Maturation State and Function of Monocyte Derived Dendritic Cells in Liver Transplant Recipients

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ABSTRACT

Background: Dendritic cells (DCs) are potent antigen presenting cells for triggering of the immune reaction post transplantation. These cells are centrally involved in the initiation of T cell-dependent immune responses. **Objective:** To compare the level of DC maturation and function in liver transplant recipients with healthy controls. **Methods:** In this study, twelve peripheral blood samples were selected from six liver transplant patients and six healthy controls. After the generation of DCs from monocytes, expression levels and mean fluorescent intensity (MFI) of several DC maturation markers were evaluated using flowcytometry. Secretion of IL-6, IL-12 and IL-23 proinflammatory cytokines was determined using ELISA. Gene expressions of TLR-2, TLR-4 and IL-23 were analyzed using real-time PCR. **Results:** DC expression markers including CD83 (p=0.007) and CD86 (p=0.02), as well as secretion of IL-6 (p=0.02) and IL-12 (p=0.007) by DCs were significantly increased in liver transplant patients compared with healthy controls. The MFI of CD86 (p=0.009) and HLA-DR (p= 0.005) expression on DCs was also higher in patients. The expression of TLR-2 transcripts in DCs of patients was higher than that of the controls (p=0.03). **Conclusion:** Based on these findings, increased frequency of DCs expressing CD83 and CD86, higher expression of CD86, HLA-DR, and TLR-2 as well as elevated secretion of proinflammatory cytokines in DCs of liver transplant recipient's point to the more mature phenotype and active function of DCs in patients compared with controls.

Shariat A, et al. Iran J Immunol. 2014; 11(3):153-165

Keywords: Dendritic Cells, Liver Transplantation, TLR

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INTRODUCTION

Liver transplantation is the only available life-saving therapy for chronic end-stage liver disease and fulminant acute liver failure (1). After the liver transplantation, the immune system responds to the foreign antigens of the donor graft in order to eliminate the graft rejection (2). Transplant rejection is the result of a complex series of interactions involving coordination between both the innate and adaptive immune systems (3).
T cells recognize antigens expressed on professional antigen presenting cells (APCs), which are mainly dendritic cells (DCs) (3). DCs, with the capacity of antigen presentation to naïve T cells, are the key effectors responsible for triggering the allospecific immune reaction after transplantation (4). DCs are involved in the graft rejection via the direct and indirect pathways (5). Therefore, DCs centrally contribute to the initiation of T cell-dependent immune responses. These cells in the graft recipient can act as the foe, stimulating rejection (6). In the context of transplantation, it is well documented that DCs are key initiators of the immune response (4).
DCs can be activated through a signaling cascade initiated by stimulation of toll like receptors (TLRs) (7). Graft mediated activation of TLRs on DCs might in turn activate the adaptive immune system through T cell maturation (8). Increased TLR-2 and TLR-4 expression on DCs may participate in acute rejection after liver transplantation (9). TLR-2 and TLR-4 also recognize endogenous ligands which are released into the circulation during the pre-transplantation period and increase the risk of allograft rejection (10,11).
TLR activation on DCs induces pro-inflammatory cytokines, which contribute to the clinical outcome of severe liver injuries (12). TLRs initiate a signaling pathway, which in DCs begins a maturation program consisting of increased expression of costimulatory molecules and the release of proinflammatory cytokines (8). In particular, TLR signaling can induce the production of IL-12, IL-23 and IL-6 from DCs (13). IL-12 production from activated DCs contributes to the generation of specific immune responses (14) and induces Th1 cell deviation. IL-23 appears to be required for amplifying and stabilizing the response of differentiated Th17 cells (15). Th17 cells and IL-6 contribute to the mechanisms of rejection after transplantation (16). Recently, a novel role for IL-6 in the development and differentiation of the Th17 cell has been reported (17). In view of the fact that DCs are involved in allograft rejection, the inflammatory reactions and DCs post liver transplantation can be targeted to generate beneficial therapeutic immunity. Therefore, in this study the state of maturation and function of DCs were investigated in liver transplant recipients in comparison with controls.

MATERIALS AND METHODS

Study Population and Data Collection. Of the patients who underwent liver transplantation surgery between 2013 and 2014 in the Transplant Center of Namazi Hospital, Shiraz, Iran, 6 transplant recipients were selected. Underlying diseases for liver transplantation in these patients included: PSC (primary sclerosing cholangitis), hypercholesterolemia, cryptogenic cirrhosis, hepatitis B virus infection, NASH (non alcoholic steato-hepatitis), autoimmune hepatitis. At day 30 post-transplantation, 20 ml EDTA-treated blood samples were taken from liver transplant patients. All patients

received a uniform triple immunosuppressive regimen with oral prednisone (20 mg/day), oral cyclosporine (100-150 mg/day) and oral cellcept (2-3 g/day). Also, in this study blood samples were taken from 6 healthy individuals. The age range was similar in both groups (median 42 years, range 24-65 years) and male to female ratio was two to one in both groups.

