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BASIC SCIENCE

Clonal restriction and predominance of regulatory T cells in coronary thrombi of patients with acute coronary syndromes

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Aims Regulatory T cells (Treg) exert anti-inflammatory and atheroprotective effects in experimental atherosclerosis. Treg can be induced against specific antigens using immunization strategies associated with clonal restriction. No data exist on Treg in combination with clonal restriction of T cells in patients with acute coronary syndromes (ACS).

Methods and results Among T cell subsets characterized by flow cytometry, Treg ($CD4^+$ $CD25^+$ $CD127^{low}$) were twice as frequent in coronary thrombi compared with peripheral blood. Treg prevailed among T cell subsets identified in coronary thrombi. To evaluate clonal restriction, genomic DNA was extracted from coronary thrombi and peripheral blood in order to evaluate T cell receptor (TCR) β chain diversity by means of Multi-N-plex PCR using a primer specific for all TCR β V gene segments and another primer specific for TCR β J gene segments. T cell receptor diversity was reduced in thrombi compared with peripheral blood (intra-individual comparisons in 16 patients) with 8 gene rearrangements in the TCR common in at least 6 out of 16 analysed coronary thrombi. Compared with age-matched healthy controls ($n = 16$), TCR diversity was also reduced in peripheral blood of patients with ACS; these findings were independent of peripheral T cell numbers.

Conclusion We provide novel evidence for a perturbed T cell compartment characterized by clonal restriction in peripheral blood and coronary thrombi from patients with ACS. Our findings warrant further studies on Treg as novel therapeutic targets aimed at enhancing this anti-inflammatory component of adaptive immunity in human atherothrombosis.

Keywords Acute coronary syndromes • Immunity • T cells

Introduction

Atherothrombosis subsequent to plaque rupture or erosion with prominent features of inflammation constitutes the underlying pathophysiology in patients with acute coronary syndromes (ACS). However, its triggers remain poorly understood and not all rupture-prone plaques culminate in atherothrombosis.[1–3] We have previously identified monocytes/macrophages as the most abundant inflammatory cell type of innate immunity in coronary atherothrombosis, co-expressing Toll-like receptor (TLR)-4.[4] Although less abundant

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in number, T cells orchestrate the antigen-specific (adaptive) immune response in atherogenesis with prominent effects mediated by distinct T cell subsets throughout the stages of atherosclerosis, including ACS.[2,3,5–8]

Among T cell subsets, regulatory T cells (Treg) exert atheroprotective effects and constitute an inherent anti-inflammatory component of adaptive immunity.[5–9] Identifying a novel mechanistic link between Treg immunity and lipid metabolism, we recently demonstrated that selective depletion of Treg (Foxp3$^+$) impacted on lipid metabolism mediating hypercholesterolaemia and increased atherosclerotic lesions.[10] Furthermore, we and others showed that atheroprotective Treg can be induced in response to immunization with antigen specificity for the apolipoprotein B-100 peptide used in the vaccine.[11–13] In humans, Treg were found in increased numbers in lipid-rich, advanced plaques, whereas reduced numbers of circulating Treg were found in patients with ACS.[14–18] From a clinical perspective, Treg may be an attractive therapeutic target in atherosclerosis due to their anti-inflammatory effects. However, a better understanding of the role of Treg in ACS is necessary.

T cells orchestrate adaptive immunity to mediate an antigen-specific immune response. Each cell expresses several copies of a single antigen receptor with a unique antigen-binding site. The great variety of antigen specificities in the T cell receptor (TCR) repertoire is generated in the thymus by random recombination of separate inherited TCR α/β gene segments termed V(D)J which encode the variable parts of the heterodimeric receptor. T cell receptor diversity is confined to the complementarity determining regions (CDRs) as the binding site for a peptide presented on the major histocompatibility complex by an antigen-presenting cell (i.e. dendritic cell).[19] Recent advances in multiplex assay technology made it possible to measure usage frequencies of gene segments V(D)J and enable detection and tracking of specific TCR sequences expressed by clonally restricted T cells. Compared with spectratyping which employs only a limited set of V and J primers, this method enables a more comprehensive analysis that avoids bias due to transcriptional regulation.[20,21]

Evidence for an antigen-specific local immune response carried by T cells in unstable atherosclerotic plaque mandates clonal restriction of T cells when compared with peripheral blood. Initial reports on T cells isolated from human atherosclerotic plaques showed a high TCR diversity indicating a polyclonal origin of T cells.[22,23] In circulating CD4$^+$ CD28^{null} T cells from patients with unstable angina compared with patients with stable coronary artery disease, clonal restriction of T cells was identified using spectratyping.[24] Clonal restriction of T cells was demonstrated using spectratyping of atherectomy specimens from coronary plaque compared with peripheral blood mononuclear cells (PBMCs) from patients with ACS and only to a lesser extent in patients with stable coronary artery disease.[25] Similarly, clonal restriction was demonstrated when comparing carotid plaques with PBMC from asymptomatic and symptomatic patients with >90% carotid artery stenosis.[26]

However, so far T cell diversity in thrombi of ACS patients has not been investigated. The aims of this study, therefore, were (i) to compare Treg counts in coronary thrombi with PBMC from patients with ACS, (ii) to compare TCR diversity in coronary thrombi with PBMC from patients with ACS, (iii) to compare TCR diversity in PBMC from age-matched healthy subjects with patients with ACS.

