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Low Blood Content of IL-10-Producing CD4$^+$ T Cells as a Risk Factor for Progression of Coronary Atherosclerosis

A. Yu. Filatova, E. A. Pylaeva, A. V. Potekhina, N. Yu. Ruleva, E. A. Klesareva, N. V. Radyukhina, V. P. Masenko, A. M. Shchinova, E. A. Noeva, S. I. Provatorov, O. I. Afanas'eva, and T. I. Aref'eva

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In a 2-year prospective study, prognostic significance of the blood content of IL-10-producing CD4$^+$ T lymphocytes for progression of coronary artery atherosclerosis was assessed. Patients with verified stable angina (n=36) admitted for scheduled coronary angiography and coronary stenting were enrolled. The blood levels of CD4$^+$FoxpP3$^+$ Treg, CD4$^+$IFNγ$^+$ Th1, CD4$^+$IL17$^+$ Th17, CD4$^+$IL10$^+$ cells, sCD25, IL-10, IL-17, C-reactive protein, and lipoprotein (a) were assayed before endovascular interventions. The blood content of CD4$^+$IL10$^+$ T cells below 3.3% was associated with progression of coronary artery atherosclerosis (OR 12.0 (2.3, 61.0), sensitivity 77%, specificity 78%, p=0.003). No differences in other immunological parameters and common atherosclerosis risk factors in the groups were revealed. We hypothesize that the content of CD4$^+$IL10$^+$ T cells can be an important predictive marker for the progression of coronary atherosclerosis.

Key Words: *atherosclerosis; inflammation; coronary arteries; T lymphocytes; IL-10*

Chronic inflammation plays a leading role in the development of atherosclerosis (AS). T lymphocytes, primarily T helpers, are involved in the regulation of inflammatory process in the atherosclerotic plaque. T lymphocyte subpopulations include cells with proinflammatory (IFNγ-producing Th1 and IL-17-producing Th17) and anti-inflammatory (regulatory T cells, Treg) properties inhibiting AS progression. Numerous clinical studies have revealed changes in the blood content and activity of Th17 and Treg in patients with AS [2,7,11,15]. The role of IL-10, a cytokine with pronounced anti-inflammatory properties, in deceleration of AS progression and inhibition of the inflammatory response and cell apoptosis during AS was demonstrated in experimental models [3]. In humans, the main producers of IL-10 among CD4$^+$ T lymphocytes are Treg and Treg1; the role of these cell populations in AS development is little studied.

Federal Medical Research Center of Cardiology, Ministry of Health of the Russian Federation, Moscow, Russia. ***Address for correspondence:*** anastasia.m088@yandex.ru. A. Yu. Filatova

The aim of this 2-year prospective study was to evaluate the prognostic significance of the blood content of IL-10-producing CD4$^+$ T lymphocyte for coronary AS progression.

MATERIALS AND METHODS

The study included 36 patients (30 males and 6 females; mean age 63 (55; 70) years) with verified coronary artery disease (CAD, stable angina functional class 2-3) admitted for scheduled coronary angiography and coronary stenting. The follow-up examination in 21 (12; 29) months included control coronary angiography. Exclusion criteria were acute coronary syndrome, stroke or interventions during the previous 6 months, neoplasms, liver or renal failure, infectious/inflammatory disease, decompensated diabetes mellitus, and the use of immunosuppressive drugs. All patients at the enrollment and during the observation period received standard therapy recommended for CAD patients with β-blockers, acetylsalicylic acid/clopidogrel, ACE inhibitors/sartans, and statins in accordance with the level of LDL cholesterol.

Coronary angiography was performed via transradial approach using a standard technique. The presence of coronary AS was assessed with the degree of the stenosis of the main coronary arteries lumen. The appearance of new stenosis of 50% or more, or an increase in the degree of the preexisting stenosis of 30% or more was considered as progression of coronary AS.

