Iraqi Journal of Biotechnology, 2015, Vol. 14, No. 1 , 63-69

Circulating Human IL-10 - Secreting Regulatory B Cells in Acute and Chronic Ischemic Heart Disease

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Received: November 24, 2014 / Accepted: May 5, 2015

Abstract: Ischemic heart disease (IHD) is a leading cause of death worldwide. Suppression of immune system after the resolution of infection or inflammation is an important process that limits immune-mediated pathogenesis, therefore, in this study for the first time in Iraq we highlights the importance of IL-10 secreting regulatory B cells (B10/Br1/B_{REG}) in ischemic heart disease immunoregulation. Peripheral blood lymphocytes were isolated from 83 patients with ischemic heart disease, then IL-10 secreting regulatory B cells were detected by using double staining immunocytochemistry (DS-ICC) with both CD19 and IL-10 monoclonal antibodies. Results showed the decreased number and mean percentage of B_{REG} in the peripheral blood of both acute and chronic ischemic heart disease in general when compared with controls, but these cells recorded higher number and mean percentage in acute than chronic ischemic heart disease, and there was a significant difference in the mean percentage of B_{REG} cells among the patient's groups. In conclusion, IL-10 secreting regulatory B cells play an important but limited role in ischemic heart disease immunoregulation due to their lower numbers, and further studies must be done for future directions correlated with how to manipulate these cells in immunoregulation with more advanced pathways.

Key words:IL-10, B cell, CIH, DS-ICC,IHD.

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Introduction

Ischemic heart disease (IHD) is a disease characterized by reduced blood supply to the heart. The coronary arteries supply blood to the heart muscle and no alternative blood supply exists, so a blockage in the coronary arteries reduces the supply of blood to heart muscle. Ischemic heart disease is caused by atherosclerosis which is characterized by the presence of atherosclerotic lesions in large and medium-sized elastic and muscular arteries, which may lead to narrowing of the vessel lumen and restriction of blood flow (1). As a result, the clinical presentation of ischemic heart disease will take place in many pictures. In general, ischemic heart disease appears as acute and\or chronic form according to the severity of disease. The acute form of IHD is characterized by acute coronary syndrome (ACS) which describes the spectrum of clinical manifestations which follow disruption of the coronary arterial plaque, complicated by thrombosis, embolization and varying degrees of

obstruction to myocardial perfusion. ACS occurs as a result of three problems (according to the appearance of electrocardiogram): ST segment elevation myocardial infraction (STEMI), non-ST segment elevation myocardial infraction (NSTEMI) or unstable angina (2). The chronic form of IHD is characterized by chronic high-grade stenosis of the coronary arteries. A transient mismatch causing reversible myocardial ischemia is the dominant feature of chronic ischemic heart disease (CIHD), which is also characterized by stable symptoms over a period of months, years, or even decades. Stable angina is the most frequent presentation of CIHD which is mainly caused by obstructive coronary atherosclerosis [3]. Suppression of immune system after the resolution of infection or inflammation is an important process that limits immune-mediated pathogenesis and autoimmunity. Several mechanisms of immune suppression have received a great deal of attention in the past three decades. One of these mechanisms is the suppression by regulatory B and T cells with different types. Many lines of evidence supporting an important role for B lymphocytes as both regulatory and killer cells in many inflammatory settings (4).

Three types of regulatory B cells are characterized in human by the production of IL-10 and TGF-β as a major regulatory cytokines or by the expression of FasL on their surface.

IL-10 – secreting regulatory B cells (B_{REG}), (Br1) or (B10), a small proportion of B cells, represent in human about (1-3) % of spleen B cells and <1% of peripheral blood B cells (5). In general, CD19 is a B-cell–specific, cell-surface protein that serves as appositive response regulator, and it gives the main phenotypical picture of B cells (6). However, peripheral blood B10 cells and B10PRO cells are highly enriched in the $CD24^{hi}CD27^{+}$ B cell subset, with approximately 60% also expressing CD38. Similar total numbers of IL-10^{+} B cells have been described in the $CD19^{+}CD24^{hi}CD38^{hi}$ and $CD19^{+}CD24^{int}CD38^{int}$ B cell subsets (7). Regulatory B cells can develop from different subsets of B cells. Whether B regulatory cells uniquely derive from a specific progenitor or originate within conventional B cell subsets is still an open question (8).