Methods

Characteristics of patients and healthy subjects

Sixteen patients referred to the University Hospital Zurich, Switzerland, for coronary angiography with the diagnosis of ACS between 08/2010 and 11/2011 were enrolled as part of a larger cohort study (SPUM-ACS, clinical trial number NCT01000701). All patients aged 18 years and older presenting within 72 h after pain onset with the main diagnosis of ST-elevation myocardial infarction (STEMI) or non-ST elevation myocardial infarction (NSTEMI) were included in the study. Patients had symptoms compatible with angina pectoris (chest pain, dyspnoea) and at least one of the following inclusion criteria: (i) persistent ST-segment elevation or depression, T inversion or dynamic ECG changes, new LBBB; (ii) evidence of positive Troponin based on high sensitive TnT measurement (≥14 ng/L) with rise and/or fall in serial TnT levels. Exclusion criteria comprised documented active autoimmune disease or neoplasm; stent thrombosis; inability to comprehend study, less than 1 year of life-expectancy (for non-cardiac reasons). The Gensini score[27] was calculated by two interventional cardiology fellows (inter-observer variability = 17.4) for each of the 16 patients using the formula severity score × segment location multiplying factor × collateral adjustment factor to determine the extent and severity of coronary artery disease in the epicardial coronary arteries. In seven patients, the Gensini score differed by more than 10 points based on a different interpretation of collateral flow/total occlusion and an expert opinion was consulted from a third experienced senior interventional cardiologist.

Samples from 16 healthy subjects were obtained from a larger cohort study (SuSa Study, AFSSAPS clinical trial number 2010- A00428-31, ImmunID) that were recruited between between 07/2010 and 12/2011 at Optimed Clinical Research, Lyon, France. Individuals were included following a detailed medical history, physical exam with vital signs, and blood draw. Exclusion criteria comprised autoimmune disease or neoplasm, inability to comprehend study, treatment with corticosteroids or other immunomodulatory therapy, or vaccination in the three preceding months. All individuals were older than 18 years of age with birth control in place for at least 2 months prior to inclusion in women of childbearing age in the absence of pregnancy or breast feeding. Informed consent was obtained from all individuals with the approval by the Kantonale Ethik-Kommission Zurich, Switzerland (EK-1688), for patients with ACS and the Comité de Protection des Personnes CPP Sud-Est III, France (Numero EudraCT : OL039/Susa), for healthy subjects.

Analyses of blood and coronary thrombi

Laboratory parameters were measured using standard protocols. Individual estimated glomerular filtration rate was calculated using the CKD-EPI formula[28] based on serum creatinine, gender, and ethnicity. Based on the published trials,[29,30] coronary thrombi were aspirated using an Export catheter (Medtronic, Tolochenaz, Switzerland) at the site of coronary occlusion from patients with ACS undergoing primary percutaneous coronary intervention (PCI) and were immediately immersed in phosphate-buffered saline (PBS)-containing vials. Peripheral blood was sampled from the inguinal arterial sheath from the same patient and stored in citrate vials. Thrombi and the corresponding peripheral blood were treated with 50 μL tissue plasminogen activator (0.001% Actilyse®; Boehringer Ingelheim Pharma GmbH & Co., Ingelheim, Germany) to remove fibrin in 1 mL 1% Accutase® (PAA Laboratories, Pasching, Austria), and rotated for 2 h at 37°C. Thrombi and blood were then gently dissociated through a 40 μm pore-size cell strainer (BD *Falcon*™) with a rubber syringe plunger and rinsing with ice-cold PBS. Half of the cell suspensions were separated for Ficoll-paque centrifugation and subsequent ImmunTraCkeR β® analysis (ImmunID,

Grenoble, France), whereas remaining cells were analysed by flow cytometry. In healthy subjects, PBMCs were separated from whole blood (5 mL EDTA) with a density gradient tube (Uni-Sep, Novamed, Jerusalem, Israel). Peripheral blood mononuclear cells were prepared and stabilized in Easy'ID® stabilization solution provided by ImmunID, Grenoble following a standard operating procedure.

