Cent. Eur. J. Med. • 6(1) • 2010 • 31-36
DOI: 10.2478/s11536-010-0056-z

VERSITA

Central European Journal of **Medicine**

Kinetic of monocytes Fcγ receptors in patients after coronary artery bypass

Research Article

Hristina Dimitrova Andreeva[1*], Steinar Lundemoen[2,3], Godfrid Greve[2,4], Rune Haaverstad[2,3], Gustav Fjaertoft[5]

[1] *Department of Immunology and Transfusion Medicine, Haukeland University Hospital, Jonas Lies V. 65, 5021, Bergen, Norway*

[2] *Department of Hearth Disease, Haukeland University Hospital, Jonas Lies V. 65, 5021, Bergen, Norway*

[3] *Unit for Cardiothoracic Surgery, Haukeland University Hospital, Jonas Lies V. 65, 5021, Bergen, Norway*

[4] *Institute of Clinical Medicine, Haukeland University Hospital, Jonas Lies V. 65, 5021, Bergen, Norway*

[5] *Department of Woman and Child Health, Karolinska Institute, 171 77 Stockholm, Sweden*

Received 28 May 2010; Accepted 4 September 2010

Abstract: Cardiopulmonary bypass is usually associated with an increased risk of post-operative infections and systemic inflammatory response syndrome.This is accompanied by a neutrophil leucocytosis and lymphopenia.Less is known about the role of monocytes and markers of monocyte activity.This study focuses on the changes of Fc-gamma receptors on monocytes in patients undergoing on-pump coronary artery bypass grafting (CABG).The surface expression of CD64,CD32 and CD16 were studied using flow cytometry in 37 patients scheduled for CABG.The antigen density and the percentage positive cells were monitored preoperatively and on day 1, 2, 3 and 7 postoperatively. CD64 and CD32 antigen density were significantly increased from day 1 to day 7($p<0.0001$). A significant increase ($p<0.0001$) in the percentage CD16+ monocytes was detected at day 1,2,and 3. The downregulation of CD16 expression on day 1 was followed be elevation at days 2,3($p<0.01$).On day 7th percentage CD16+ monocytes and density were not returned to baseline values.Only the baseline levels of CD64 was lower compared to controls($p<0.05$).The results suggest that on-pump CABG induces dynamic changes in the expression of Fc-gamma receptors on monocytes as late as 7 days.We observed significant upregulation in the expression of CD64 and CD32 and "to phases" distribution of CD16 in the post-CABG period.

Keywords: *Cardiothoracic surgery • Flow Cytometry • Monocytes • Fcgamma- reseptors*

1. Introduction

Cardiac surgery involving cardiopulmonary bypass (CPB) is associated with pre and postoperative inflammatory responses. The exposure of blood to non-physiological surfaces, ischemia and reperfusion, cardioplegia, the surgical trauma, and the anesthesia, trigger the systemic inflammatory response syndrome (SIRS). SIRS is caused be several immunological events such as enhanced production of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-8), activation of endothelial cells, neutrophils, monocytes, complement cascade and platelets [1]. These events are thought to be associated with postoperative organ dysfunction or even organ failure [2].

Among cells involved in SIRS, monocyte-macrophages are the richest source of pro-inflammatory cytokines upon activation. Activated monocytes up- or down regulate various surface antigens, receptors for the Fc domain of IgG (Fcγ R), for complement, lipopolisaccharide receptor-CD14 as well as generate

* *E-mail: hrandreeva@yahoo.com*

oxidative specimens and phagocytose [3].

Fcγ receptors I (CD64), II (CD32) and III (CD16) are transmembrane glycoproteins that bind IgG and are expressed on leukocytes, including monocytes. They mediate receptor-specific phagocytosis of IgG-opsonized particles or endocytosis of immune complexes [4]. All three receptors have been investigated by leukocytes in different study design by different patient's groups. The key goal was either to identify patients at the risk of development of overwhelming inflammatory response or to implicate the causative agent of such a response, for example, bacterial infection [5-7]. In a previous study we found neutrophil CD64 expression to be a specific marker for the detection of bacterial infections after a major surgical trauma [8].