Mononuclear cells were isolated by centrifugation in Ficoll—verographin density gradient (ρ=1.077) and activated in the presence of phorbol myristate acetate, ionomycin, and monensin (all reagents from Sigma). Surface antigens and intracellular proteins were identified using fluorescence-labeled antibodies (BD Biosciences, eBioscience, and BeckmanCoulter). For identification of intracellular proteins, kits for cells fixation and permeabilization were used (all reagents from eBioscience). The cell fluorescence was measured by flow cytometry (FACSCalibur, Becton Dickinson Immunocytometry Systems). Lymphocytes were gated by light scattering and CD45 expression. Treg were identified as $CD4^+FoxpP3^+$, and Th1 и Th17 as $CD4^+IFN\gamma^+$ and $CD4^+IL17^+$, respectively. IL-10-producing T helpers were identified as $CD4^+IL\text{-}10^+$ cells.

The blood content of sCD25 was assessed by chemiluminescent method on an Immunlite 1000 analyzer (Siemens); IL-10 and IL-17 were assayed by ELISA (Bender Medsystems kits), CRP (hsCRP) were measured by nephelometry (Bering Marburg GmbH), and lipoprotein (a) was measured by ELISA [1].

All measurements were performed prior to endovascular interventions.

The results were processed using Statistica 9.0; ROC analysis was performed using PRISM software. The Mann—Whitney *U* test was used for paired comparisons. Fisher exact *p* two-tailed test was used for paired comparisons of qualitative parameters. As the data distribution did not fit the normal law, the results were presented as the median and 25 and 75% percentiles. The differences were significant at $p<0.05$.

RESULTS

Progression of coronary AS was observed in 13 (36%) patients. The initial clinical characteristics (age, body mass index, smoking status, history of arterial hypertension and myocardial infarction) and medication (β-blockers, antiaggregants, ACE-inhibitors/sartans, and statins) did not differ in patients with and without progression of coronary AS (Table 1). The severity of coronary AS at enrollment was higher in patients who demonstrated AS progression after 2 years (Table 1).

The initial content of $CD4^+IL10^+$ T lymphocytes and IL-10 in the blood were lower in patients who developed progression of coronary AS ($p=0.03$, $p=0.06$, respectively, Table 1). Other subpopulations of $CD4^+$ lymphocytes, concentrations of hsCRP, sCD25, IL-10, and IL-17 in the blood, and lipid spectrum parameters (total cholesterol, LDL, and lipoprotein (a)) did not differ in the groups of patients, which can be a result of small number of patients included in the study.

The blood content of $CD4^+IL10^+$ T cells below 3.3% of all $CD4^+$ T cells was associated with progression of coronary artery atherosclerosis (OR 12.0 (2.3, 61.0), sensitivity 77%, specificity 78%, $p=0.003$). For evaluation of the prognostic value of the content of $CD4^+IL10^+$ T cells for progression of coronary AS, we performed ROC analysis; the area under the curve was 0.80 (0.66, 0.95), $p=0.002$ (Fig. 1).

IL-10 is a cytokine with an anti-inflammatory properties participating in the reactions of innate and adaptive immunity. IL-10 is produced by B, NK cells, monocytes [10], and a variety of T-cell subpopulations ($Foxp3^+$Treg, Treg1, Th1, Th2, and Th17) depending on stimulation and microenvironment. The main producers of IL-10 among T lymphocytes are Treg and Treg1 [14]. IL-10 inhibits effector lymphocytes, natural killers, macrophages, and antigen-presenting cells, down-regulates the expression of MMPs and production of proinflammatory cytokines [14]. Suppression of the inflammatory and immune responses via IL-10-dependent mechanisms as well prevention of the formation of atherosclerotic plaque and stabilization of existing plaques were demonstrated in animals [4]. IL-10 deficient mice are characterized by accelerated AS development, while exogenous IL-10 suppressed plaque formation [8]. The data of clinical studies are contradictory. In CAD patients with unstable angina and multivessel CAD, the blood level of IL-10 was shown to be elevated [6,9]. The symptomatic and progressive CAD is also

