Journal of the Egyptian Society of Parasitology, Vol. 47, No. 1, April 2017
J. Egypt. Soc. Parasitol. (JESP), 47(1), 2017: 207- 210

DOWN REGULATION OF CLASSICAL MONOCYTES SUBSET IN PATIENTS WITH HCV RELATED LIVER FIBROSIS

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Abstract

Chronic liver disease is a worldwide common pathology characterized by inflammatory and fibrotic processes that may lead to progressive evolution from chronic hepatitis to cirrhosis. Peripheral blood monocytes may play an important role in the pathogenesis and resolution of liver fibrosis. These cells may offer new approaches for better understanding the pathogenesisof liver fibrosis.

This work defined the proportion of circulating classical monocyte subset with hematopoietic origin in peripheral blood to establish the possible potential role of this subset as non-invasive biomarker of liver fibrosis in patients with HCV related chronic liver disease. Forty patients with HCV induced chronic liver disease were classified according to the stage of liver fibrosis after METAVIR score into 4 groups, patients with stages F1, F2, F3 & F4 liver fibrosis (10 patients each) and 10 healthy subjects served as normal controls. Flowcytometric analysis for immunophenotypic characterization for identification of levels of circulating peripheral blood classical monocytes subset in different groups studied was carried out using monoclonal antibodies anti-CD45, anti-CD14 and anti-CD16.

The results: data demonstrated a significant down regulation ($p< 0.01$) in the proportion of classical monocytes subset ($CD45^{+ve}$, $CD14^{+ve}$ and $CD16^{-ve}$) in patients with chronic hepatitis C related liver disease compared to healthy subjects. Data also demonstrate that down regulation of the expression of classical monocyte subset paralleled the worsening severity of liver disease and the progression of liver fibrosis.

Key words: Monocytes, Chronic liver disease, HCV, Liver fibrosis.

Introduction

Circulating monocytes are committed precursors with the capacity to differentiate into a variety of phagocytes, including macrophages and dendritic cells, there is growing evidence that these monocytes can differentiate into other cell types as well, including cells with the typical characteristics of endothelial cells and fibroblasts (Mattoli*et al.*, 2009; Seta and Kuwana, 2010). They are commonly defined and discriminated by the extent of their cell surface expression of CD14 and CD16, with associated differences in function and phenotype to the intensity of expression of these markers (Appleby *et al*, 2013). Accumulating evidences from murine models indicated that monocyte infiltration into the liver is a major pathogenic factor for chronic hepatic inflammation and fibrosis (Imamura *et al*, 2005; Karlmark*et al*, 2009; Mitchell *et al*, 2009; Seki *et al*, 2009).

Monocytes are able to act as first responders' to inflammatory signals, infiltrating inflamed tissues shortly after the onset of injury. Based on a distinct pattern of chemokine receptors expressed on their surface, monocyte subsets show fundamentally different patterns in their migratory behaviour (Tacke, 2012). Two major subsets of peripheral blood monocytes are recognized;the classical monocytes ($CD14^{++}CD16^{-}$) subset and non-classical monocytes ($CD14^{+}CD16^{++}$) subset (Rao *et al*, 2009).

The study aimed to define the proportion of circulating classical monocyte subset with hematopoietic origin in circulating peripheral blood and to assess the possible potential role of this subset as non-invasive biomarker of liver fibrosis in patients with HCV related

chronic liver disease.

Patients and Methods

In the present work, forty patients with HCV induced chronic liver disease were studied.

Patients were classified according to METAVIR score for hepatic activity index for scoring of necroinflammatory activity in chronic hepatitis into 4 stages of fibrosis; patients with stage F1 fibrosis (10 cases), stage F2 fibrosis (10 cases), stage F3 fibrosis (10 cases) and stage F4 fibrosis (10 cases); 10 healthy individuals served as normal controls. Assessment of percentage of circulating peripheral blood monocytes of hematopoietic origin in different groups studied was carried out by immunophenotype characterization by flow cytometric analysis (COULTER, EPICS® XL-MCL, Brea, CA, USA), using fluorochromelabeled mouse anti-human monoclonal antibody (mAb) CD45 conjugated with ECD (EPICS®, Coulter, Marseille, France), fluorescence labelled mouse anti-human mAb for CD14 conjugated with FITC (EPICS®, Coulter, Marseille, France), Fluorochrome mouse anti-human mAb for CD16 conjugated with PE (EPICS®, Coulter, Marseille, France).