In addition, during the first part of our project we could confirm that on-pump coronary artery by-pass grafting (CABG) does not increase neutrophil CD64 expression to the levels seen during bacterial infection (Lundemoen S, Corbascio M, Segedal L, Andreeva H, Fjaertoft G, presented at SATS&SCANSECT, Reykjavik, Iceland, 16 to 19 August, 2006). However, it still remains difficult to discriminate uncomplicated SIRS from SIRS associated with infections. Yet, this is an important issue in terms of prophylactic and therapeutic antibiotic treatment during the pre and postoperative course. In addition to the conventional markers of inflammation, such as C-reactive protein (CRP), white blood cells counts (WBC) and SIRS criteria, much effort has been spent on finding new markers that predict the patient's outcome [9].

The second part of our project aimed to investigate the kinetics of Fcγ receptors expression on monocytes by the same patients undergoing on-pump CABG. The effect of extracorporeal circulation on Fcγ receptors, CD64 (FcγRI), CD32 (FcγRII) and CD16 (FcγRIII) on monocytes were investigated using flow cytometry prior to surgery and in the postoperative period. Furthermore, because CRP has a pivotal role in monocyte recruitment and proliferation [10], changes of CRP concentrations in the plasma were measured in parallel.

2. Material and Methods

2.1. Patients

Thirty-seven patients scheduled for elective on-pump CABG without clinical signs of resent of ongoing infection or inflammatory disease, with CRP <5mg/L, leukocyte count <11.0x10^9/L, not using Non-steroidal Anti-Inflammatory Drugs (NSAIDs) or steroids preoperatively, were included. Median age was 68 years (range 38-79 years). Median cutting time was 169 minutes (range 94-264), median extracorporeal circulation time 82 minutes (range 38-172), median Euroscore (logistic) 2.11% (range 0.88-12.40%), median number of anastomoses onto the heart 4 (range 2-6). Cefalotin was administered intravenously by introduction of anesthesia, when on extracorporeal circulation and after skin closure, 2g, 1g, and 2g, respectively. The informed consent was obtained from all patients to accepted enrollment in the study. Healthy adults (n=50) were used as a control group (HA). Our project had written approval from the Ethics Committee of the Haukeland University Hospital, Bergen.

2.2. Methods

The surface expression of CD64, CD32 and CD16 was monitored by flow cytometry on gated monocytes preoperatively (day 0) and on day 1, 2, 3, and 6/7 postoperatively. Leukocytes were prepared from heparinzed venous blood as previously described [8]. Briefly, 50 μl leukocyte suspensions were incubated for 30 min. at 4°C with the following monoclonal antibodies (MoAbs): CD64 FITC (Immunotech SA, France), CD32 FITC (BD Pharmingen, USA), CD16 FITC, CD14PE (Dako Cytomation, Denmark) as well as with negative isotype matched controls for mouse IgG_1 and IgG_2 (DakoCytomation, Denmark). Flow cytometric analysis was performed on an EPICS XL-MCL flow cytometer (Coulter, USA).

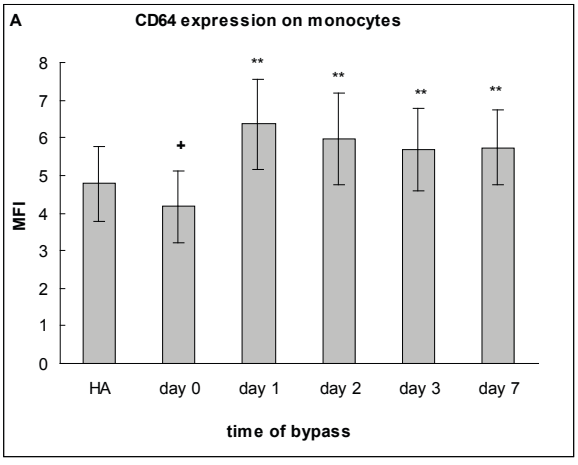
Routinely 2500 $CD14^+$ cells were analyzed. Data were expressed as mean fluorescence intensity (MFI) of CD64, CD32 and CD16 expression, and as relative number of positive cells (%), defined as the relative number of cells that expressed the antigen to a higher extent than the isotype-defined background. Percentage CD64 and CD32 positive monocytes were higher than 95% (data not shown).