Generation of Monocyte Derived DCs (MoDCs). Twenty ml fresh whole blood was obtained from liver transplant patients and healthy individuals by venous puncture and collected in sterile tubes containing EDTA. Peripheral blood mononuclear cells (PBMCs) were isolated and DCs were generated from monocytes as previously described (18). Briefly, CD14+ monocytes were isolated from PBMCs by positive selection using a MACS system (Miltenyi Biotech, Bergisch Gladbach, Germany), according to the manufacturer's instructions. Monocytes were cultured in six-well tissue culture plates (5×10^5 cells/ml) in fresh complete RPMI-1640 medium (Invitrogen, USA) supplemented with 10% heat-inactivated fetal bovine serum (Life technologies, USA), 4mM L-glutamine (Life technologies, USA), 100 IU/ml penicillin (Life technologies, USA), 1% sodium pyruvate (Bioidea, Iran), 1% non essential amino acid (Life technologies, USA), 1000 IU/ml recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF; R&D Systems, UK) and 500 IU/ml recombinant human interleukin-4 (IL-4; R&D Systems, UK). The cultures were fed with fresh medium and cytokines every 3 days. To induce maturation of DCs, the immature DCs were further incubated with 1000 IU/ml recombinant human tumor necrosis factor-α (TNF-α; R&D Systems, UK) for an additional 48 hours. After 7 days of culture in a 37 °C humidified incubator with 5% CO2, the cells were harvested and counted.

Detection of Maturation Markers by Flowcytometry. The markers (CD83, CD86, CD1a and HLA-DR) of MoDCs were determined with dual-color cytometry. Cell suspensions were incubated for 45 minutes on ice in the dark with the fluorescein isothiocyanate (FITC) and phycoerythrin (PE)-conjugated mAbs (PE-anti-CD14, FITC-anti-CD83, FITC-anti-CD86, FITC-anti-CD1a and FITC-anti-HLADR) (eBiosciences, USA). The stained cells were washed with PBS and resuspended with 300 µL PBS.

Assay of Cytokine Secretion by ELISA. After 7 days of culture and maturation of MoDCs, the supernatants were removed and the concentrations of IL-6, IL-12 and IL-23 were determined by ELISA using commercial human IL-6, IL-12p70 and IL-23 ELISA Ready-SET-Go assay (eBioscience, USA), according to the manufacturer's instructions.

RNA Extraction and cDNA Synthesis. According to the routine protocol for isolation of RNA from cells, MoDCs were lysed and total RNA was isolated by RNX plus (Cinnagen, Iran). The purity and integrity of RNA were determined by measuring the optical density ratio in 260 and 280 nm and agarose gel (1%) electrophoresis. Traces of the residual genomic DNA were removed by DNase digestion from DNA set (Roche, Germany). Subsequently one microgram of each RNA sample was reversely transcribed to cDNA by Reverse Transcriptase (Vivantis, Malaysia) and random hexamer. The cDNA synthesis was performed in two steps. First, RNA (1 µl/µg), dNTPs (1 µl/10 mM), and random hexamer (1 µl/0.2 µg) were mixed and incubated at 65°C for 7 minutes and then on ice for 2 minutes. Second, M-Mulv reverse transcriptase enzyme (1 µl/200 Unit), RT-buffer (2 µl/10x), RNase inhibitor (1.3 µl/ 60 Unit), and nuclease free water were mixed and added to product of the first step. Then the final mix was incubated at 45°C for 90 minutes and 85°C for 5 minutes.

Design of Primers. The primers were designed by primer blast software of NCBI for transcripts of TLR-2 (NM_003264.3), TLR-4 (NM_ 003266.3), IL-23 (NM_016584.2)

and β-actin (NM_001101.3) as the internal control and evaluated by version 7 of Oligo software（MBI Company, USA). The primer sequences used in this study are listed in Table 1.

Table 1. Primer sequences were used in Real time-PCR.

Gene	Primer	Sequense (5' -> 3')
TLR-2	Forward	TCCGCCTCTCGGTGTCGGAA
	Reverse	AAACGGTGGCACAGGACCCC
TLR-4	Forward	TCAAGCCAGGATGAGGACTG
	Reverse	CAGCAATGGCCACACCGGGA
IL-23	Forward	AGTGGAAGTGGGCAGAGATTC
	Reverse	CAGCAGCAACAGCAGCATTAC
B-actin	Forward	GGCGGCACCACCATGTACCC
	Reverse	GACGATGGAGGGGCCCGAC

TLR-2: Toll like receptor 2; TLR-4: Toll like receptor 4; and IL-23: Interleukin 23.

