Human Immunology 72 (2011) 553-557

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

$CD4^+CD25^{high}CD127^{low}$ regulatory T cells in patients with stable angina and their dynamics after intracoronary sirolimus-eluting stent implantation

Alexandra V. Potekhina [a,*], Sergey I. Provatorov [a], Vladimir O. Sokolov [a], Ekaterina A. Pylaeva [a], Valery P. Masenko [a], Elena A. Noeva [a], Nadezhda B. Kukhtina [a], Tatiana L. Krasnikova [a], Tatiana I. Arefieva [a]

[a] Russian Cardiology Research Center, Third Cherepkovskaya str., 15A, Moscow 121552, Russia

ARTICLE INFO

Article history:
Received 11 December 2010
Accepted 31 March 2011
Available online 14 April 2011

Keywords:
Regulatory T cells
$CD4^+CD25^{high}CD127^{low}$ T cells
Coronary stenting
Sirolimus (rapamycin)-eluting stent

ABSTRACT

Rapamycin contributes to the expansion of regulatory T cells (Tregs) *in vitro*. We investigated $CD4^+CD25^{high}CD127^{low}$ Treg level dynamics as well as the major parameters of cell immunity and sCD25 and highly sensitive C-reactive protein (hsCRP) concentrations in the blood of patients after coronary stenting (CS) with sirolimus (rapamycin)-eluting stents (SES; $n = 43$). The relation between initial Treg values and the severity of coronary atherosclerosis was observed. Treg and sCD25 levels were increased 1 month after CS versus baseline values and versus data in the control group (coronary angiography [CA], $n = 20$). A positive correlation between Treg and sCD25 levels was reported, whereas no relation was observed with the length of SES implanted. HsCRP level was increased during the first 7 days and returned to baseline values 1 month after CS/CA. Treg content is lower in patients with multivessel CAD. Elevated levels of Tregs and sCD25 after SES implantation might occur because of the immunomodulating effect of rapamycin.

1. Introduction

Coronary stenting (CS) has become a conventional therapeutic strategy for coronary artery disease (CAD). Stent implantation can effectively expand the lumen of the vessel, but an inflammatory reaction occurs in response to vessel wall injury, which can lead to the formation of restenosis. The use of drug-eluting stents results in a significant reduction in the incidence of restenosis [1]. Sirolimus (rapamycin)-eluting stents (SES) are among the most prevalently used stents for interventional cardiology. Rapamycin is a macrocyclic lactone antibiotic with immunosuppressive activity. Rapamycin inhibits the proliferation of fibroblasts, endothelial and smooth muscle cells, B lymphocytes, and most T-lymphocyte populations, which play a crucial role in the inflammatory process induced by stent implantation and consequently in the growth of neointima. An opposite action of rapamycin was demonstrated in a population of regulatory $CD4^+$ $FOXP3_+$ T lymphocytes (Tregs), contributing to their expansion in the culture of lymphocytes [2,3] and in the blood of patients after oral administration [4,5]. Several alternative intracellular signaling pathways in Tregs that interfere with the cell cycle arrest and apoptosis caused by rapamycin have been discovered [6].

Tregs are distinct from the T-helper 1 and 2 subsets of lymphocytes; they possess anti-inflammatory action and maintain self-tolerance by contact-dependent suppression or releasing of anti-inflammatory cytokines (interleukin [IL]-10 and transforming growth factor-β1) [7]. Tregs were reported in atherosclerotic plaques of carotid [8] and coronary arteries in humans [9]. Naturally occurring Tregs constitute 5 to 10% of $CD4^+$ T cells and are characterized by a high density of membrane expression of the IL-2 receptor (identified by α-subunit CD25) and low expression of the IL-7 receptor (identified by α-subunit CD127). A key marker of Tregs is the expression of forkhead helix transcription factor (FOXP3), which is required for their development and function [10]. In the context of human coronary atherosclerosis, CAD and its complications may relate to Treg disturbances [11–15].

A gradual release of rapamycin within 3 to 4 weeks after SES implantation suppresses the development of inflammatory reaction, migration, and proliferation of smooth muscle cells in the vessel wall and thus significantly slows neointimal growth [16].