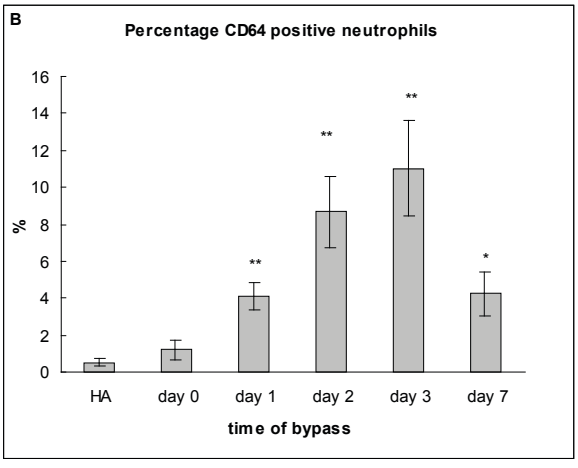
Serum concentration of CRP was measured by an immunoturbidimetry assay at the Department of Clinical Chemistry, Haukeland University Hospital, Bergen. A level lower than 5 mg/L (serum) was considered normal.

2.3. Statistical analysis

Statistical analysis of data was performed using the SPSS Advanced Statistics 10.0. The results are expressed as the mean ±SD. Values at all time points were compared with pre-CABG baseline levels using Paired T–test for multiple comparisons. Samples taken from healthy volunteers were considered as reference. A probability (p) value < 0.05 was considered as statistically significant.

Figure 1. CD64 expression on peripheral blood leukocytes before and after CABG. Results, presented as antigen density for monocytes (A) and percentage of positive cells for neutrophils (B) are compared with controls (HA). Statistical significance: within-group comparison with baseline: * $p<0.05$, ** $p < 0.0001$; comparison between controls and patients: † $p<0.05$.



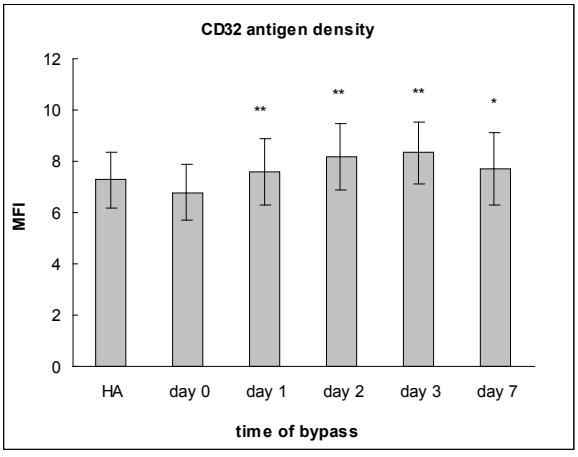


3. Results

3.1. CD64

CD64 exhibits normal distribution on monocytes and expression was therefore evaluated only as MFI. Preoperative levels of the CD64 molecule on monocytes were slightly lower compared to controls ($p<0.05$). The antigen density rose significantly on day 1 postoperatively ($p<0.0001$) and did not returned to baseline or controls levels by the 7th postoperative day (Figure 1A). Postoperatively, the neutrophil CD64 expression was only moderately increased (Figure 1B). Maximum neutrophil CD64 expression was reached on day 3 ($p<0.001$), median 11.03±2.57% and then gradually declined. Comparing CD64 dynamics patterns on both cells group, the maximum of CD64 expression on monocytes (day 1) preceded the maximum of CD64 expression on granulocytes with 2 days.

Figure 2. CD32 expression on peripheral blood monocytes before and after CABG. Results are compared with controls (HA). Statistical significance: within-group comparison with baseline: * $p<0.01$, ** $p < 0.0001$.



3.2. CD32

CD32 exhibits normal distribution on monocytes and expression was therefore evaluated only as MFI. The Fcγ R II antigen density was significantly upregulated in the postoperative period on day 1 as well as on day 2 and 3 with maximum increase on day 3 ($p<0.0001$), and did not return to baseline values by the 7th postoperative day ($p<0.01$). Baseline levels on CD32 were slightly but not significantly decreased compared to values from healthy controls (Figure 2).

3.3. CD16

FcyR III on monocytes exhibited changes both in the percentage positive cells and in MFI. The downregulation of CD16 expression on day 1 was followed be significant ($p<0.01$) upregulation on days 2, 3 after surgery (Figure 3A). Significantly elevated percentage $CD16^+$ monocytes were detected during the all postoperative period with maximum levels on day 1 ($p<0.0001$), (Figure 3B). On day 7 both percentage $CD16^+$ monocytes and their MFI were not completely returned to the preoperative values ($p<0.01$).