Analysis of T cell receptor diversity

Human TCR diversity was measured using ImmunTraCkeRβ® tests (ImmunID Technologies, Grenoble, France) and performed as described.[31] Peripheral blood mononuclear cells and thrombus samples were frozen and analysed by ImmunID laboratories. Genomic DNA was extracted using standard techniques and Multi-N-plex PCR was performed using an upstream primer specific for all functional members of a given T cell receptor β V segment and a downstream primer specific for a given T cell receptor β J segment (international ImMunoGeneTics information system, www.imgt.org) encoding for the CDR 3 in the human TCR β chain. This assay allows the simultaneous exhaustive detection of V(D)J rearrangements in the same reaction expressed as percentage based on the detected V(D)J combinatorial diversity divided by the expected maximal diversity (276 gene segments) which corresponds to the theoretical combinatorial diversity obtained with all rearrangements of all functional V(D)J genes within the genome. Each Vx–J1, J2, J3, J4, Jn product was separated as a function of its size. Constel'ID® software (ImmunID Technologies) was used for further analytical studies, including generation of three-dimensional repertoire illustration. Numeration and diversity of T cells is presented together through NDL® scoring which enables to determine a correlation between lymphocyte counts and T cell diversity. A decreased percentage (i.e. a decreased TCR diversity) defines a state of divpenia (www.divpenia.com). Normal values for TCR β-chain combinatorial diversity were defined based on a cohort of 16 healthy volunteers extracted from a larger study (SuSa Study, AFSSAPS clinical trial number 2010- A00428-31, ImmunID; age range, 42.8–74.9 years; 6 females and 10 males).

Flow cytometry

For flow cytometry, thrombus and peripheral blood-derived cell suspensions obtained from patients with ACS were centrifuged at 200G or 10 min and resuspended in 1 mL of ice-cold FACS buffer (PBS, 1% foetal calf serum, 0.05% EDTA) with FcR block (Human TruStain FcX, Biolegend, San Diego, CA, USA). Cells were labelled for 1 h at 4°C with monoclonal antibodies for extracellular staining of CD3, CD4, CD8, CD28, and a three-color reagent (CD4, CD25, CD127) for identification of Treg (all from BD Biosciences, San Jose, CA, USA) and analysed on a FACScalibur (BD Biosciences). Subpopulation analyses were performed using SSC/FSC scatters, differentiating lymphocyte, monocytes, and granulocyte gates (quadrant analysis). Mouse IgG1 was used as isotype control and the proportion of positive cells per lymphocyte gate were determined.

Statistical analysis

For comparisons of continuous and categorical data, we used the non-parametric Mann–Whitney *U*-test and the Pearson Chi-square test, respectively. Intra-individual comparisons of distributions of combinatorial diversity and percentages of cell counts are the result of Wilcoxon signed rank test for paired data. Unless otherwise indicated, data are shown as mean ± SEM or relative and absolute frequencies. Pearson's correlation coefficient was determined for the Gensini score (mean) and TCR diversity in PBMC and thrombi, respectively. A two-sided *P*-value of <0.05 was considered significant.

Results

Clinical characteristics of patients with acute coronary syndromes and healthy subjects

Pairs of coronary thrombi and peripheral blood were obtained from 16 patients with ACS using an aspiration catheter as part of the PCI for a native coronary culprit lesion. Among these, 12 patients were diagnosed with STEMI, 4 patients with NSTEMI, 11 patients were symptomatic for <24 h, 4 patients had symptoms for 24–48 h and 1 patient for 48–72 h. For the evaluation of TCR β chain diversity in peripheral blood, 16 patients with ACS were compared with 16 healthy age-matched subjects. The clinical, demographic, and laboratory characteristics of patients with ACS and matched healthy subjects are summarized in *Table 1*. Compared with healthy subjects, patients with ACS

Table 1 Clinical, demographic, and laboratory characteristics of patients and healthy subjects

	ACS	Healthy	*P*-value
Age (years)	58.9 ± 2.3	58.7 ± 2.3	n.s.
Male gender (%)	15 (94)	10 (63)	n.s
Caucasian ethnicity (%)	16 (100)	16 (100)	n.s.
Leucocyte count ($10^3/\mu L$)	11.6 ± 1.5	5.8 ± 0.3	$P \leq 0.001$
Lymphocyte count ($10^3/\mu L$)	3.2 ± 1.2	1.6 ± 0.08	n.s.
Erythrocytes ($10^6/\mu L$)	4.3 ± 0.2	4.5 ± 0.1	n.s.
Platelets ($10^3/\mu L$)	279 ± 25	219 ± 8	$P < 0.05$
Total cholesterol (mmol/L)	5.1 ± 0.4	5.6 ± 0.3	n.s.
Baseline glucose (mmol/L)	9.0 ± 1.7	5.2 ± 0.08	$P \leq 0.01$
Serum creatinine (μmol/L)	79.9 ± 6.3	72.1 ± 2.7	n.s.
eGFR (mL/min)	90.1 ± 4.9	92.3 ± 2.4	n.s.
BMI (kg/m^2)	28.4 ± 2.0	24.6 ± 1.1	n.s.
Gensini Score	39.7 ± 5.7	0	$P \leq 0.001$
Cardiovascular risk factors			
Hypercholesterolaemia	8 (50)	0	$P \leq 0.01$
Hypertension (%)	4 (25)	0	$P < 0.05$
Diabetes (%)	2 (13)	0	n.s.
Smoking (current, %)	7 (44)	0	$P \leq 0.01$
Previous medical history			
Previous MI	3 (19)	0	n.s.
Previous PCI	2 (13)	0	n.s.
Medications			
Aspirin (%)	16 (100)	0	$P \leq 0.001$
Statins (%)	4 (25)	0	$P < 0.05$
ACE-I/ARB (%)	2 (13)	0	n.s.
β-Blockers (%)	3 (19)	0	n.s.
Nitrates (%)	15 (94)	0	$P \leq 0.001$
Calcium antagonists (%)	0	0	–
Diuretics (%)	1 (6)	0	n.s.