In general, regulatory B cells exert their regulatory functions both by secretion of cytokines, predominantly IL-10, and by direct cell-cell contact, in which CD80 and CD86 play a pivotal role. The important cellular targets include CD4+ T cells, CD8+ T cells, Tregs, monocytes, NK, Th17, antigen presenting cells, and even effector B cells (9). This study highlights the importance of IL-10 – secreting regulatory B cells in acute coronary syndrome by detecting their mean percentage in the peripheral blood of patients with acute coronary syndrome.

Materials and Methods

Forty-five patients with acute coronary syndrome and 38 patients with chronic ischemic heart disease under coronary artery bypass grafting surgery (CABG) were selected from Ibn-Al-Bitar hospital for cardiac surgery, and 5 ml of the peripheral blood were taken from these patients before receiving thrombolytic treatment for ACS patients and before surgery in the case of CIHD patients. Lymphocyte separation medium (Isopaque-Ficoll Mixture or Lymphoprip) was used for lymphocyte separation by density gradient centrifugation, then the

separated lymphocytes were fixed on positive charge slides in smears. Double-staining immunocytochemistry (DS-ICC) was used for the detection of IL-10 – secreting regulatory B cells by using rabbit anti-human CD19 and mouse anti-human IL-10 monoclonal antibodies (MyBioSource, USA). Staining was occurred by using ready – to - use immunohistochemistry (I H C) / immunocytochemistry (ICC) detection kit (Biotin free), One-Step HRP Polymer anti-Mouse, Rat and Rabbit IgG (H+L) with DAB (the first stain), (BioVision, USA). Benzidine dihydrochloride (BDHC) (UCD Company, Belgium) was used as the second stain. Negative control slides were included to determine the signal specificity. Finally, Slides were examined under the light microscope at 400X magnification power. The evaluation of IHC occurred by applying the following equation when considered +, <10%; ++, 10 to 50%; and +++, >50% as positive cells: The percentage of positive cells

= ***The number of positive cells / The number of total cells X 100***.

Results

The results showed that there was a difference in the mean percentage of B_{REG} positive cells among studied groups (figure 1). ACS patients of high risk (HR) and low risk (LR) with first ACS attack were exhibited high numbers of B_{REG} cells before aspirin treatment ($_{FNO}$) (AHR_{FNO} and ALR_{FNO}) with a mean of 0.75% and 0.62% respectively, whereas other ACS groups of patients showed lower mean percentage of B_{REG} especially those with regular using of aspirin AHR_{RUA} (0.38%) and ALR_{RUA} (0.36%).

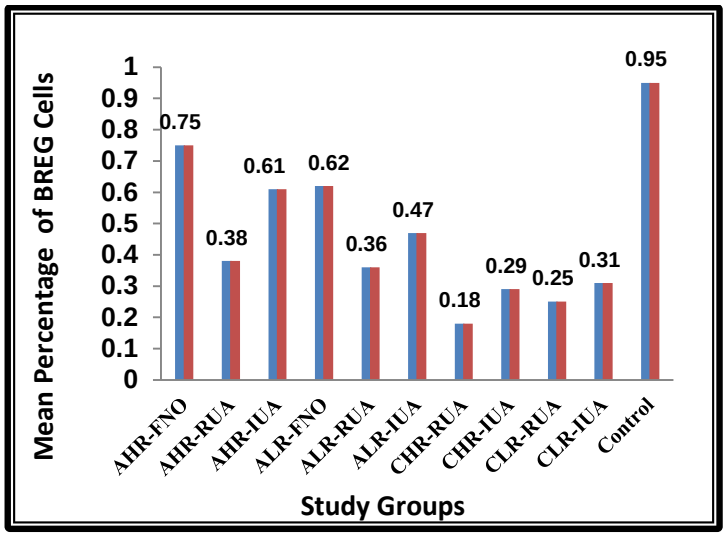

Figure (1): Mean percentage of B_{REG} cells among different study groups. (AHR-FNO, acute high risk first attack and no therapy; AHR-RUA, acute high risk and regular using of aspirin; AHR-IUA, acute high risk and irregular using of aspirin; ALR-FNO, acute low risk first attack and no therapy; ALR-RUA, acute low risk and regular using of aspirin; ALR-IUA, acute low risk and irregular using of aspirin; CHR-RUA chronic high risk and regular using of aspirin; CHR-IUA, chronic high risk and irregular using of aspirin; CLR-RUA chronic low risk and regular using of aspirin; CLR-IUA, chronic low risk and irregular using of aspirin)**.**

CIHD patients also showed differences in the number and mean percentage of B_{REG} cells among high and low risk, under regular or irregular using of aspirin. Significant ($P < 0.05$) difference was found between AHR_{FNO} and AHR_{RUA} (figure 2).