3.4. CRP

Serum concentrations of C-reactive protein were monitored routinely every day during the stay in the hospital. The values for CRP were significantly increased on the first day after surgery, with maximum level on day 2, median CRP=211.5±78mg/L ($p<0.0001$) and remained significantly higher during the follow-up (Figure 4).

Figure 3. CD16 expression on peripheral blood monocytes before and after CABG. Results, presented as MFI (A) and percentage positive cells (B) are compared with controls (HA). Statistical significance: within-group comparison with baseline: * $p<0.01$, ** $p < 0.0001$.

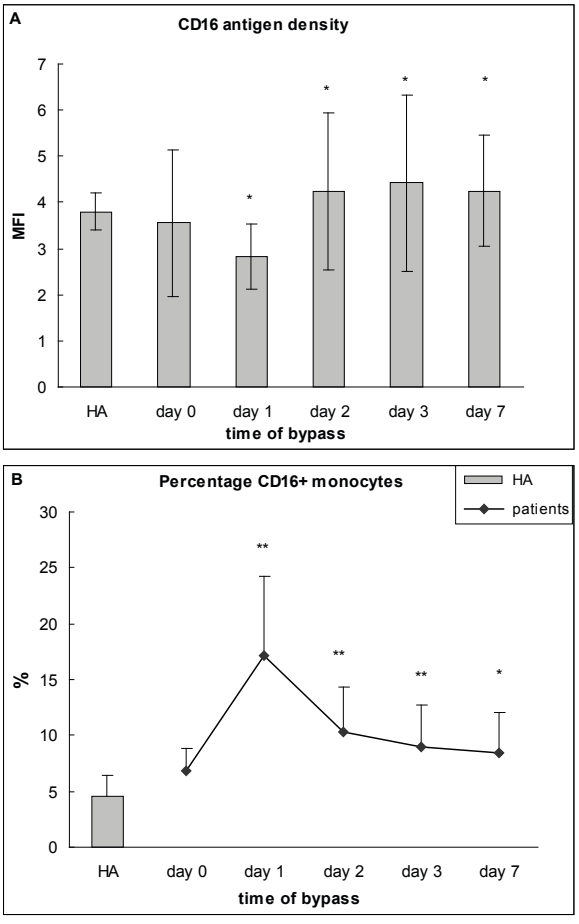
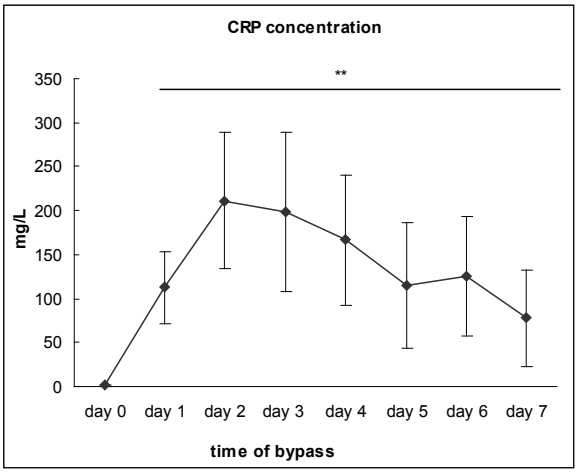


Figure 4. Changes in CRP levels before and after CABG. Statistical significance: within-group comparison with baseline: ** $p < 0.0001$.



4. Discussion

FcγR I (CD64), II (CD32) and III (CD16) are presented on neutrophils, monocytes and macrophages, and their expression is linked to their state of activation. The objective of this study was to evaluate the degree of activation of monocytes by determination of time-dependent changes in Fcγ receptors before and after CABG.

The results presented here reveal that CABG induces changes in the monocytes Fcγ receptors expression on the 1 after the surgery and during the whole studied period.

