Changes in number of NK cells after one year from coronary artery bypass graft

ŁUKASZ HAK[1], JOANNA WIĘCKIEWICZ[1], JOLANTA MYŚLIWSKA[1], KRZYSZTOF SZYNDLER[2], JANUSZ SIEBERT[3], JAN ROGOWSKI[2]

[1]Department of Immunology, Medical University of Gdańsk, Poland; [2]Clinic of Cardiosurgery, Medical University of Gdańsk, Poland; [3]Department of Family Medicine, Medical University of Gdańsk, Poland

Abstract

Natural Killer cells (NK cells) are components of nonspecific immune response involved in the defense against viral and bacterial infections. Basing on the CD56 cell surface antigen density human NK cells may be divided into two distinct subpopulations: CD56dim – "cytotoxic subset" and CD56bright – "regulatory subset". Our previous work revealed that coronary heart disease (CHD) patients are characterized by a reduction in absolute values and percentage of the CD3-CD56dim and CD3-CD56+ cells. CHD is inflammation dependent disease. Therefore we decided to estimate NK cells status in CHD patients after one year from coronary artery bypass graft (CABG). This procedure improves the heart and vascular status in patients and also remove inflammatory burden.

Blood was collected from eighty five patients from the Clinic of Cardiosurgery of the Medical University of Gdańsk before CABG. Blood from eleven CHD patients was examined ones more after one year from CABG. Seventy seven people, of similar age, with excluded coronary heart disease were enrolled into the study as a control group. Percentage of the CD3-CD56+, CD3-CD56dim and CD3-CD56bright cells were analyzed in all samples by flow cytometry.

Before CABG blood of the CHD patients was characterized by a low percentage of the CD3-CD56+ and CD3-CD56dim subsets in comparison to control group. However when number of cells were analyzed within one year after CABG we observed a rise of the CD3-CD56+ and CD3-CD56dim cells percentage. The increase of cell percentage was relevant when data were compared to control group and to the results obtained from CHD group before CABG.

It may be concluded that a lower NK cells number in CHD is a consequence of inflammation and further that this number may be treated as marker of inflammation.

Key words: *NK cells, coronary heart disease, coronary artery bypass graft.*

(Centr Eur J Immunol 2009; 34 (2): 86-89)

Introduction

The established risk factors for atheriosclerosis such as hypertension, smoking, diabetes mellitus and hyperlipidemia do not explain clinical and epidemiological features of coronary heart disease (CHD). CHD has been considered as an inflammatory process [1]. Increased production of the C-reactive protein (CRP), serum amyloid A, interleukin-6 (IL-6) which are considered as inflammatory markers correlates with the increased risk of future cardiovascular events [2]. Elevated serum levels of proinflammatory cytokines, such as tumour necrosis factor (TNF), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-12 (IL-12) and interferon-γ (IFN-γ) were also associated with the progression of atherosclerosis and its complications [3, 4]. Chronic infections are etiological factors, which link inflammation and atherogenesis. Particularly cytomegalovirus (CMV), *Chlamydia pneumoniae* and *Helicobacter pylori*.

Natural Killer cells (NK cells) are components of nonspecific immune response involved in the defense against viral and bacterial infections [5]. Basing on the CD56 cell surface antigen density human NK cells may be divided into two distinct subpopulations: CD56dim and CD56bright [6]. The CD56dim cells are called "cytotoxic

Correspondence: Łukasz Hak, Department of Immunology, Medical University of Gdańsk, Dębinki 1 Str., 80-210 Gdańsk, Poland.
Phone number: +48 58 349 14 32, Email: lhak@amg.gda.pl

NK subset" and comprise about 90% of the CD56 positive circulating cells. They exert the cytotoxic effect through perforins and granzymes [5]. In contrast, the CD56bright cells are involved in the regulation of innate immune response, through secretion of an array of cytokines, such as IFN-γ, IL-10, IL-13 and GM-CSF, which modulate immune response [6]. Thus the CD3-CD56bright cells are so called "NK regulatory subset".

Our previous work revealed that CHD patients are characterized by a reduction in absolute values and percentage of the CD3-CD56dim and CD3-CD56+ cells in comparison to age matched control group. Furthermore cytotoxic activity of NK cells from CHD patients was also lower. We concluded that disregulation of NK cells compartment may provide a gate for infections and contribute to CHD pathogenesis [7]. However, still remains a question if impairment of NK all functions is a cause or consequence of CHD. Therefore we decided to estimate NK cells status in CHD patients after one year from coronary artery bypass graft (CABG). This procedure improves the heart and vascular status in patients. So, we may expect that is also has an important influence on the NK cells.

Material and Methods

Patients

Eighty five patients undergoing elective first time coronary artery by-pass grafting from the Clinic of Cardiosurgery of the Medical University of Gdańsk were selected.