Values are shown as mean ± SEM or absolute and relative frequencies (in brackets with respect to $n = 16$ patients), respectively.
$N = 12–16$; n.s. non-significant. ACE-I/ARB, angiotensin converting enzyme-inhibitor/angiotensin receptor blocker; aspirin comprises oral and intravenous formulation; BMI, body mass index; eGFR, estimated glomerular filtration rate; MI, myocardial infarction; PCI, percutaneous coronary intervention.

had increased leucocyte and platelet counts, elevated glucose concentration in peripheral blood, presence of coronary artery disease, cardiovascular risk factors, and received medications.

Increased Treg counts in coronary thrombi compared with peripheral blood in patients with acute coronary syndromes

To evaluate T cell subset distribution, flow cytometry was performed for phenotypic characterization of coronary thrombi compared with PBMC from patients with ACS. Overall $CD3^+$ T cell content was unaltered and no difference was detected for T helper cells ($CD4^+$ $CD3^+$ $CD8^-$), but for a trend to numerical reduction vs. peripheral blood (*Figure 1A* and *B*). In contrast, Treg ($CD4^+$ $CD25^+$ $CD127^{low}$) were significantly increased by 2.2-fold in coronary thrombi (*Figure 1C*). Cytotoxic T cells ($CD8^+$ $CD3^+$ $CD4^-$), $CD4^+$ $CD3^+$ $CD28^{null}$ T cells and double negative ($CD4^-$ $CD8^-$ $CD3^+$) T cells were found in similar numbers in coronary thrombi and PBMC (*Figure 1D–F*). Supplementary material online, *Figures S1–S6* show representative graphs on how FACS analysis for individual cell subsets was performed. Supplementary material online, *Figure S7* shows a representative immunostaining of $CD3^+$ T cells.