We have discussed clinical usefulness of neutrophil CD64 determination in the detection of bacterial infections after a surgical trauma [8]. We reported earlier that on-pump CABG (by the same studied group) did not increase neutrophil CD64 expression to the level seen during bacterial infection (S. Lundemoen, M. Corbascio, L. Segedal, H. Andreeva, G. Fjaertoft, presented at SATS&SCANSECT, Reykjavik, Iceland, 16 to 19 August, 2006). When comparing the CD64 changes on monocytes, we observed that maximum levels on monocytes were reached 2 days earlier in comparison to granulocytes. This observation implies that FcγR I density on monocytes is upregulated by a different cellular mechanism during surgical trauma. Monocyte expression of CD64 has also been investigated by other studies as a potential marker for the diagnosis of bacterial infections by patient undergoing CABG [6,7]. There are, however, diverging results on whether monocytes CD64 is up-regulated or not by on-pump CABG and when the earliest changes appear. In the present study the monocyte expression of CD64 has been found significantly increased on the 1st postoperative day by all studied patients (n=37). We could not fine a statistically difference in monocyte CD64 dynamics pattern by a subgroup of patients (n=8), who developed postoperatively SIRS associated with infections. These results correspond well with earlier report [5], and indicate that activation of CD64 on monocytes is a more unspecific phenomenon than “de novo” synthesis of this receptor on neutrophils. It also confirms the conclusion that in the presence of proinflammatory stimuli the upregulation of CD64 on monocytes and neutrophils at least are regulated by different mechanisms [11].

CD 32 and CD16 bind IgG with a low affinity and mediate phagocytosis and cytotoxicity [12]. It has been previously reported that CD32 expression on monocytes by patients undergoing CABG either remained unchanged [6] or suppressed [7]. In contrast to Hiesmayr et al. [7], we found CD32 still increased with

13% up until 7 days after intervention. However, only 7 patients were enrolled in the study with follow up period up to day 1 after CABG. Furthermore, in our study CD32 expression was up-regulated simultaneous to the CRP rise, suggesting that CD32 time-dependent changes is CRP-influenced. In the current study, the observed elevation of CD64 and CD32 on monocytes parallels the kinetics of CRP serum changes during the study period. These findings correspond well with the report that CRP influences monocytes recruitment and proliferation via FcγR I and FcγR II but not FcγR III activation [13]. Several lines of evidence indicate also that in monocyte-macrophages CRP augments reactive oxygen species, tissue factor and cytokines production [10].

CD16 expression exhibited "to phases" distribution during the follow-up. The first phase was identified on day 1 after CABG. A 21 percent decrease in the antigen density coupled with elevated percentage $CD16^{+}$ monocytes was found. This is due to the rise of the main population of monocytes ($CD14^{++}/CD16^{+/-}$). This cell subset has been shown to lack IL-10 synthesis, and is expanded in several acute and chronic inflammatory diseases [7,15]. Previously reports regarding monocyte CD16 expression in cardiac surgical patients have also shown the same phenomenon [7,16]. The second phase was characterized by enhancing of CD16 antigen density with 20% on day 2, 3, and 7 after CBP. The percentage of positivity remained also significantly increased at the same time points.

The observed shift in the cell phenotype was due to a group of monocytes expressing low $CD14^{+}$ and high $CD16^{++}$ levels. An expansion of CD14+/$CD16^{++}$ monocytes has been reported in several inflammatory diseases, in surgical patients with high APACHE II score or in patients undergoing cardiothoracic surgery [14]. These subpopulations of monocytes have a profile very similar to mature committed alveolar macrophages and are considered to possess anti-inflammatory properties under SIRS [16]. It is unclear why CD16 have such time dependent "to phases" changes during CABG. Several reasons are possible: first CD16 undergo shedding from the cell surface and release into the circulation in soluble form; second, internalization into the cell with possibility for re-expression [12] and last, mobilization and influx of CD14+/$CD16^{++}$ monocytes with anti-inflammatory properties as a part of the stress response. The results obtained from subgroup of our patients (n=8), who developed postoperatively SIRS associated with infections showed additional increase (not statistically significant; data not shown) in the percentage of these monocytes when comparing to the others 29 patients. Our findings conformed the suggestion by other reports [7,14] that monitoring of CD14+/$CD16^{++}$ monocytes may be implicated as a helpful marker for discriminate uncomplicated SIRS from SIRS associated with infections.

Limitations of this study are the same as in other similar studies [5,6]. The present study is limited by the size of the studied populations. Our study utilized a small sample, and its results should be viewed as preliminary. Second, additional monocyte receptors should be conducted to determine whether the prevailing profile of monocytes within such groups of patients will provide us with valuable information about protracted activation to CABG. These facts naturally limit the generalization of results. However, in keeping with our previous data showing that on-pump CABG does not increase neutrophil CD64 expression, the limitations of the present study do not allow the potential clinical relevance of our preliminary findings to be ignored.

