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Disease complexity in acute coronary syndrome is related to the patient's immunological status ☆

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ABSTRACT

Background: Our aim was to investigate whether patients with acute coronary syndrome (ACS) display an overall T cell immunosenescence that could be contributing to worsening the stage of the disease.
Methods and results: We compared the immunological status of 52 ACS patients, 21 controls with absence of coronary artery disease (CAD) (C1), and 50 healthy individuals (C2). We characterized leukocyte and T lymphocyte subpopulations by flow cytometry. CAD was classified according to SYNTAX score, number of diseased coronary vessels, previous episodes of ACS and left ventricular ejection fraction (LVEF). ACS patients showed an increased number of total leukocytes, neutrophils and monocytes ($p < 0.001$), but a decreased number of lymphocytes ($p < 0.05$). ACS patients had significantly higher levels of NK cells and CD8+ T-cells ($p < 0.05$). ACS was associated with high differentiation in CD4+ and CD8+ T-lymphocytes. Frequencies of naïve, naïve CD31+, EM1, and pE1 subsets were significantly reduced in ACS patients ($p < 0.05$), while EM3, EM4 (in CD4+), and E (in CD8+) subsets were increased ($p < 0.05$). Aging of T-lymphocyte subpopulations was associated with a worse SYNTAX score ($p < 0.05$), and aging of CD4+ T-lymphocytes with a larger number of affected vessels, larger number of previous ACS episodes and lower LVEF, in ACS patients ($p > 0.05$). Furthermore, the proliferation ability of CD4+ and CD8+ T-lymphocytes was significantly impaired in ACS patients ($p < 0.05$), although they had increased activation ($p < 0.05$).
Conclusions: We conclude that ACS patients show a higher degree of T-lymphocyte immunosenescence than healthy controls, which could contribute to disease impairment through a compromised adaptive immune response.

1. Introduction

CAD continues to be the leading cause of death in the developed world. Recent studies have demonstrated that CAD results from an uncontrolled immune response, and T-lymphocytes play a central role in the development and progression of the disease [1,2]. Activated inflammatory cells have been found in the coronary plaques as well as in the peripheral blood of patients with acute coronary syndromes (ACSs) [3]. These patients have an increased frequency of CD4+ T-lymphocytes characterized by defective cell surface expression of CD28, a major costimulatory molecule critically involved in determining the outcome of antigen recognition by T-lymphocytes. $CD4+CD28^{null}$ T-cells are expanded in the peripheral blood of patients with ACS, where they undergo clonal expansion, probably triggered by specific antigens [4,5].

During aging and in patients with chronic infections and autoimmune diseases [6,7], a global immunosenescence process takes place, but CD4+ T-cells are the last cells to be affected. CD4+ T-cells are more resistant to age-related phenotypic and functional changes than CD8 T-cells [8,9]. The unusually high frequency of $CD4+CD28^{null}$ T-cells in patients with inflammatory syndrome and chronic infections is disproportionate to their age and may represent the result of a repeated stimulation by a chronic antigen that leads to continuous immune activation. Several antigens have been identified in atherosclerotic

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plaques: *Chlamydia pneumoniae*, CMV, oxidized LDL, hHSP60 and β2-glycoprotein Ib [10,11], however, the target antigen for CD4+CD28^{null} T-cells is not yet clear.

Loss of CD28 expression is a hallmark of the age-associated decline in T-cell function. As CD28 provides a pivotal role during T-cell activation, such as inducing cytokine production (IL-2) and promoting cell proliferation, a lack of this co-stimulatory signal during activation results in partial activation or even an anergic state in T-cells [12]. In this way, the accumulation of CD28^{null} T-cells, is associated with immunosenescence, a reduced overall immune response to pathogens and vaccines [13]. The aged immune system displays a decline in numbers of naïve T-cells in peripheral blood and lymphoid tissues [14]; in contrast, they have a marked increase in the proportion of highly differentiated effector and memory T-cells like CD28^{null} T-cells [15,16]. An Immune Risk Profile (IRP) was defined using a cluster analysis approach [17]. In agreement with a higher IRP, higher 2-year mortality was observed in a population of very old Swedish individuals who had an inverted CD4/CD8 ratio, an accumulation of CD28^{null} T-cells, and CMV infection [18]. In summary, considerable evidence suggests a clear association between immune function, the development and evolution of chronic pathologies and the longevity of individuals [19]. All of these findings prompted us to evaluate the degree of differentiation of immune system T-lymphocytes in ACS patients and its association with their disease status.

2. Methods

2.1. Study population

One hundred and twenty-three participants (54 women and 69 men) were recruited to the study. Individuals in the study belonged to three different groups: healthy controls with known non-obstructive CAD (C1) (n = 21), healthy general population controls (C2) (n = 50), and patients with a recent diagnosis of ACS (n = 52). For the C1 group, we selected consecutive patients with chest pain, without electrocardiogram (EKG) anomalies and with normal values of troponin in serial determinations until 12 h after the onset of pain. Coronary angiography showed non-obstructive coronary arteries. The C2 group was recruited from the Centro de Transfusiones del Principado de Asturias (Oviedo, Spain). The ACS group comprised patients who were hospitalized due to an acute coronary syndrome with or without ST-segment elevation, according to the current ESC guidelines [20,21]. ACS patients were classified according to the number of diseased vessels, previous ACS, and left ventricular ejection fraction (LVEF). The synergy between percutaneous coronary intervention (PCI) with TAXUS and cardiac surgery (SYNTAX score) [22] was calculated in 49 ACS patients.