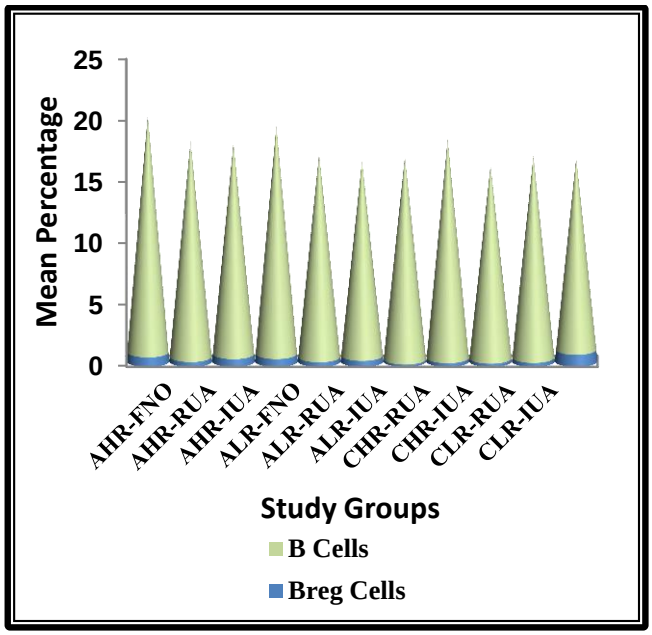

Figure (2): Mean percentage of B_{REG} and B cells among different study groups. (AHR-FNO, acute high risk first attack and no therapy; AHR-RUA, acute high risk and regular using of aspirin; AHR-IUA, acute high risk and irregular using of aspirin; ALR-FNO, acute low risk first attack and no therapy; ALR-RUA, acute low risk and regular using of aspirin; ALR-IUA, acute low risk and irregular using of aspirin; CHR-RUA chronic high risk and regular using of aspirin; CHR-IUA, chronic high risk and irregular using of aspirin; CLR-RUA chronic low risk and regular using of aspirin; CLR-IUA, chronic low risk and irregular using of aspirin)**.**

The results (figure 2) showed correlations with highly significant ($P < 0.01$) differences between (ALR_{RUA} and ALR_{IUA}), (CHR_{RUA} and CHR_{IUA}), and a significant ($P < 0.05$) difference between (CLR_{RUA} and CLR_{IUA}). Also, B_{REG} cells were recorded highly significant difference ($P < 0.01$) between ACS-LR and CIHD-LR under regular using of aspirin (ALR_{RUA} and CLR_{RUA}) with a mean of 0.36% and 0.25% respectively. Also, according to the risk factors, there was shown a highly significant $P < 0.01$ differences among patients with high risk and low risk groups (ALR_{RUA} 0.36% and AHR_{RUA} 0.38%), (AHR_{FNO} 0.36% and ALR_{FNO} 0.30%).

B cells also detected according to their CD19 expression which was appeared with high mean percentage in all study groups. A significant ($P < 0.05$) difference was found between (AHR_{FNO} and AHR_{RUA}) and (CHR_{RUA} and CHR_{IUA}), whereas highly significant ($P < 0.01$) correlation was recorded between ALR_{RUA} and ALR_{IUA}, but there was no difference between ACS-HR patients in correlation with regular or irregular using of aspirin. Also, there was no difference between CLR_{RUA} and CLR_{IUA}.

Discussion

Regulatory B cells appear to play an important role in controlling the pathogenic processes during atherosclerosis in ischemic heart disease. This study revealed that human regulatory B cells have immunoregulatory pathway according to their ability to secret the immunosuppressive/anti-inflammatory cytokine IL-10, but the role of these cells was restricted due to their very low percentage which represents < 1% of peripheral Blood mononuclear cells in healthy individuals, also, circulating regulatory B cells were recorded significant decrease in its number when compared with control (figure 1).