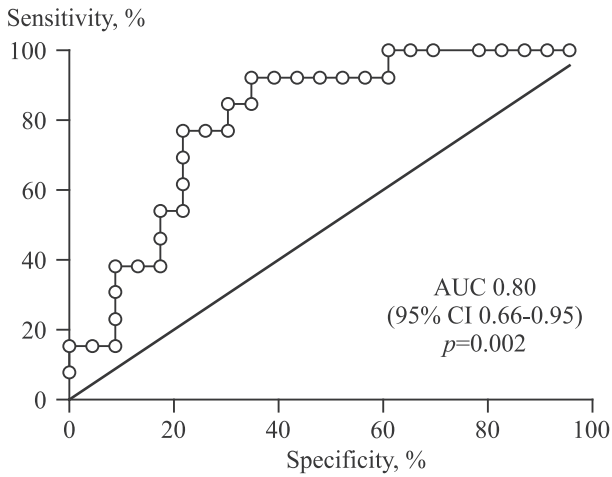

Fig. 1. ROC curve for evaluation of diagnostic value of the blood content of IL-10-producing $CD4^+$ T lymphocytes for coronary AS progression.

TABLE 1. Angiography and Laboratory Data

Parameter	Patients without AS progression (*N*=23)	Patients with AS progression (*N*=13)
Coronary arteries stenosis, %		
1-vessel lesion	52	7
2-vessel lesion	4	31
3-vessel lesion	4	54
Leukocytes, mln/ml	6.5 (5.5; 7.2)	6.9 (4.8; 8.5)
Total cholesterol, mmol/liter	4.3 (3.8; 5.4)	4.4 (4.2; 4.7)
LDL cholesterol, mmol/liter	2.1 (1.5; 2.7)	2.3 (1.7; 2.9)
Triglycerides, mmol/liter	1.3 (1.1; 1.7)	1.5 (1.2; 1.7)
Glucose, mmol/liter	5.4 (5.1; 5.9)	5.1 (5.0; 6.0)
hsCRP, mg/liter	1.2 (0.6; 4.5)	2.8 (1.1; 6.2)
Lipoprotein (a), mg/dl	12.7 (3.7; 25.9)	16.4 (4.6; 58.1)
Lymphocytes, %		
leukocytes	28.5 (21.0; 32.0)	29.5 (27.0; 33.0)
$CD4^+$, % lymphocytes	38.0 (35.0; 43.5)	34.5 (30.0; 43.5)
$CD4^+Foxp3^+$Treg	8.1 (6.0; 10.0)	8.8 (7.9; 11.3)
Th1	18.8 (11.9; 27.7)	15.4 (7.3; 20.8)
Th17	0.8 (0.7; 1.4)	1.2 (0.6; 2.3)
$CD4^+IL10^+$ T cells	4.1 (3.3; 6.0)	2.7 (1.8; 3.2)*
sCD25, U/ml	558.0 (429.0; 1064.0)	796.0 (664.0; 906.0)
IL-10, pg/ml	3.0 (2.1; 3.9)	2.0 (1.5; 2.7)$^+$
IL-17, pg/ml	1.2 (1.0; 3.0)	1.4 (1.0; 9.0)

Note. Content of T helper lymphocyte subsets and Treg are expressed as % $CD4^+$ lymphocytes. *p<0.05, ^+p=0.06.

associated with elevated IL-10 content. In patients with acute coronary syndrome, the concentration of IL-10 and blood contents of $CD8^+IL10^+$ T cells were significantly higher than in healthy volunteers [13]. However, serum concentration of IL-10 in patients with unstable angina was lower than in patients with stable CAD course [12]. In patients with stable CAD and a history of at least two myocardial infarctions, the values of Treg and IL-10 in the blood were lower than in patients without myocardial infarctions despite of comparable risk of cardiovascular complications and CAD severity [5]. We hypothesize that these contradictory data can be explained by different time form the onset of CAD destabilization to blood assays, effects of medication, and the influence of stress factors on cell immunity.

Thus, the baseline low content of IL-10-producing $CD4^+$ lymphocytes and low IL-10 level in blood were associated with the progression of coronary AS in stable CAD patients. The amount of IL-10 producing T cells was more sensitive and specific parameter than blood concentration of IL-10.

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