TLR-2, TLR-4 and IL-23 mRNA Expressions Determined by Real Time PCR. Real time PCR was performed for the quantitative analysis of TLR-2, TLR-4 and IL-23 mRNA expression profiles in MoDCs of liver transplant patients and healthy volunteers. The PCR mix contained: SYBR green Premix (10 µl) by Ex-taq (Takara, Japan), SYBR Green Dye (0.4 µl), forward and reverse primers (1 µl) and template cDNA (2 µl). Experimental samples were run in duplicate with the same concentration of cDNA per reaction. The PCR thermo-cycling condition included: one cycle 95°C for 2 minutes, followed by 40 cycles of 95°C for 30 seconds and 65°C for 20 seconds using Step One Plus Real-Time instrument (ABI, applied biosystems Step One plus, USA). The specificity of amplification reaction was confirmed by a melting-curve analysis. The results for the target genes were measured as fluorescent signal intensity and normalized to the internal standard gene β-actin. A cycle threshold (Ct) value was calculated for each sample; sample values were averaged. The mean Ct value of target genes in each sample was normalized using β-actin gene Ct value to give a ΔCt value.

Statistical Analysis. All results were expressed as mean ± standard error (SE). All samples were analyzed using GraphPad Prism 5.0 software (GraphPad Software, USA). Differences between groups were evaluated by non-parametric Mann-Whitney *U-test*. A *p* value of 0.05 or less was considered statistically significant.

RESULTS

Generation of MoDCs and Changes in the Expression Levels of DC Maturation Markers in Liver Transplant Recipients and Healthy Individuals. In groups of liver transplant recipients and healthy individuals, CD14+ monocytes isolated by MACS system (with >95% purity) (Figure 1A) were cultured with GM-CSF and IL-4 (Figure

1Band 1C). TNF-α was added to induce the terminal maturation of DCs. Analysis by flowcytometry showed the absence of CD14 positive cells after 7 days of culture and monocytes were differentiated into mature DCs (Figure 1D).

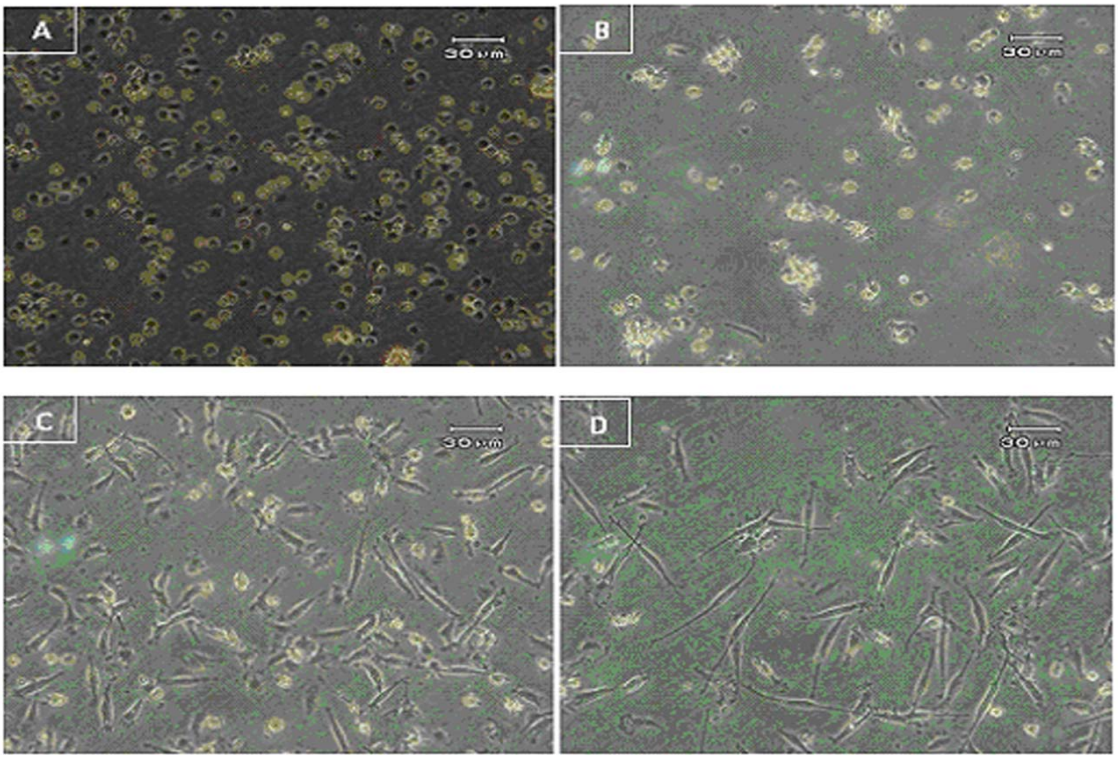

Figure 1. Monocytes cultured with GM-CSF/IL-4/TNF-α after 7 days differentiate into mature dendritic cells. Monocytes isolated by MACS system (A), Immature dendritic cells cultured with GM-CSF/IL-4 for 3 and 5 days (B,C) respectively, and mature dendritic cells after adding TNF-α at 7 days (D). Magnification 20X.