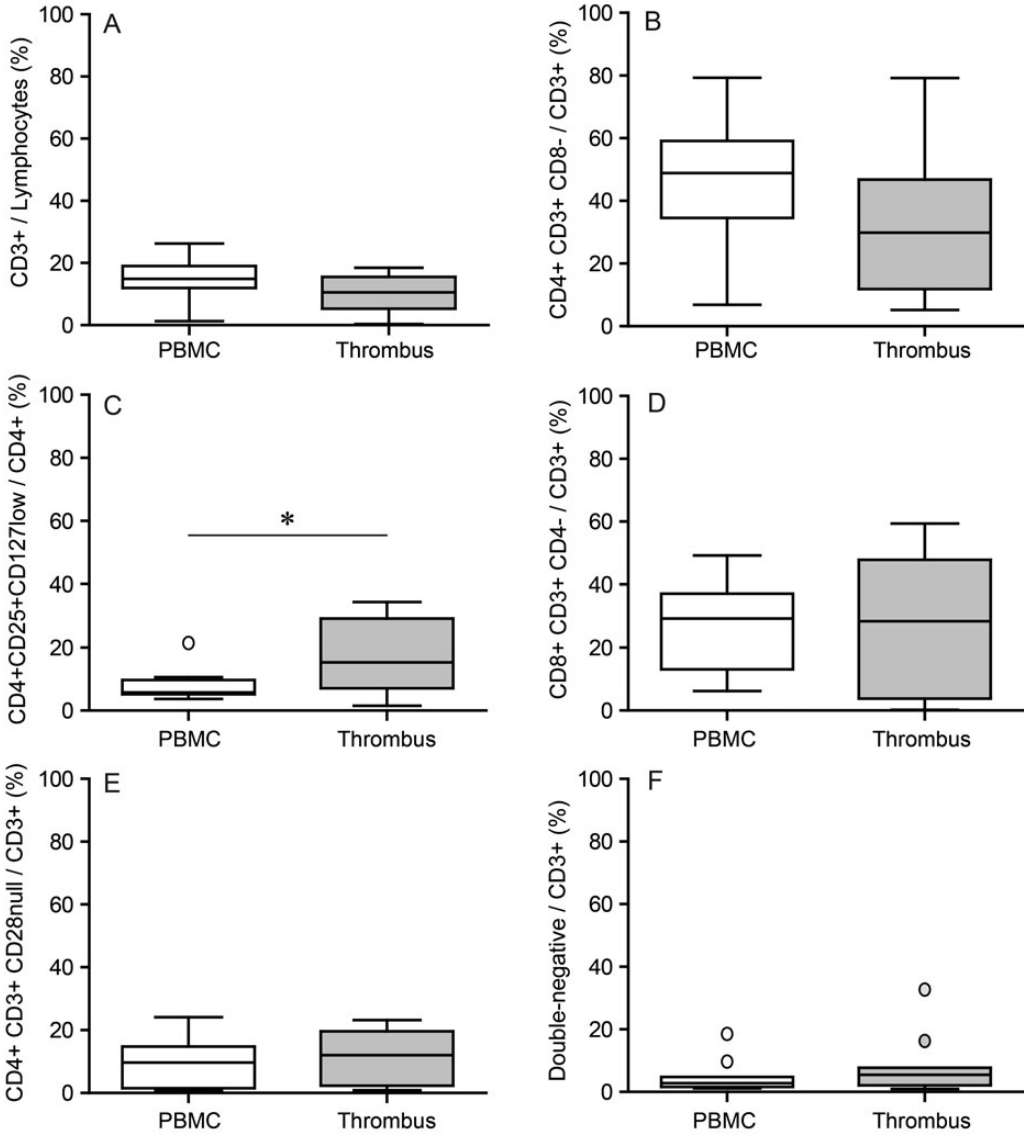

Figure 1 Treg as prominent T cell subset in coronary thrombi vs. peripheral blood from patients with ACS. Flow cytometry of coronary thrombus and PBMC from patients with ACS. Double-staining for T cell subtypes (CD3 with CD8 or CD4, respectively). (*A*) T cells ($CD3^+$). (*B*) T helper cells ($CD4^+$ $CD3^+$ $CD8^-$). (*C*) Regulatory T cells ($CD4^+$ $CD25^+$ $CD127^{low}$). (*D*) Cytotoxic T cells ($CD8^+$ $CD3^+$ $CD4^-$). (*E*) $CD4^+$ $CD3^+$ $CD28^{null}$ T cells. (*F*) Double-negative T cells ($CD4^{null}$ $CD8^{null}$ $CD3^+$). The bottom and top of the box represent the first and third quartiles (Q1, Q3), and the band inside the box is the median. The whiskers extend to the most extreme data point which is no more than 1.5 times the interquartile range (IQR = Q3 − Q1) from the box, with individual outliers shown beyond the whisker; $n = 9–12$; $^*P = 0.0098$.

Decreased T cell receptor diversity in coronary thrombi compared with peripheral blood in patients with acute coronary syndromes

Pairwise intra-individual comparisons of coronary thrombi demonstrated a markedly reduced TCR diversity in coronary thrombi vs. PBMC (reduction by 0.28-fold), shown in *Figure 2A–C*. Among V(D)J gene segments identified in coronary thrombi from 16 patients with ACS, 8 rearrangements were found in common in at least 6 thrombi from individual patients (*Table 2*). These gene rearrangements are Vbeta18-J2.3, which is the most frequent (8/16); Vbeta05-J2.7 and Vbeta25-J2.6, both found in common in 7/16 samples; Vbeta27-J2.7, Vbeta24-J2.3, Vbeta24-J2.5, Vbeta15-J2.4, and Vbeta19-J2.5 were found in common in 6/16 samples (IMGT nomenclature). In turn, in peripheral blood from patients with ACS, Vbeta05-J2.7 was the most frequently detected gene rearrangement (13/16). Interestingly, Vbeta05-J2.7 was less common in coronary thrombi compared with PBMC (*Table 2*). T cell receptor diversity was only weakly correlated with the extent and severity of epicardial coronary artery disease as assessed by the Gensini score (Supplementary material online, *Figure S8*). Pearson's correlation coefficient for the comparison of the Gensini score (mean) with TCR diversity in PBMC was −0.418 and −0.058 for the comparison of the score with TCR diversity in coronary thrombus, respectively. These associations were not significant.

Reduced T cell receptor diversity in circulating T cells of patients with acute coronary syndromes compared with healthy subjects

T cell receptor diversity was measured using genomic DNA isolated from PBMCs from 16 healthy subjects and PBMCs from 16 patients with ACS. Compared with matched healthy subjects, patients with ACS had a profoundly reduced TCR diversity in peripheral blood (reduction by 0.36-fold), shown in *Figure 3A–C*. Importantly, reduced TCR diversity did not correlate with overall lymphocyte cell