Basic characteristics of the patients are shown in Table 1. Hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, or patient's history of anti-hypertensive treatment verified at admission. Diabetes was defined as fasting glucose ≥ 126 mg/dl, non-fasting glucose ≥ 200 mg/dl, or patient's history of diabetes verified at admission. Coronary angiography was performed in all patients. Patients were qualified for coronary artery by-pass grafting (CABG) if they had at least one stenosis with diameter of ≥ 75% in the right coronary artery (RCA) and/or left-anterior descending artery (LAD) and/or left circumflex artery (CX). Blood from eleven CHD patients was examined ones more after one year from CABG.

Seventy seven people, of similar age, with excluded coronary heart disease were enrolled into the study as a control group. The disease was excluded on the base of physical examination, lack of clinical symptoms of CHD and normal resting and exercise-related electrocardiogram. The written informed consent was obtained from all participants. The present study was approved by the Ethics Committee of the Medical University of Gdańsk.

Specimen collection

Venous blood samples (10 ml) were collected between 9.00 and 10.00 am aseptically into the tubes with anti-coagulant (EDTA tubes Medlab, Austria).

Table 1. Basic clinical data

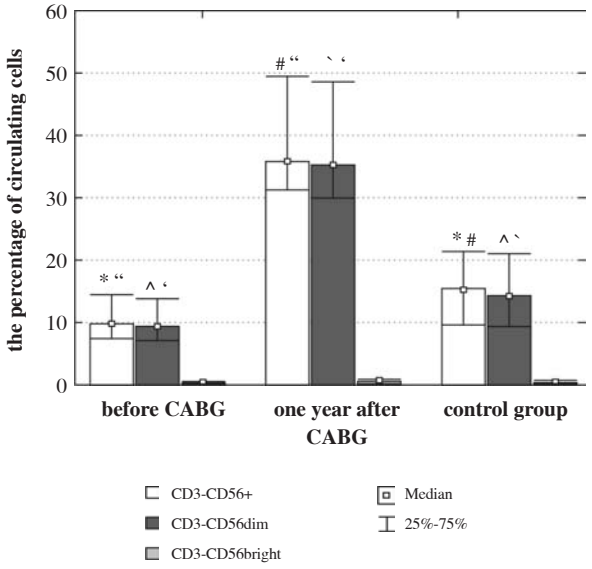
	CHD patients (N = 85)	Control grup (N = 77)	*p*
Age (years), (median, 25th percentile/ 75th percentile)	61.6, 56/67	63.13, 57.5/68	0.24
Men (%)	56.99	42.86	0.11
Hypertension (%)	79.27	31.17	**0.00**
Diabetes (%)	34.15	3.89	**0.00**
Current Ex-smokers Non-smokers	16.44 56.16 27.40	8.06 43.55 48.39	0.3
Medical treatment			
β-blockers (%)	90	9.52	**0,00**
Ca-blockers (%)	15	14.29	0.47
Nitrates (%)	60	0	–
Diuretics (%)	20	19.05	0.46
Oral anti-diabetics	20	0	–
ACE inhibitors	76.25	0	–
Statins	91.25	0	–

Staining of CD3- and CD56+ NK cells

The samples of venous blood were aliquoted into plastic tubes (Falcon, Becton Dickinson Company, USA), 100 µl per tube. Cells were stained with anti-CD3 (IgG2a,κ mouse PE-Cy5, Clone: HIT3a, PharMingen, Becton Dickinson Company, USA), anti-CD56 (IgG1,κ mouse PE, Clone: B159, PharMingen, Becton Dickinson Company, USA) human antibodies (20 µg/test). For each set, appropriate isotypic control was done (IgG2b,κ mouse PE-Cy5, Clone: 27-35, IgG1k mouse PE, Clone: MOPC-21, PharMingen, Becton Dickinson Company, USA). After 30 min incubation in the dark at a RT samples were fixed using Immuno-prep reagents (Immunotech, USA) with Q-prep Immunology Workstation (Coulter, USA).

Acquisition and analysis of flow cytometry data

Listmodes were acquired on Epics XL flow cytometer (Coulter, USA) and analyzed using Winlist, version 5.0, software. Dead cells were excluded by forward (FSC) and side (SS) angle scattered light window. The region containing lymphocytes was generated on the basis of using their forward versus right angle light scatters. A lymphocytes gate was used to measure the proportion of NK cells subsets in the sample. Typically, 10 000 events were acquired in this region.



* p = 0,0005, ^ p = 0,0003, " p = 0,03, ` p = 0,03, # p = 0,0001, ` p = 0,0001.
The results are presented as median, 25th percentile/75th percentile.

Figure 1. Percentage of circulating NK cell subsets. The results were analyzed by the U Mann Whitney test (CHD group vs control group) and Wilcoxon matched pairs test (CHD group before CABG vs CHD group after one year after CABG)

Statistical analysis

Data were computed using program Statistica 8.0 (Statsoft, Poland). Parametric and non-parametric distributions was assessed by W Shapiro-Wilka test. The analysis was based on non-parametric statistic U-Mann-Whitney and Wilcoxon matched pairs test as indicated by data distribution.

Results

Basic clinical data

Basic clinical differences between studied group are presented in Table 1. As we expected, differences were mainly dependent on health status of included patients.