There are multiple factors that affect the function of regulatory B cells lead to cytokine secretion blocking or cytokine function inhibition. IL-10 secreted by regulatory B cells may be inhibited by another cytokine like interferon-γ (IFN-γ) (pleiotropic cytokine produced by T lymphocytes and natural killer cells) or the cells undergo IL-10 resistance by secretion more amounts of IFN-γ, thus IL-10 function will be restricted. A study by Yamana *et. al*., 2004 showed that peripheral blood CD4+ T cells from patients with active rheumatoid arthritis (RA) were able to produce greater amounts of interferon gamma after CD3 and CD28 costimulation in the presence of 1 ng/ml IL-10 than were normal control CD4+ T cells, although their surface expression of the type 1 IL-10 receptor was increased (10). Also, IFN-γ is generally antagonistic to TGF-β in the regulation of hematopoietic development and immune cell functions such as inflammation, also IFN-γ interfere with transforming growth factor beta signaling through direct interaction of Y box-binding protein YB-1with Smad 3 (11).

Aspirin has the ability to inhibit IL-4 which has an important role in the development and differentiation of B cells in general. As well as aspirin had the opposite effect of what might be expected and was clearly acting in a completely novel way. In mapping the gene responsible for the production of IL-4, they found that aspirin targets part of a complex of DNA binding proteins that form on the IL-4 promoter, the region that regulates the quantity of protein manufactured (12). Ischemic heart disease patients with diabetic mellitus have B cell function impairment (13).

Many factors may interfere with regulatory B cells function and development to make them inactive and abrogate their suppressive function, and according to the presence results, there was no correlation between disease severity and the lower percentage of regulatory B cells when compared with patients clinical and physical parameters because the severity of disease as explained previously may return to more than one risk factor affect the disease activity and according to this fact chronic ischemic heart disease patients could be divided to high risk and low risk groups.

The percentage of circulating regulatory B cells were significantly high in the peripheral blood of CIHD patients, while higher percentage of peripheral regulatory B cells were recorded in acute coronary syndrome patients which may be due to increase the cellular proliferation in response to the inflammatory conditions. There was no difference in the percentage of circulating regulatory B cells in ACS and CIHD patients. In acute coronary syndrome, peripheral B cells were found in high mean percentage in all groups under

study (figure 2), whereas, regulatory B cells recorded lower mean percentage when compared with controls. The cause underlining these results is that the immune system will normally switch on the anti-inflammatory factors that lead to increase the cellular proliferation of B cells in order to overcome the inflammatory damaged effect.

In spite of that, regulatory B cells were showed to move in an opposite direction due the negative effects of the multiple factors mentioned above. regular/irregular using groups of aspirin and the mean percentage of regulatory B cells was recorded a significant association because the results showed that there was a highly significant ($P < 0.01$) differences between (ALR_{RUA} and ALR_{IUA}), (CHR_{RUA} and CHR_{IUA}), and significant difference ($P < 0.05$) between (CLR_{RUA} and CLR_{IUA}) which revealed the important effect of aspirin, and this may be due to its effect on IL-4 as explained above (12).

Any difference in the mean percentage of regulatory B cells for one or two patients outside the expected range of the studied group was return to the difference in the number and the type of risk factors that predispose to ischemic heart disease which determine the severity of disease and as a result, it will determine the prevalence and the cellular type included in the mechanisms of pathogenesis or immunoregulatory mechanisms in ischemic heart disease.

According to the effects of risk factors, there was a highly significant $P < 0.01$ differences (figure 2) among patients with high risk and low risk groups (ALR_{RUA} 0.36% and AHR_{RUA} 0.38%), (AHR_{FNO} 0.36% and ALR_{FNO} 0.30%).

In conclusion, B_{REG} cells showed lower numbers in all groups under study included both ASC and CIHD.

Acknowledgment

Thanks for all the medical and technical staff in Al-Nahrain University – College of Medicine, Ibn-Al-Bitar Hospital for Cardiac Surgery/Iraq-Baghdad, and Al-Kadhymia Teaching Hospital/Iraq-Baghdad.

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