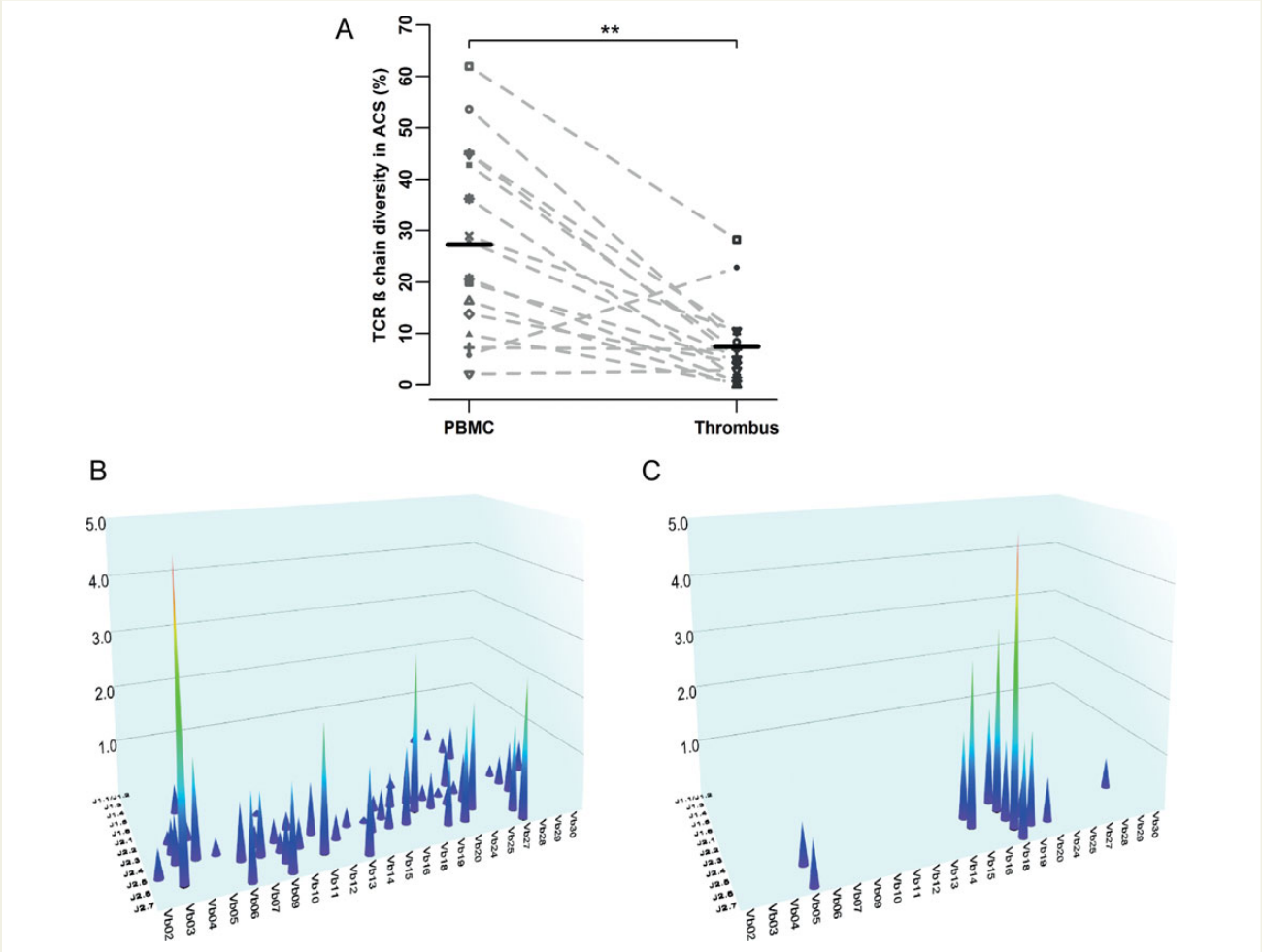

Figure 2 Reduced T cell receptor diversity in coronary thrombus vs. peripheral blood from patients with ACS. Intra-individual comparisons of TCR diversity expressed as percentage of 276 possible V(D)J gene segment rearrangements in the human TCR β chain (hTRB) in coronary thrombus vs. PBMC (*A*). Bars indicate means; $^{**}P = 0.001$. Representative 3-D plots of human TCR β chain diversity in an individual patient with ACS derived from PBMC (*B*) and coronary thrombus (*C*).

Table 2 T cell receptor β gene segment rearrangements in patients with an acute coronary syndrome

V-J rearrangement	PBMC	Thrombus	*P*-value
Vbeta18-Jβ2.3	7 (44)	8 (50)	n.s.
Vbeta05-Jβ2.7	13 (81)	7 (44)	$P = 0.028$
Vbeta25-Jβ2.6	3 (19)	7 (44)	n.s.
Vbeta27-Jβ2.7	11 (69)	6 (38)	n.s.
Vbeta24-Jβ2.3	10 (63)	6 (38)	n.s.
Vbeta24-Jβ2.5	8 (50)	6 (38)	n.s.
Vbeta15-Jβ2.4	6 (38)	6 (38)	n.s.
Vbeta19-Jβ2.5	3 (19)	6 (38)	n.s.
Vbeta04-Jβ2.3	9 (56)	5 (31)	n.s.
Vbeta05-Jβ2.5	8 (50)	5 (31)	n.s.
Vbeta24-Jβ2.4	7 (44)	5 (31)	n.s.
Vbeta20-Jβ2.6	3 (19)	5 (31)	n.s.

All values are shown as absolute and relative frequencies (in brackets with respect to $n = 16$ patients), respectively. n.s., non-significant by the Pearson Chi-square test.

number in peripheral blood from patients with ACS and healthy subjects (*Figure 3D*).

Discussion

The present study confers the following key findings: first, we identified Treg as the major T cell subset in aspirated coronary thrombus adjacent to the culprit lesion in patients with ACS. Second, we found a restricted TCR diversity within coronary thrombi compared with peripheral blood of patients with ACS. Third, we show a markedly reduced TCR diversity also in peripheral blood of patients with ACS compared with healthy age-matched subjects.