Peripheral blood samples were drawn from all subjects for hematological and immunological analyses, and blood was drawn again at hospital discharge in ACS patients. All subjects underwent a physical examination and answered a standardized questionnaire to assess their medical history, current illnesses, and any medication they were taking. Exclusion criteria included all conditions that might influence the immune system, such as a recent or current infection, autoimmune disease or tumor, malnutrition, abnormal laboratory data (hemoglobin < 12 g/dL, leukopenia < 3500 cells/μL, neutropenia < 1500 cells/μL, leukocytosis > 15,000 cells/μL and thrombocytopenia < 10^5 cells/μL), and pharmacological interference. Informed consent was obtained from all volunteers before participation in the study. The study was approved by the ethics committee of the Hospital Central de Asturias (Oviedo, Spain).

Table 1
Clinical characteristics and biological parameters of patients and healthy individuals.

	Acute coronary syndrome (ACS) (n = 52)	Controls, Non-obstructive coronaries (C1) (n = 21)	Controls, General population (C2) (n = 50)	p value ACS vs C1	p value ACS vs C2	p value C1 vs C2
Age ± SD (years)	57.6 ± 9.3	58.0 ± 5.9	59.6 ± 7.1	NS	NS	NS
Male (%)	45 (86.5)	7 (33.3)	17 (34)	0.001	0.001	NS
Risk factors, n (%)						
Smoking status, current (%)	36 (69.2)	8 (38.1)	19 (36.5)	0.03	0.002	NS
Hypertension (%)	31 (59.6)	6 (28.6)	4 (8.0)	0.025	0.001	0.035
Hypercholesterolemia (%)	30 (57.7)	6 (28.6)	7 (14.0)	0.02	0.008	0.047
Diabetes mellitus (%)	31 (59.6)	2 (9.5)	0 (0)	0.001	<0.001	0.006
Medications						
β-Blockers	42 (80.7)	3 (14.3)	2 (4.0)	<0.001	<0.001	0.03
Aspirin	49 (94.2)	0 (0.0)	0 (0.0)	NA	NA	NA
ACE inhibitors	20 (38.5)	4 (19.0)	2 (4.0)	<0.001	<0.001	<0.001
Clopidogrel	30 (57.7)	0 (0.0)	0 (0.0)	NA	NA	NA
Statins	49 (94.2)	6 (28.6)	7 (14.0)	<0.001	<0.001	0.037
Atorvastatin	34 (65.4)	3 (14.3)	3 (6)	NA	NA	NA
Rosuvastatin	14 (26.9)	2 (9.5)	2 (4)	NA	NA	NA
Other	1 (1.9)	1 (4.8)	2 (4)	NA	NA	NA
Biochemistry variables (mean and SD)						
Total cholesterol (mg/dL)	170.5 ± 34.9	189.7 ± 34.8	176.8 ± 47.7	0.034	NS	NS
LDL (mg/dL)	129.7 ± 33.9	113.0 ± 33.7	119.1 ± 36.0	NS	NS	NS
HDL (mg/dL)	42.1 ± 10.25	57.8 ± 13.3	57.2 ± 9.8	<0.001	<0.001	NS
Triglycerides (mg/dL)	158.8 ± 103	95.52 ± 30.0	119.0 ± 38.5	0.002	0.045	NS
Troponin T (ng/L)	4331 ± 4430	ND	ND	NA	NA	NA
Hematological variables (mean and SD)						
WBCs (10^3/μL)	9.46 ± 3.7	6.3 ± 2.15	6.36 ± 1.7	<0.001	<0.001	NS
Neutrophils (10^3/μL)	6.68 ± 3.6	4.4 ± 1.8	3.48 ± 1.0	<0.001	<0.001	NS
Neutrophils (%)	67.7 ± 10.9	56.5 ± 7.6	54.2 ± 8.4	<0.001	<0.001	NS
Lymphocytes (10^3/μL)	1.8 ± 0.8	2.2 ± 0.5	2.51 ± 0.8	0.01	0.003	NS
Lymphocytes (%)	21.9 ± 8.5	31.8 ± 7.0	35.1 ± 8.8	<0.001	<0.001	NS
Monocytes (10^3/μL)	0.64 ± 0.2	0.44 ± 0.1	0.45 ± 0.1	<0.001	0.001	NS
Monocytes (%)	7.4 ± 2.6	7.6 ± 2.3	7.2 ± 1.8	NS	NS	NS

WBCs, white blood cells; SD, standard deviation.
NA, not applicable.
ND, not done.
NS, not significant.