In conclusion, our results propose that monocytes in on-pump CABG patients showed time dependent changes in the expression of FcγR, indicating a state of activation as late as 7 days postoperatively. This assumption was supported by the observed substantial dynamics in the expression of CD64 and CD32 and by the "to phase" distribution of CD16 in the post-CABG period. The results discussed here provide us with valuable information about systemic inflammation and CABG in general. Further research into the role of the monocytes during CABG is needed to establish the importance of Fc gamma receptors cross-linking in relation to phagocytosis and oxidative burst activity.

Acknowledgements

This study has been supported by FFHB – Oslo.

References

[1] Hall RI, Smith MS, Rocker G. The systemic inflammatory response to cardiopulmonary bypass: pathophysiological, therapeutic, and pharmacological considerations. Anesth Analg, 1997, 85, 766-782

[2] Dybdahal B, Wahba A, Haaverstad R, Kirkeby-Garstad I, Kierulf P, Espevik T. et al. On-pump versus off-pump coronary artery bypass grafting: more heat. shock protein 70 is released after on-pump surgery. Eur J Cardiothorac Surg, 2004, 25, 985-992

[3] Rutherford MS, Witsell A, Schook LB. Mechanisms generating functionally heterogeneous macrophages: chaos revisited. J Leukoc Biol, 1993, 53, 602-18

[4] Davis W, Harrison PT, Hutchinson MJ, Allen JM. Two distinct regions of Fc gamma RI initiate separate signaling pathways involved in ednocytosis and phagocytosis. EMBO J, 1995, 14, 432-41

[5] Kolachkova M, Kudlova M, Kunes P, Lonsky P, Mandak J, Andrys C., et al. Early Expression of FcγRI (CD64) on monocytes of cardiac surgical patients and higher density of monocytes anti-inflammatory scavenger CD163 receptor in “on-pump” patients. Mediators of Inflammation, 2008, 1-6

[6] Stefanou DC, Asimakopoulos G, Yagnik DR, Haskard DO, Anderson JR, Philippidis P., et al. Monocyte Fc gamma receptor expression in patients undergoing coronary artery bypass grafting. Ann Thorac Surg, 2004, 77, 951-955

[7] Hiesmayr MJ, Spittler A, Lassnigg A, Berger R, Laufer G, Kocher A., et al. Alterations in the number of circulating leucocytes, phenotype of monocyte and cytokine production in patients undergoing cardiothoracic surgery. Clin Exp Immunol, 1999, 115, 15-323

[8] Fjaertoft G, Hakansson LD, Pauksens K, Sisask G, Venge P. Neutrophil CD64 (FcγRI) expression is a specific marker of bacterial infection: A study on the kinetics and the impact of major surgery. Scand J Infect Dis, 2007, 39, 525-535

[9] Parolari A, Camera M, Alamanni F, Naliato M, Polvani GL, Agrifoglio M, et al. Systemic inflammation after on-pump and off-pump coronary bypass surgery: a one-month follow-up. Ann Thorac Surg, 2007, 84, 823-828

[10] Devaraj S, Yun JM, Duncan-Staley C, Jialal I. C-reactive protein induces M-CSF release and macrophage proliferation. J Leukoc Biol, 2008, 13, 1-6

[11] Quereshi SS, Lewis SM, Gant VA, Treacher D, Davis BH, Brown KA. Increased distribution and expression of CD64 on blood polymorphonuclear cells from patients with the SIRS. Clin&Exp Immunol, 2001, 125, 258-265

[12] Heijnen IAFM, van de Winkel JGJ. Human IgG Fc receptors. Int Rev Immunol, 1997, 16, 29-55

[13] Devaraj S, Dasu MR, Singhu U, Rao V, Jialal I. CRP stimulates superoxide anion release and tissue factor activity in vivo. Atherosclerosis, 2009, 203, 67-74

[14] Fingerle-Rowson G, Auers J, Kreuzer E. et al. Expansion of CD14+/CD16+ monocytes in critically ill cardiac surgery patients. Inflammation, 1998, 22, 367-79

[15] Andreeva H, Petrova P, Yonov M. Functional and phenotypic heterogeneity in monocyte subpopulation in neonates and adults. Clin Appl Immunol, 2000,1,36-45

[16] Tsuchida M, Watanabe H, Watanabe T, Hirahara H, Haga M, Ohzeki H. et al. Effect of cardiopulmonary bypass on cytokine release and adhesion molecule expression in alveolar macrophages. Preliminary report in six cases. Am J Resp Crit Care Med, 1997, 156, 932-938