In this study, we sought to investigate the dynamics of $CD4^+CD25^{high}CD127^{low}$ Tregs as well as the major parameters of cell immunity, including $CD4^+CD25^{low}CD127^{high}$ activated T effectors ($aCD4^+$) in the peripheral blood of patients after intracoronary SES implantation. Because both $aCD4^+$ and Tregs express the IL-2 receptor, we also estimated the dynamics of the plasma concentration of the soluble α-subunit of the IL-2 receptor (sCD25), which is shed from cell membranes because of proteolytic cleavage. Highly sensitive C-reactive protein (hsCRP) was evaluated as a marker of inflammatory reaction resulting from stent implantation.

* Corresponding author.
E-mail address: potehina@gmail.com (A.V. Potekhina).

0198-8859/11/$32.00 - see front matter
doi:10.1016/j.humimm.2011.03.015

2. Subjects and methods

2.1. Patients

This study was approved by the Institutional Ethics Committee. Written consent was obtained from each patient. We enrolled consecutive patients with stable CAD who faced the need for coronary angiography (CA) with possible one-stage SES implantation from March 2009 to September 2009. Of the 67 patients enrolled, 4 were excluded: 2 because of non-SES implantation and 2 because only balloon angioplasty was performed. The remaining 63 patients formed 2 groups: 43 patients underwent CS with SES implantation and 20 patients underwent CA only. CA was performed by the transfemoral approach using a standard technique. The results of all coronary angiograms were evaluated by 1 experienced interventional cardiologist who was single blinded to the study. The severity of coronary stenosis was assessed with a worst view projection. The percentage of luminal narrowing was recorded according to the American Heart Association reporting system [17]. The extent of coronary atherosclerosis was defined as coronary 1, 2, or 3 vessel disease corresponding to the number of affected main vessels with hemodynamic relevant stenosis greater than 50%. Lesions in the left main trunk were not observed in the present study. The clinical characteristics of the patients are summarized in Table 1. The exclusion criteria were myocardial infarction, surgery or major trauma, coronary stenting during past 6 months, acute coronary syndrome, left ventricle dysfunction with ejection fraction <40%, neoplasms, advanced liver disease, renal failure, infectious/inflammatory disease, and current use of immunosuppressive drugs.

2.2. Blood samples

Blood samples were obtained from all patients before CA/CS and at 24 and 48 hours, 7 days, and 1 and 3 months after the procedure. The main lymphocyte subpopulations (T cells, $CD4^+$ T-lymphocytes, $CD3^+CD8^+$ T-cytotoxic cells, natural killers [NK], B-lymphocytes and NKT cells) were assayed in 30 patients (15 in the CS group and 15 in the CA group) at all time points. Activated $CD4^+$ T cells and Tregs were studied in all 63 patients before and 1 month after CS/CA. The serum concentration of sCD25 was assayed in all 43 patients in the CS group at all time points and in all 20 patients in the CA group before CA/CS and 7 days and 1 month after the procedure. The serum level of hsCRP was measured in all patients at every time point.

Table 1
Clinical data of patients

Characteristics	CS ($n = 43$)	CA ($n = 20$)	p
Men, n (%)	38 (88.4)	16 (80)	0.38
Age (years)	61.1 ± 8.2	58.3 ± 8.3	0.22
Anamnesis of MI, n (%)	21 (48.8)	9 (45)	0.78
Hypertension, n (%)	31 (72.1)	17 (85)	0.26
Diabetes mellitus, n (%)	8 (18.6)	4 (20)	0.90
Obesity, body mass index > 30 kg/m^2, n (%)	14 (32.6)	6 (30)	0.84
Current smoking, n (%)	12 (27.9)	6 (30)	0.86
Previous CS, n (%)	9 (20.9)	6 (30)	0.43
Total cholesterol (mM)	4.83 ± 1.2	4.87 ± 1.22	0.90
Glucose (mM)	5.71 ± 0.95	5.78 ± 1.44	0.83
Leukocytes (mln/mL)	7.66 ± 2.33	7.12 ± 2.24	0.45
Number of atherosclerosis-affected coronary arteries, n (%)	one – 18 (41,7) two – 21 (48,8) three – 4 (9,3)	no – 5 (25) one – 7 (35) two – 5 (25) three – 3 (15)	0.11
Number of SES implanted, n (%)	one – 29 (67,4%) two – 9 (20,9%) three – 5 (11,6%)		
Medication			
β-blockers, n (%)	42 (97.7)	18 (98)	0.18
Aspirin, n (%)	43 (100)	20 (100)	1.0
Statins, n (%)	43 (100)	20 (100)	1.0
ACE inhibitors, n (%)	30 (69.8)	13 (65)	0.71
Calcium antagonists, n (%)	10 (23.3)	6 (30)	0.57