Data in figures 2 and 3 showed the absence of CD14+ cells and the presence of CD83+/ CD86+/ CD1a+/ HLA-DR+ cells (Figure 1D). These results indicated that monocytes were differentiated into DCs. Table 2 shows the expression levels for surface markers including: CD83, CD86, CD1a and HLA-DR of MoDCs in each studied liver transplant patient by flowcytometry. However, no significant differences were found in the expression of each DC maturation markers between 6 evaluated transplant patients.

The expression levels of CD1a, CD83, CD86 and HLA-DR in MoDCs of liver transplant recipients were compared with that of healthy individuals (Figures 2 and 3). Table 3 shows an average mean of fluorescence intensity (MFI) for surface markers of MoDCs in liver transplant recipients and healthy individuals.

Results showed the expression level of CD1a in liver transplant recipients had no significant difference in comparison with that of healthy individuals ($p=0.1$; median value of 39.6% versus 30.4%, respectively, Figures 2 and 3B).

Table 2. The expression levels of maturation markers on MoDCs in studied patients by flowcytometry.

Patients	CD83(%)	CD86(%)	CD1a(%)	HLA-DR(%)
1	68	92	39	85
2	50	85	40	84
3	60	90	35	86
4	45	80	43	85
5	50	87	36	86
6	45	85	38	84

Also, the expression level of CD83 in liver transplant recipients was significantly increased compared to that of healthy individuals (p=0.007; median value of 68.5% versus 21.9%, respectively, Figures 2 and 3C).

Table 3. Mean fluorescence intensity (MFI) of MoDC surface markers in liver transplant recipients and healthy individuals. The data are shown as means ± SE.

Surface Marker of MoDCs	Liver Transplant Patients	Healthy Individuals
	MFI±SE	MFI±SE
CD38	30± 0.3	30.7± 0.3
CD86	106±3.1	74± 6.7
CD1a	78.5± 6.5	74 ±2
HLA-DR	72.3± 1.8	50± 0.8

Furthermore, the expression level of CD86 in liver transplant recipients was significantly increased in comparison with that of healthy individuals (p=0.02; median value of 92.9% versus 78.3%, respectively, Figures 2 and 3D). Also, the MFI for CD86 was significantly increased in liver transplant recipients compared to that of healthy individuals (p=0.009, Table 3).

However, the expression level of HLA-DR in liver transplant recipients had no significant difference in comparison with that of healthy individuals (p=0.5; median value of 85.6% versus 87.8%, respectively, Figures 2 and 3E). Whereas, the MFI for HLA-DR was significantly increased in liver transplant patients compared to that in healthy individuals (p=0.005; Table 3).

Comparison of the Cytokine Secretions of MoDCs in Liver Transplant Recipients and Healthy Individuals. In liver transplant patients and healthy individuals, IL-6 concentrations were 352.3 ± 4.6 pg/ml and 312 ± 1.08 pg/ml, respectively. Results showed a significant difference in IL-6 secretion by MoDCs in liver transplant patients and healthy individuals (p=0.02; Figure 4A).

