1. Clinical Characteristics and Biological Parameters of Patients and Healthy Individuals.

Figure 1



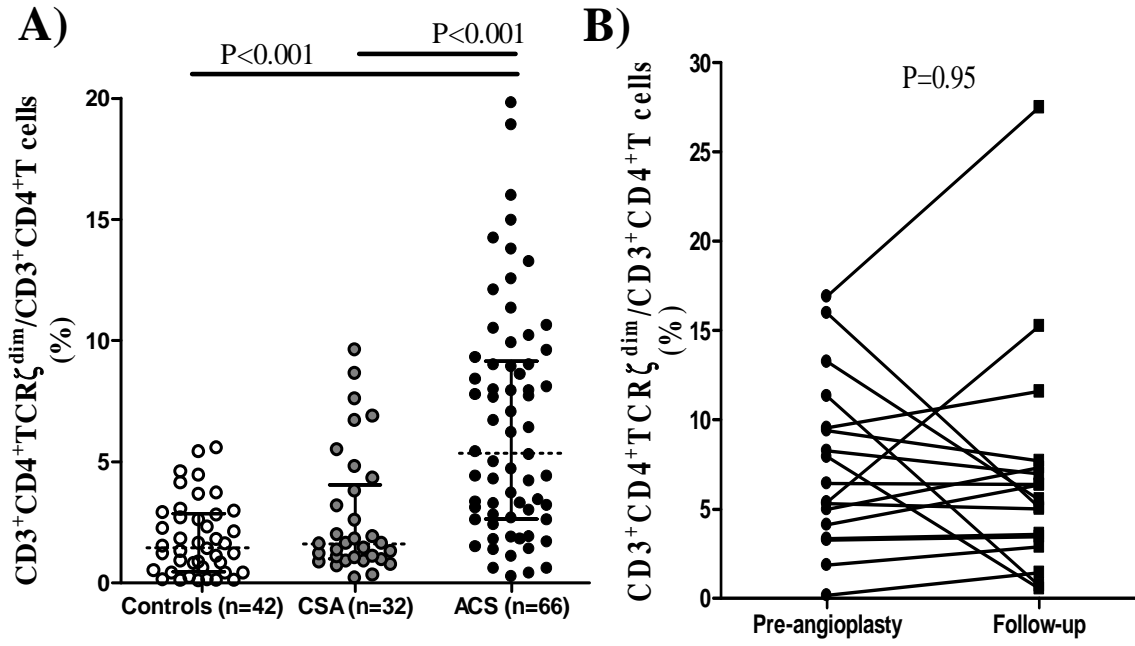


Figure 2