2.3. Flow cytometry

Whole blood from each patient was collected in a sodium citrate anticoagulated vacutainer tube. Samples were stained and fixed within 2 hours of collection. The following antibodies and reagents were used: CD3-FITC/CD19-PE, CD3-FITC/CD4-PE, CD3-FITC/CD8-PE, CD3-FITC/CD(16+56)-PE, CD4-FITC (Becton Dickinson Immunocytometry Systems, Heidelberg, Germany), CD25-PC5, CD127-PE (Beckman Coulter, High Wycombe, UK), and lysing and fixing solutions (Becton Dickinson Immunocytometry Systems). The samples were processed in accordance to the manufacturer's manuals. Total $CD3^+$ T cells, $CD19^+$ B cells, $CD4^+$ T-lymphocytes, $CD3^+CD8^+$ T-cytotoxic cells, CD3-$CD(16+56)^+$ NKT, and $CD3^+$ $CD(16+56)^+$ NK were measured. Tregs were typed as $CD4^+CD25^{high}$ as previously described [18]. For more appropriate typing of Tregs, staining with antibodies against CD127 was performed. Activated and regulatory T cells were identified as $CD4^+CD25^{low}CD127^{high}$ and $CD4^+CD25^{high}CD127^{low}$ as previously established [19,20]. Stained cells were analyzed with a FacsCalibur flow cytometer equipped with CellQuest software (Becton Dickinson Immunocytometry Systems).

2.4. hsCRP and sCD25 analysis

The hsCRP concentration was detected in serum by nephelometry (Behring nephelometer, Marburg GmbH). The sCD25 level in serum was measured by chemiluminescence using an Immulite 1000 (DPC–Siemens) analyst according to the manufacturer's manual.

2.5. Statistical analysis

Data in the tables are presented as means ± SD or as medians (25th–75th percentile) for normal and abnormal distributions, respectively. Wilcoxon W and Mann–Whitney U tests were used when comparing the data in small groups (from 30 patients after CS/CA) and for hsCRP data analysis. Student's t tests for dependent and independent variables were applied for intra- and intergroup comparisons, respectively. Spearman's correlation was used in case of abnormally distributed variables and Pearson's correlation was applied for normally distributed variables. The relationship between categorical variables was analyzed by χ^2 test. Differences were considered statistically significant at $p < 0.05$.

3. Results

Clinical and anamnestic characteristics, biochemical markers, and parameters of cell immunity determined before the interventions did not differ significantly in both (CS and CA) groups of patients (Tables 1–3).

No correlations were observed between the initial values of Tregs and CAD risk factors (age, sex, arterial hypertension, obesity, current smoking, total cholesterol, or hsCRP levels). We reported a negative correlation between the initial values of circulating Tregs in patients with atherosclerotic lesions in native coronary arteries (without anamnesis of previous percutaneous coronary interventions, $n = 48$) and the number of atherosclerosis-affected coronary vessels ($r = -0.42$, $p = 0.004$).

No changes in whole leukocytes, relative and absolute levels of lymphocytes, main lymphocyte subpopulations (T, B, and NK), NKT cells, and $aCD4^+$ were observed after CS/CA at all time points (Fig. 1B and Tables 2 and 3).

By exploring the dynamics of $CD4^+CD25^{high}CD127^{low}$ regulatory T-lymphocyte content in peripheral blood, we revealed a significant increase in Treg level on the 7th day with a peak 1 month