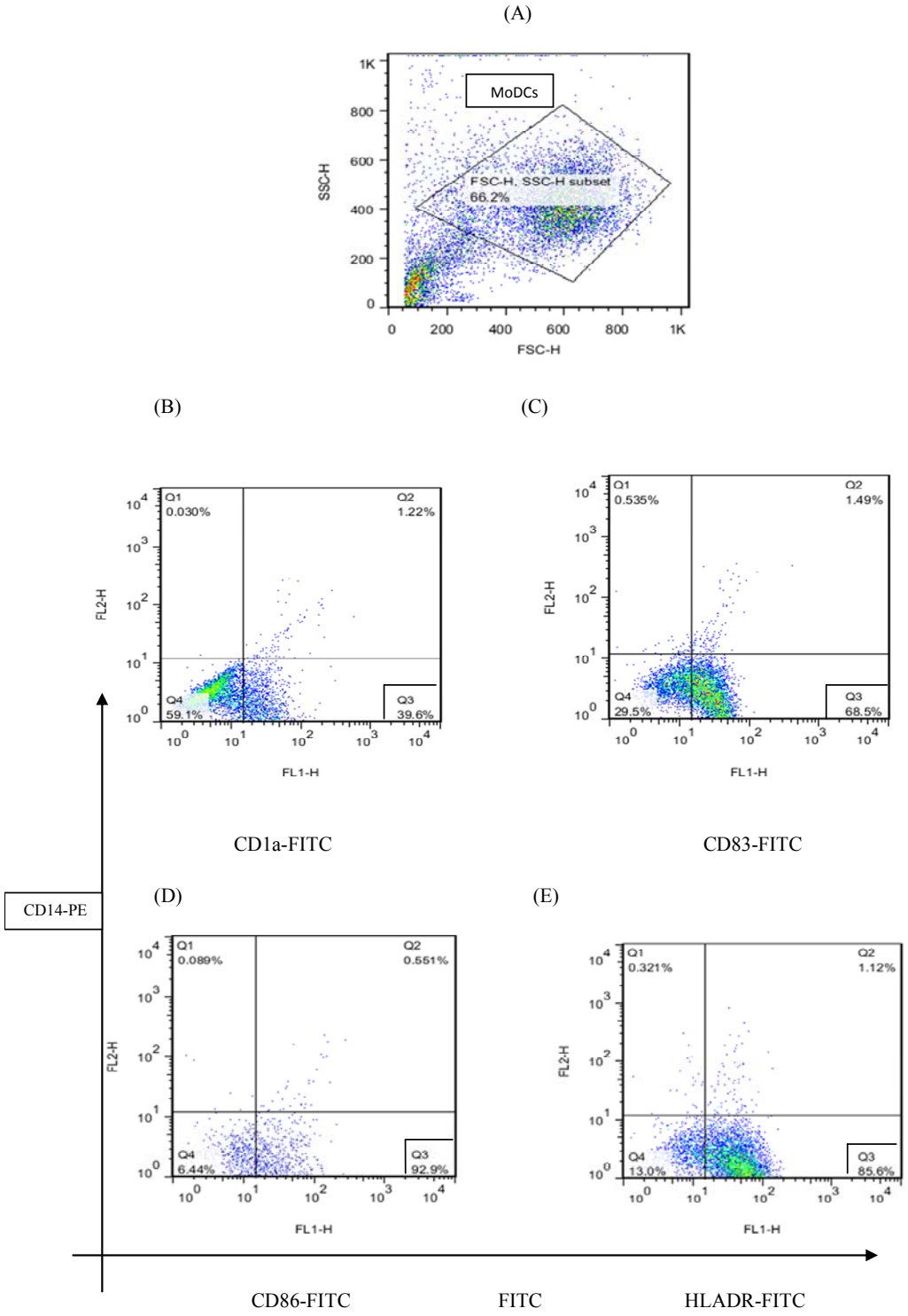

Figure 2. The cell surface phenotype of dendritic cells in liver transplant patients was examined by dual-color cytometry. FSC vs. SSC profile, gating of monocyte derived dendritic cells (MoDCs) (A). Expression of surface markers: CD1a (39.6%) (B). CD83 (68.5%) (C). CD86 (92.9%) (D), and HLA-DR (85.6%) (E) on monocyte derived DCs in liver transplant patients and absence of CD14 positive cells after seven days in the presence of GM-CSF, IL-4 and TNF-α. CD14-PE, phycoerythrin-conjugated CD14; CD1a-FITC, fluorescein isothiocyanate-conjugated CD1a; CD83-FITC, fluorescein isothiocyanate-conjugated CD83; CD86-FITC, fluorescein isothiocyanate-conjugated CD86; HLADR-FITC, fluorescein isothiocyanate-conjugated HLA-DR.

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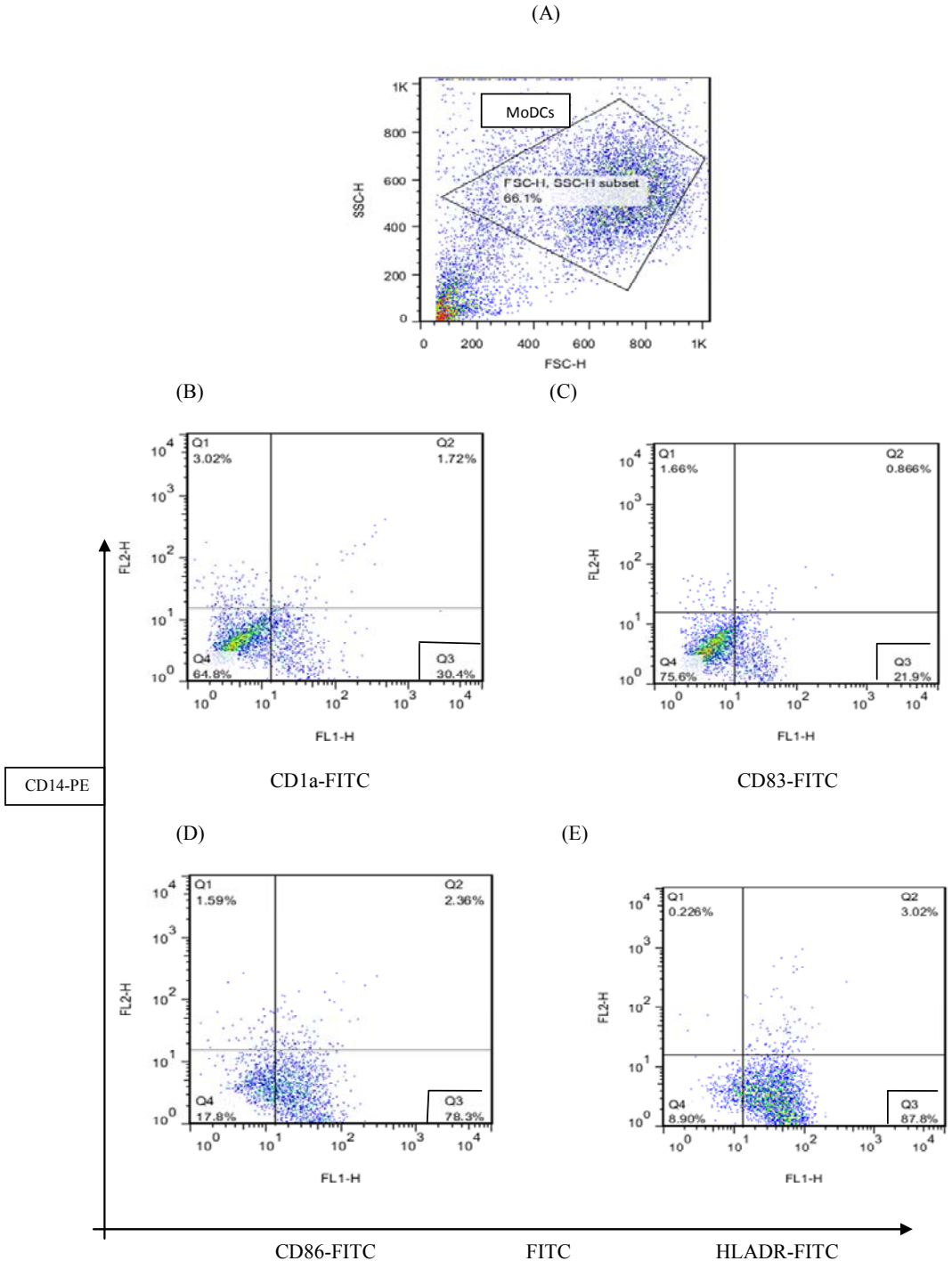