Percentage of NK cells

Our approach aimed at analysis of NK cells number in peripheral blood. Percentage of the CD3-CD56+ (whole NK cells population) and CD3-CD56dim subsets were lower in the CHD patients before CABG compared to control group.

Further analysis were performed on eleven patients, in which NK cells data were obtained after one year from CABG. We noticed an enormous increase of the percentage of NK cells (CD3-CD56+) and particularly of the CD3-CD56dim subsets in comparison to the values before CABG and also to control group (Fig. 1).

Discussion

Blood from CHD patients was taken in two time points: before coronary artery bypass graft (CABG) and one year after. All obtained results were compared to age matched control group.

Before CABG blood of the CHD patients was characterized by a low percentage of the CD3-CD56+ and CD3-CD56dim subsets. However when number of cells were analyzed within one year after CABG we observed a rise of the CD3-CD56+ and CD3-CD56dim cells percentage. The increase of cell percentage was relevant when data were compared to control group and to the results obtained from CHD group before CABG.

Our previous study showed that the number and cytotoxic activity of NK cells in blood of CHD patients is low [7]. We suggest that if the CHD is reported as an infection dependent disease than the lower NK cells number may be a possible gate for pathogen entrance. However, we did not exclude other hypothesis in which inflammation related processes may impair NK cells response. The CHD development is underlied by an inflammatory process. Initiation of the CHD is caused by the injury of vessel wall by chemical, biological or genetical factors. The injury induces an immune response. The activated macrophages together with T-cells form the initial lesion of atherosclerosis, the fatty streak. This site becomes thrombogenic and smooth muscle cells migration and proliferation is induced. Important stimuli to the migration and proliferation are cytokines (TNF, IL-6) and chemokines (IL-8), thus inflammation is initiated. The aforementioned events, which are initiated by endothelial cells injury, culminate in the formation of fibrous plaque composed of smooth muscle cells, foamy macrophages and T-cells embedded in a collagenous matrix of connective tissue [8]. Fibrous plaque is chopping blood vessel and directly cause ischemia of heart muscle. Furthermore ischemia also cause an enormous proinflammatory cytokine release. Our previous paper showed that NK cytotoxic activity are negatively correlated with the TNF (tumor necrosis factor) concentration [9]. It is seem that NK cells number may be treated as a marker of inflammation. Further proof for this hypothesis was a study performed in patients with type 1 diabetes (DM1). DM1 is also connected with high values of proinflammatory cytokines such as: TNF or IL-6 [10]. Not surprisingly, the number of NK cells in blood of DM1 patients was lower in comparison to control group [11]. The CABG procedure probably has removed inflammatory burden, that is why we observed a rise of NK cells number within one year from the CABG. From this point of view we may answer the question if decrease of NK cells number is a cause or consequence of CHD. It may be concluded that a lower NK cells number is a consequence of inflammatory process in the CHD and further that this number may be treated as marker of inflammation.

References

1. Ross R (1999): Atherosclerosis is an inflammatory disease. Am Heart J 138: 419-420.
2. Ridker PM, Hennekens CH, Buring JE, Rifai N (2000): C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 342: 836-843.
3. Kishikawa H, Shimokama T, Watanabe T (1993): Localization of T lymphocytes and macrophages expressing IL-1, IL-2 receptor, IL-6 and TNF in human aortic intima. Role of cell-mediated immunity in human atherogenesis. Virchows Arch A Pathol Anat Histopathol 423: 433-442.
4. Libby P, Sukhova G, Lee RT, Galis ZS (1995): Cytokines regulate vascular functions related to stability of the atherosclerotic plaque. J Cardiovasc Pharmacol 25 (Suppl 2): S9-S12.
5. Garcia-Penarrubia P, Koster FT, Kelley RO et al. (1989): Antibacterial activity of human natural killer cells. J Exp Med 169: 99-113.
6. Cooper MA, Fehniger TA, Caligiuri MA (2001): The biology of human natural killer-cell subsets. Trends Immunol 22: 633-640.
7. Hak Ł, Myśliwska J, Wieckiewicz J et al. (2007): NK cell compartment in patients with coronary heart disease. Immun Ageing May 4: 3.
8. Streblow DN, Orloff SL, Nelson JA (2001): Do pathogens accelerate atherosclerosis? J Nutr 131: 2798S-2804S.
9. Myśliwska J, Bryl E, Trzonkowski P, Myśliwski A (2000): Compensatory effect of TNFalpha on low natural killer activity in the elderly. Acta Biochim Pol 47: 301-311.
10. Zorena K, Myśliwska J, Myśliwiec M et al. (2007): Serum TNF-alpha level predicts nonproliferative diabetic retinopathy in children. Mediators Inflamm 2007: 92196.
11. Hak Ł, Więckiewicz J, Katarzyna Z, Myśliwska J (2008): Komórki NK u pacjentów z cukrzycą typu 1 (NK cells compartment in patients with type 1 diabetes). Fam Med Prim Care Rev 10: 430-432.