Results

The surface expression of CD14 and CD16 of peripheral blood monocytes population were detected on gated $CD45^+$cells (Fig. 1), by flow cytometric analysis of peripheral blood monocytes in different groups of patients with chronic HCV related liver fibrosis.

In the current study, the proportion of the percentage of classical monocytes ($CD45^+$ $CD14^+$ $CD16^-$) subset,by flow cytometric analysis in the healthy subjects, range from 80.8-92% of total circulating peripheral blood monocytes (Tab. 1). Data also demonstrated that the percentage of this classical monocytes subset was down regulated (P<0.01) in all studied patients groups compared to the controls. Moreover, this down regulation of classical monocytes ($CD45^+$ $CD14^+CD16^-$) subset in patients with HCV related chronic liver disease was found to match the progression of the disease and the stages of liver fibrosis.

The marked significant decrease (P<0.01) of the percentage of the classical monocytes ($CD45^+$ $CD14^+$ $CD16^-$) subset was mostly noticed among patients with stage F4 liver fibrosis compared to those with other stages of liver fibrosis.

Table 1: Percentage of classical monocytes subset ($CD45^+$ $CD14^+$ $CD16^-$) in healthy subjects and patients with HCV-related chronic liver disease.

	Controls (n=10)	Liver Fibrosis(n=10)			
		F1	F2	F3	F4
Range	80.8-92	74.5-85.7	49-70.5	46.8-54.8	0.8-9.1
Mean±SD	88.01±3.879	79.63±3.831[a]	60.95±6.688[ab]	50.62±2.598[abc]	4.95±3.504[abcd]

[a]p<0.01: Controls *vs.* other groups, [b]p<0.01: Stage F1 *vs.* other groups, [c]p<0.01: Stage F2 *vs.* other groups, [d]p<0.01:Stage F3 *vs.* other groups, [d]p<0.01:Stage F4 *vs.* other groups

Discussion

In the current study, flowcytometric analysis revealed that the proportion of classical monocytes ($CD45^+CD14^+CD16^-$) subset, in circulating peripheral blood of healthy subjects range from 80.8-92%. Our findings agree with those of others who found that the classical monocytes ($CD45^+CD14^+$ $CD16^-$) subset represents the largest population of the circulating peripheral blood monocytes and is an important scavenger cells (Stansfield and Ingram, 2015).

The present study also revealed that the percentage of the classical monocytes subset was down regulated in different groups of patients compared to controls. The down regulation of classical monocytes ($CD45^+$ $CD14^+CD16^-$) subsetnoticed in patients with HCV related chronic liver disease was found to match the progression of the disease and the stages of liver fibrosis. These findings agreed with those of Liaskou *et al.* (2013)

who reported that the classical monocytes constitute approximately 80% of peripheral blood monocytes and their percentage were significantly down-regulated in all liver diseases compared to control. Furthermore, the marked decrease of the percentage of the classical monocytes ($CD45^+CD14^+CD16^-$) subset was mostly noticed among patients with stage F4 fibrosis compared to those with other stages of liver fibrosis.

This finding coincided with those of others who demonstrated a strong shift towards the non-classical monocytes $CD14^+CD16^+$ subset in patients with chronic liver disease, especially in patients with established cirrhosis and suggested that the down regulation of classical monocytes may be due to differentiation of some classical monocytes into non-classical monocytes (Zawada *et al*, 2011; Stansfield and Ingram, 2015), or to the recruitment of classical monocytes into injured liver in response to chemokine MCP-1/CCR2 pathway (Zimmermann *et al*, 2010).

Conclusion

The shift of classical monocyte subset towards the non-classical monocyte subset may serve as predictive biomarkers and a non-invasive tool for diagnosis of progression of liver disease and development of liver fibrosis. MCP-1 and CCR2 play a critical role in fibrogenesis and may trigger monocytes recruitment to the injured liver promoting their further differentiation into collagen type I producing monocytes, suggesting that monocytes may become a novel target for anti-fibrotic therapy.

Acknowledgment

This study was kindly supported by Theodor Bilharz Research Institute Internal Research Project Grant #101T.