Figure 3. The cell surface phenotype of dendritic cells in healthy individuals was examined by dual-color cytometry. FSC vs. SSC profile, gating of monocyte derived dendritic cells (MoDCs) (A), Expression of surface markers: CD1a (30.4%) (B), CD83 (21.9%) (C), CD86 (78.3%) (D) and HLA-DR (87.8%) (E) on monocyte derived DCs in control group and absence of CD14 positive cells after seven days in the presence of GM-CSF, IL-4 and TNF-α. CD14-PE, phycoerythrin-conjugated CD14; CD1a-FITC, fluorescein isothiocyanate-conjugated CD1a; CD83-FITC, fluorescein isothiocyanate-conjugated CD83; CD86-FITC, fluorescin isothiocyanate-conjugated CD86; HLADR-FITC, fluorescein isothiocyanate-conjugated HLA-DR.

Whereas, in liver transplant patients and healthy individuals IL-12 contents were 2.33 ± 0.1 pg/ml and 1.54 ± 0.03 pg/ml, respectively. IL-12 secretion by MoDCs in liver transplant patients was significantly increased compared to that of in healthy individuals (p = 0.007; Figure 4B).

And also, in liver transplant patients and healthy individuals IL-23 contents were 12.66 ± 1.1 pg/ml and 13.1 ± 0.1 pg/ml, respectively. There was no significant difference in the IL-23 levels produced by MoDCs of liver transplant recipients and healthy individuals in vitro (p=0.7; Figure 4C).

Analysis of Gene Expressions of TLR-2, TLR-4 and IL-23 by Real Time PCR. To quantify the expression level of TLR-2, TLR-4 and IL-23 transcripts in MoDCs of liver transplant patients and healthy individuals, we used real time-PCR.

In liver transplant recipients and healthy individuals, mean ΔCt values for TLR-2 mRNA expression were 5.4 ± 1.4 and 3.56 ± 0.4, respectively. Gene expression of TLR-2 was significantly increased in liver transplant patients compared to that of healthy individuals (p=0.03) so that in liver transplant recipients, TLR-2 mRNA was expressed about 1.5 fold more than that in healthy individuals (Figure 5).