We previously showed an increased immune response at the site of coronary occlusion in patients with ACS compared with peripheral blood, demonstrating elevated levels of serum amyloid A, interleukin (IL)-6,[32] myeloid-related protein 8/14,[33] and expression of TLR-4 on monocytes as part of innate immunity.[4] The current study expanded these previous findings to T cells. Analysis of adaptive immunity carried by T cell subsets in patients with ACS was performed by comparing coronary thrombi with peripheral blood intra-individually and peripheral blood from healthy subjects.

Treg were reported in increased numbers in lipid-rich, advanced plaques, whereas reduced numbers of circulating Treg were found in patients with an ACS.[14–18] This decrease in the circulating Treg pool is currently unclear as it may be due to either a global Treg defect or increased redistribution between the blood and local sites of inflammation.[34] Interestingly, the latter study[18] found reduced circulating Treg in patients with unstable angina, but increased circulating Treg in patients with acute myocardial infarction (AMI). This may be attributable to insufficient Treg recruitment to the site of the culprit lesion in patients with AMI unlike in unstable angina where sufficient influx of Treg is maintained to control the inflammatory reaction in the lesion.[34] In contrast, we herein demonstrated a 2.2-fold increase in Treg counts in coronary thrombus adjacent to the ruptured plaque compared with peripheral blood, suggesting efficient redistribution of the circulating Treg pool to inflammatory sites in patients with ACS. Furthermore, we found 8 TRB VJ rearrangements common in at least 6 of the 16 analysed thrombus samples. Treg accumulation in non-lymphoid tissues (coronary thrombus in our study) is shaped by several mechanisms including migration and retention of circulating Treg as well as expansion of Treg clones specific for tissue-specific antigens.[35] The local chemokine and cytokine milieu and expression of antigens specific for the tissue promote chemotaxis and clonal expansion of Treg. Temporal changes in these factors may impact on the amount of Treg detected by flow cytometry in our study. It is possible that the increase in Treg in coronary thrombi found in the current study reflects a local compensatory response to attenuate inflammation in the surrounding pro-inflammatory milieu characterized by elevated concentrations of pro-inflammatory cytokines in coronary blood distal to the occluding coronary thrombus.[4] However, intra-individual comparison of TCR diversity in coronary thrombus vs. peripheral blood was reduced in all but three patients in the present study (*Figure 2A*), demonstrating a consistent pattern of clonal restriction in thrombus-resident T cells, the majority of which are likely Treg. T cell receptor diversity both in peripheral blood and coronary thrombi, respectively, was not associated with the extent and severity of coronary artery disease in our study as assessed by the Gensini score. This finding suggests that the observed restricted TCR diversity in coronary thrombi may reflect differential trapping of antigen-primed T cells from the circulating T cell pool, unrelated to the burden of underlying atherosclerotic disease. To provide definitive cues to the origin of Treg in coronary thrombi, future work should analyse Treg in atherothrombosis using a suitable experimental model such as the DEREG mouse model[10] to track labelled Treg in secondary lymphoid organs, atherosclerotic lesions, and thrombus, respectively. Along those lines, our data are in favour of a *post hoc* alteration of T cell subsets as a consequence of the ACS rather than a predisposing factor reflecting an *a priori* immune imbalance.

The concept of expanding antigen-specific Treg to diminish vascular inflammation and prevent atherothrombotic clinical events by immunotherapy[7] is appealing. Using an immunization strategy, we and others identified antigen-specific Treg as a critical component of atheroprotection in mice.[11–13] However, more data on the origin, recruitment, and kinetics of Treg during myocardial infarction are needed before such an approach can be evaluated in a clinical trial. The recently completed phase II GLACIER study (NCT01258907) reported no change in inflammatory activity in an index arterial vessel after 12 weeks of treatment with a monoclonal antibody targeting oxidized forms of LDL compared with controls, as measured by FDG-PET/CT imaging ([18*F*]-2-deoxyglucose positron emission-tomography/computed tomography but showed a good safety profile. Pending full publication of the data, the choice of primary endpoint, treatment duration, and patients appear pivotal for future trials to address efficacy of immunotherapy in atherosclerotic disease.[7]

Limitations

We could not perform cell-sorting and subsequent repertoire analysis for TCR diversity in Treg only as coronary thrombi are very small with very few T cells present.