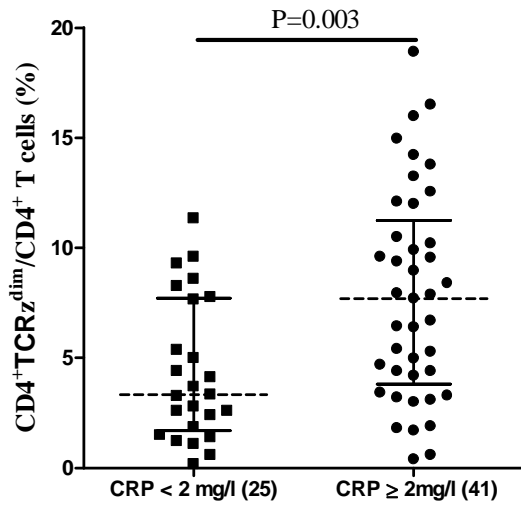


Figure 3

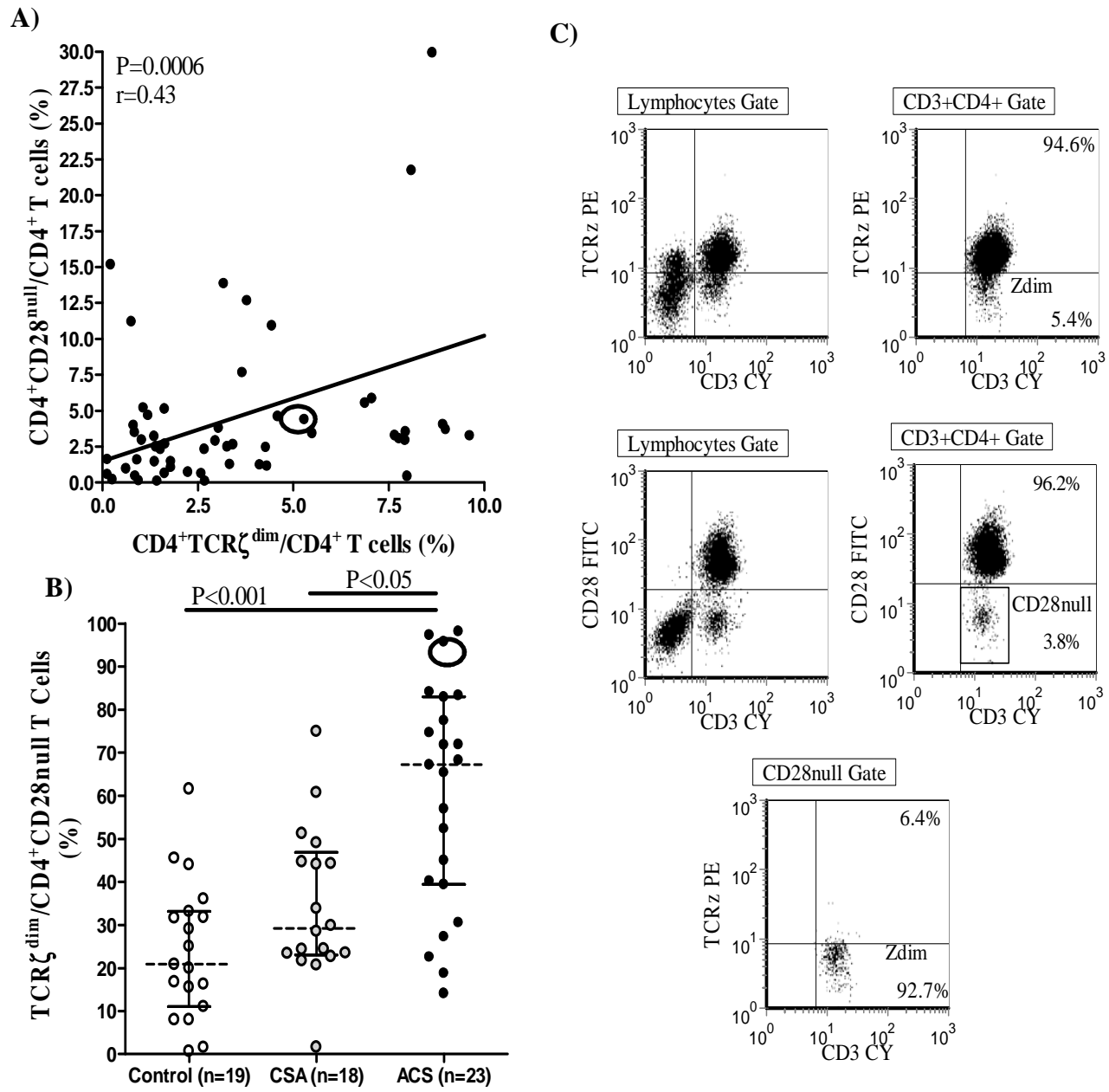


Figure 4

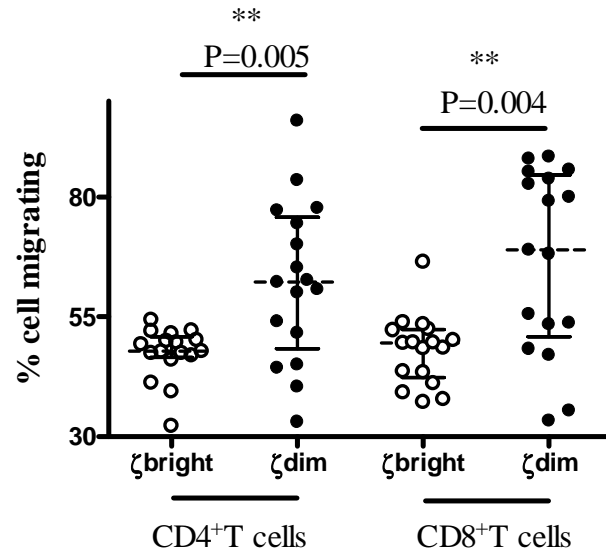


Figure 5