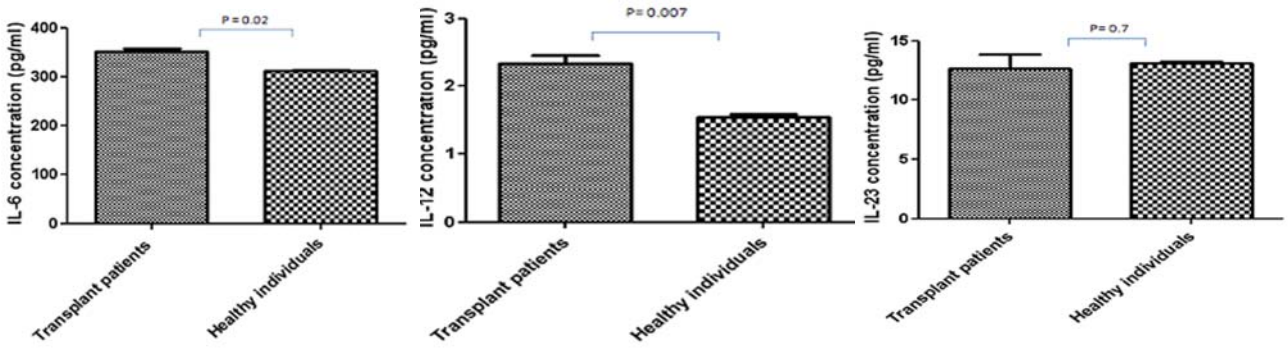

Figure 4. Levels of cytokines secreted by monocyte derived DCs. The IL-6 (A), IL-12 (B) and IL-23 (C) concentrations in the supernatants were measured by ELISA. Productions of IL-6 and IL-12 in liver transplant patients were statistically increased compared to that of healthy individuals (A and B).IL-23 secretion had no significant difference in liver transplant patients compared to healthy individuals (C). P values less than 0.05 is considered statistically significant. The data are shown as mean ± SE.

Also, in liver transplant recipients and healthy individuals, mean ΔCt values for TLR-4 mRNA expression were 3.52 ± 0.7 and 3.54 ± 0.97, respectively. Results showed no significant difference in the expression of TLR-4 between liver transplant patients and healthy individuals (p=0.9; Figure 5).

Furthermore, in liver transplant patients and healthy individuals, the mean ΔCt values for IL-23 mRNA expression were 7.02 ± 0.4 and 7.18 ± 0.1, respectively. The expression of IL-23 showed no significant difference in transplant patients compared to healthy individuals (p=0.8; Figure 5).

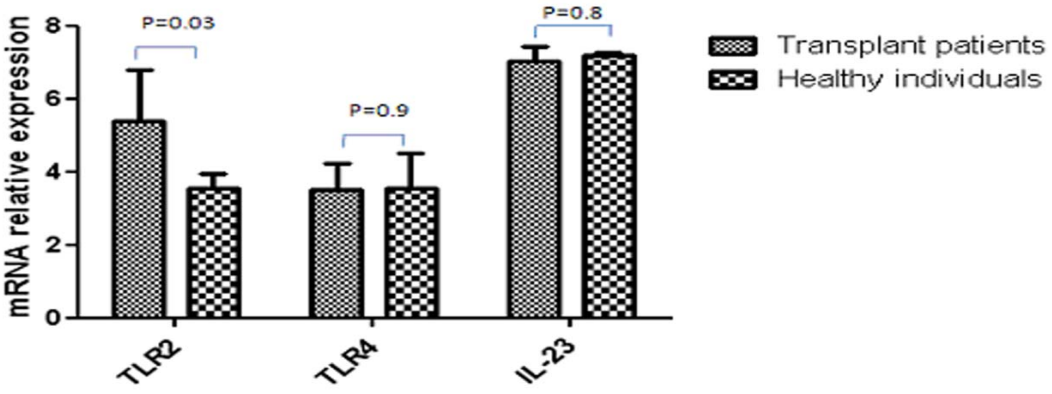

Figure 5. Mean ΔCt values for mRNA relative expressions of TLR-2, TLR-4 and IL-23 were determined by real-time PCR. Expression of TLR-2 gene was significantly increased in liver transplant patients compared to healthy individuals. Expression of TLR-4 and IL-23 genes had no significant differences between the two groups. P values less than 0.05 is considered statistically significant. The data are shown as mean ± SE.

DISCUSSION

DCs are potent antigen-presenting cells that can stimulate both B and T lymphocytes. In addition, DCs are key effectors, mainly responsible for triggering the specific immune reaction after transplantation (4). Through antigen presentation to naïve T cells, DCs play a major role in allograft rejection (4). The goal of this study was to investigate the expression level of markers involved in the maturation and function of DCs in liver transplant recipients compared with healthy individuals. This is the first study that dissects the state of maturation and function of DCs in liver transplant patients facing the risk of allograft rejection.

In this study, we evaluated expression levels of markers (CD83, CD86, CD1a and HLADR) in MoDCs in the above-mentioned groups. During DC maturation, CD83 is rapidly upregulated together with the costimulatory molecules (19). CD83 plays a pivotal role in the mediation of DC/T cell interaction and induction of T-cell activation (20). Elkord *et al*. (21) suggested that the elevated expression of CD83, as a marker of functional maturation, correlates with the ability of DCs to produce increased amounts of immunomodulatory cytokines. In this study there was a significant difference in the expression level of CD83 in transplant patients and healthy individuals.

Although the role of CD1a expression on DC function has not been fully explored, CD1a expression is considered more than just a phenotypic marker of a unique DC subset (22). CD1 molecules are known to present lipid antigens to activate T cells. In humans, CD1a self-reactive T cells can induce DC maturation and polarization (22). Thus, although the specific role of CD1a in DC function is not known, it is possible that these DCs can interact with specific T cells *in vivo* that may further influence their function (23). In this study, the expression level of CD1a in transplant recipients had no significant difference in comparison with that of healthy individuals.