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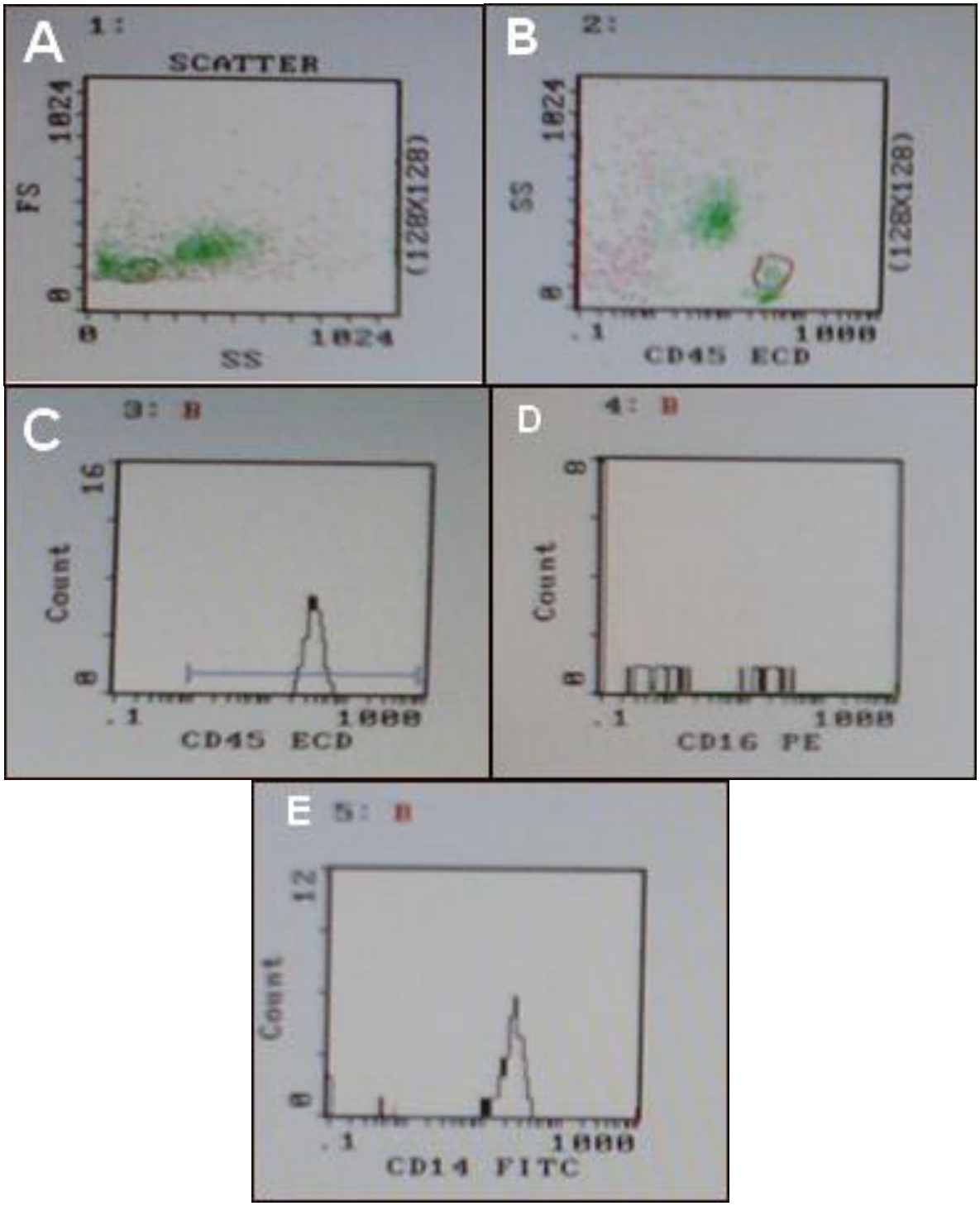

Fig.1: Flow cytometric analysis of peripheral blood monocyte using a combination of multicolour (four colour) flow cytometry, multi-parameter. a: Represents different peripheral blood leukocytes subsets according to size and granularity. B: Reveals the gating on $CD45^{+}$ monocytes (red circle in right lower corner of figure). C: Shows the percent of CD 45^{+} monocytes. d: Reveals percent of $CD16^{+}$gated onCD 45^{+} monocytes. E: Shows percent of $CD14^{+}$ gatedon CD 45^{+} monocytes.