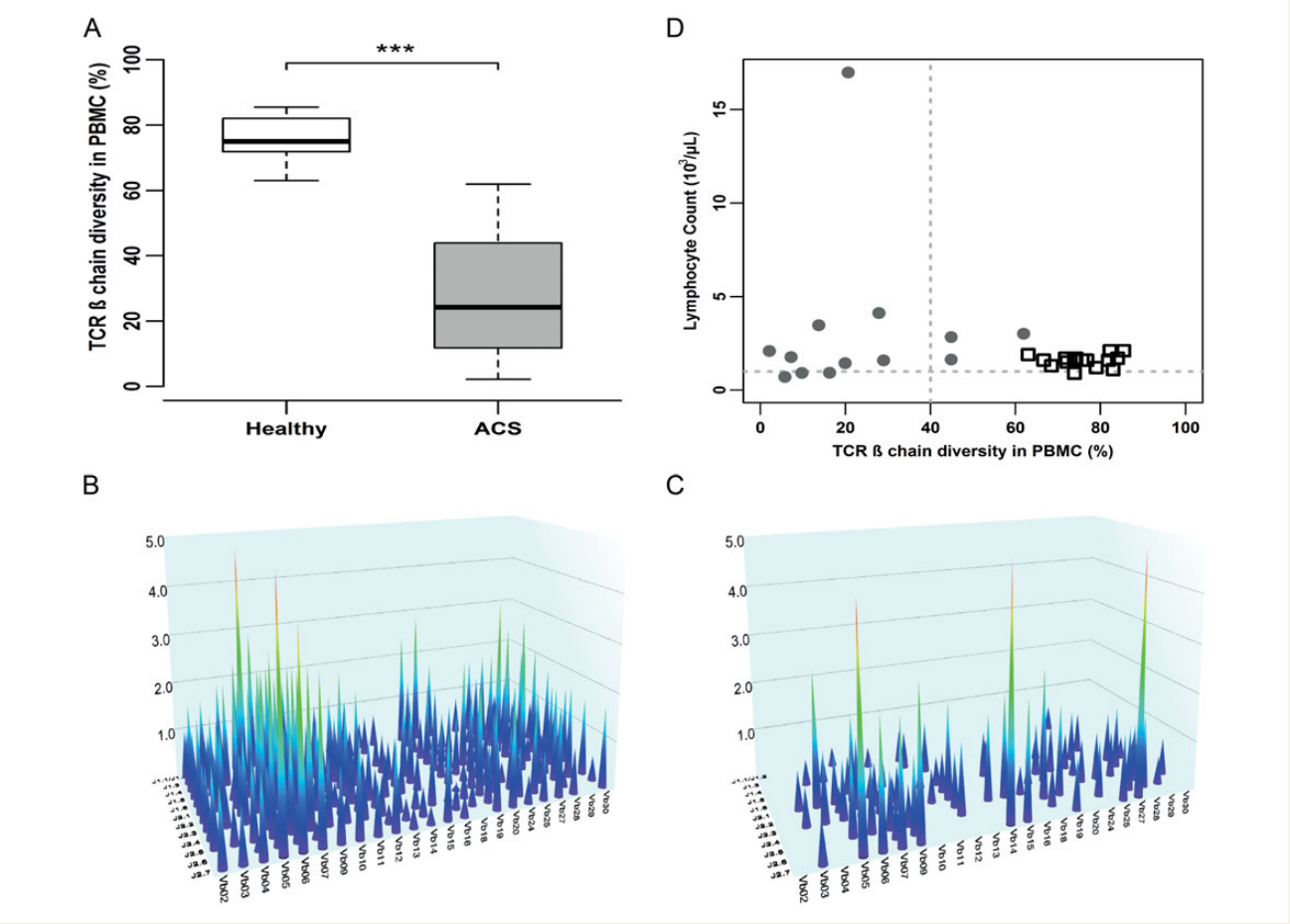

Figure 3 Reduced T cell receptor diversity in peripheral blood from patients with ACS vs. healthy subjects. Human TCR diversity expressed as percentage of 276 possible V(D)J gene segment rearrangements in the human TCR β chain (hTRB) measured in PBMCs from healthy subjects and patients with ACS (*A*). $N = 16$ in each group; $^{***}P < 0.0001$. The bottom and top of the box are the first and third quartiles (Q1,Q3), and the band inside the box is the median. The whiskers extend to the most extreme data point which is no more than 1.5 times the interquartile range (IQR = Q3 − Q1) from the box. Representative 3-D plots of hTRB diversity derived from PBMC from a healthy subject (*B*) and a patient with ACS (*C*). Graph linking peripheral lymphocyte count to human TCR diversity in the human TCR β chain (*D*). Individual samples are shown. PBMC from healthy subjects (open squares) and patients with ACS (grey circles).

Summary and conclusions

Our study is the first to report a restricted TCR diversity in a markedly increased cell pool of leucocytes in peripheral blood from patients with ACS when compared with age-matched healthy subjects. Together with the profound reduction in TCR diversity identified in coronary thrombi, these findings imply an antigen-specific immune response carried by T cells in patients with ACS. Interestingly, patients with rheumatoid arthritis also show a reduced TCR diversity in circulating T cells compared with healthy individuals.[36] Our finding adds to the shared features between rheumatoid arthritis and clinical atherosclerosis, suggesting similar autoimmune features in the pathogenesis of atherothrombosis.

In conclusion, we demonstrate a perturbed T cell compartment characterized by clonal restriction of T cells in both peripheral blood and coronary thrombi of patients with ACS. Our data provide novel evidence for antigen-specific adaptive immunity in atherothrombosis with Treg as the prominent T cell subset.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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Conflict of interest: A.G., A.C., and N.P. are employed by ImmunID, Grenoble, France.

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