Furthermore, DCs present antigen-derived peptides bound to MHC class II molecules for recognition by CD4-positive T lymphocytes (24). Following transplantation, T cells

recognize processed forms of donor HLA proteins (25). Our results showed that the MFI of HLA-DR in transplant recipients was significantly increased compared to that of healthy individuals.

Furthermore, CD86 is a costimulatory molecule that participates in the regulation of T-cell lymphocytes activation (26). Haanstra *et al*. (27) showed that using anti-CD86 antibody might be an effective approach to prevent graft rejection. In this study, the expression level and MFI for CD86 were significantly increased in transplant patients compared to healthy individuals.

On the other hand, TLRs are crucial links in activating DC maturation programs that induce immune responses and ligation of DC-expressed-TLR leads to increased expression of costimulatory molecules (12). TLR activation on DCs induces proinflammatory cytokine cascades, which contribute to the pathophysiology and clinical outcome of severe liver injuries (12). TLR activation by graft transplantation might turn on the adaptive immune system through T cell maturation, which results in allograft rejection (28). Deng *et al*. (9) reported that elevated levels of TLR-2 and TLR-4 might be used for early prediction of acute rejection after liver transplantation. Also, Hoffmann *et al*. (10) suggested that TLR-2 is markedly up-regulated in both experimental and human acute renal allograft rejection. Furthermore, signaling through TLR-4 may be responsible for the early activation of alloimmune T-cells, favoring allograft rejection (11). Our data showed that gene expression of TLR-2 in MoDCs is significantly increased in liver transplant patients compared to healthy individuals. Indeed, signaling via TLR-2 can induce DCs to mature in a MyD88 dependent or independent manner, which ultimately results in release of proinflammatory cytokines (1). But the gene expression of TLR-4 was not significantly different in transplant recipients versus healthy individuals.

Secretion of proinflammatory cytokines such as IL-6, IL-12 and IL-23 by DCs is essential for the activation of antigen-specific T lymphocytes (18). Korn *et al*. (17) reported a novel role for IL-6 in the development and differentiation of the Th17 cell subset. Th17 cells contribute to the mechanisms of rejection after transplantation (16). IL-6 is essential for the differentiation of interleukin 17-producing human T helper cells (29). Similarly, in this study IL-6 secretion by DCs in liver transplant patients were statistically increased compared to healthy individuals.

It was reported that anti-IL-12/23 can significantly prolong survival of murine skin and vascularized cardiac allografts (30). IL-12 production from DCs stimulates the induction of a Th1/Tc1 response and increases the generation of specific immune responses (31). Filatenkov *et al*. (32) found that IL-12 is important for T helper-dependent immune responses and the ability of IL-12 to replace CD4 T cell help to promote graft rejection. Similarly, our results showed a significant increase of IL-12 secretion by MoDCs in liver transplant patients compared to healthy individuals. IL-12 induces Th1 cell development and inflammatory processes in transplant patients (33).

Furthermore in separated studies, Fábrega and Liu *et al*. (34,35) found that IL-23 is temporarily up-regulated during acute liver rejection. In other studies it is reported that increased level of IL-23 amplifies and stabilizes the response of differentiated Th17 cells (15). Results of the present study revealed that in liver transplant patients, IL-23 secretion and expression by MoDCs is not significantly higher than that of healthy individuals.

Despite using of immunosuppressive drugs in liver transplant patients, DCs are involved in the stimulation of inflammatory reactions post-transplantation. Ligation of

TLRs by endogenous (like heat shock proteins) and exogenous (such as pathogenic microorganisms) ligands can stimulate DCs for antigen presentation to T cells and inducing the immune responses while increases the expression DC markers (7). Furthermore, TLR activation on DCs induces pro-inflammatory cytokines, which contribute to the inflammatory process after transplantation (12). Also, TLR signals on DCs promote Th17 cells, which contribute to the inflammation and allograft rejection (23). Finally, different etiologies have a role in the introduction and induction of inflammatory events post liver transplantation. Ischemia reperfusion injury which occurs post transplant surgery, import of donor immune mediators to recipient, primary and/or recurrent reactivation of different microbial infections in transplant recipient, and also other multiple unknown risk factors can impress efficiency of immunosuppressant post liver transplantation (34,35).

In conclusion, these results highlight the fact that in spite of the use of immunosuppressive conditioning regimens in liver transplant patients, expression of the DC maturation and inflammatory markers including CD83 and CD86, HLA-DR, IL-6, IL-12 and TLR-2 were increased in liver transplant recipients compared with controls. These elevations can trigger the specific immune reactions, activation of T cell–dependent immune responses, and increase the risk of allograft rejection in these patients; however, the details need to be confirmed in larger groups of patients.

ACKNOWLEDGEMENTS

The study was financially supported using a grant from Iran National Science Foundation (INSF).

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