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World J Gastroenterol 2006 March 7; 12(9):1447-1451
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Expression of ICAM-1, HLA-DR, and CD80 on peripheral circulating CD1 α DCs induced *in vivo* by IFN-α in patients with chronic hepatitis B

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Received: 2005-11-02 Accepted: 2005-12-22

Abstract

AIM: To explore the effects of interferon-α (IFN-α) application on peripheral circulating CD1α dendritic cells (DCs) in patients with chronic hepatitis B, and the expression of HLA-DR, CD80, and ICAM-1 on CD1α DCs in order to explore the mechanism of immune modulation of IFN-α.

METHODS: By flow cytometry technique, changes of CD1α DCs were monitored in 22 patients with chronic hepatitis B treated with IFN-α and in 16 such patients not treated with IFN-α within three months. Meanwhile, the expression of HLA-DR, CD80, and ICAM-1 on CD1α DCs was detected.

RESULTS: In the group of IFN-α treatment, the percentage of CD1α DCs in peripheral blood mononuclear cells was increased after three months of therapy. In patients who became negative for HBV-DNA after IFN-α treatment, the increase of DCs was more prominent, while in control, these changes were not observed. Increased expression of HLA-DR, CD80, and ICAM-1 on CD1α DCs was also observed.

CONCLUSION: CD1α DCs can be induced by IFN-α *in vivo*, and the immune related molecules such as HLA-DR, CD80, and ICAM-1 are up-regulated to some degree. This might be an important immune related mechanism of IFN-α treatment for chronic hepatitis B.

Key words: Chronic hepatitis B; DC; Immune costimulatory molecules; Immunotherapy; IFN-α

Yu YS, Tang ZH, Han JC, Xi M, Feng J, Zang GQ. Expression of ICAM-1, HLA-DR, and CD80 on peripheral circulating CD1 α DCs induced *in vivo* by IFN-α in patients with chronic hepatitis B. *World J Gastroenterol* 2006; 12(9): 1447-1451

http://www.wjgnet.com/1007-9327/12/1447.asp

INTRODUCTION

Dendritic cells (DCs) play a key role in the process of immune response. They are involved in pathogenesis of tumor and virus infection, and show prospect in immunotherapy[1-3]. Recent studies suggest that DCs are involved in the development of chronic hepatitis B in some extent, and more attentions have been paid to the relation between DCs and hepatitis B[4,5]. Patients with chronic HBV infection have the defective function and immature phenotype of DCs, which may be associated with the inability of efficient presentation of HBV antigens to host immune system for the clearance of HBV[6]. How to increase the levels of DCs and improve their function is important in patients with chronic HBV infection. Patients in the immune active phase are candidates for antiviral therapy.

Interferon-α (IFN-α) is an important therapeutic agent in non-cirrhotic hepatitis patients with mild to moderate disease activity. The primary goal of therapy for chronic hepatitis B is suppression of viral replication, which has been shown to reduce hepatic necroinflammation and retard progression of hepatic fibrosis. Long-term suppression of serum HBV DNA is likely to reduce progression to cirrhosis and hepatic decompensation and may also decrease the risk of hepatocellular carcinoma, but the efficiency of IFN-α is not satisfactory, and further study on its mechanism in the treatment of chronic hepatitis B is necessary.

Many kinds of cytokines such as GM-CSF, IL-4 can induce DCs *in vitro*, and the expression of immune molecules can be up-regulated[7]. Some recent studies suggested that GM-CSF induce DCs *in vivo*[8]. It has been shown that IFN-α could exert the anti-tumour immune modulation action through inducing DCs *in vitro*[9], but the mechanisms of IFN-α to induce DCs *in vivo*, especially to affect immune molecules on the surface of DCs are unclear. CD1α is an important surface marker on DCs[7].

The aim of this study was to evaluate the immune modulation function of IFN-α on DCs besides the direct

Table 1 Effects of IFN-α on CD1 α DC in peripheral blood with chronic hepatitis B

Group	*n*	Percentage of CD1 α DC		*P*
		Pre-therapy	Post-therapy	
IFN-α	22	0.70±0.29%	1.27±0.38%	<0.05
Control	16	0.63±0.17%	0.72±0.22%	>0.05

anti-HBV action of IFN-α. The CD1 α DC and the expression of ICAM-1, HLA-DR, and CD80 on the cells were determined in the peripheral blood of patients with chronic hepatitis B. Whether DCs can be induced *in vivo* in patients with chronic hepatitis B treated with IFN-αwas investigated. Our study will shed new light on the mechanism of enhanced immune response by IFN-α.

MATERIALS AND METHODS

Subjects

Thirty-eight patients with chronic viral hepatitis B were randomly divided into IFN-α group ($n=22$, 16 males, 6 females, aged from 24-48 years, mean 34±6.67) and control group ($n=16$, 11 males, 5 females, aged from 25-45 years, mean 35±5.40). Patients were diagnosed with chronic hepatitis B according to the Programme of Prevention and Treatment for Viral Hepatitis revised by Chinese Society of Infectious Disease and Parasitology, Chinese Society of Hepatology, Chinese Medical Association in 2000. Those patients who were diagnosed with chronic hepatitis B were seropositive for HBV markers (HBsAg, HBeAg/anti-HBe, anti-HBc) for more than 6 months, characterized by an intermittent pattern of disease activity with elevations of alanine aminotransferase (ALT) values preceded, and in most instances, by an increase of HBV-DNA levels. The enrolled patients were diagnosed with chronic hepatitis B, in whom serum markers of HBV (HBsAg, HBeAg, HBcAb, HBV-DNA) were positive, ALT level between two and five times normal limit, total bilirubin below two times normal limit, who could bear treatment with IFN-α. Patients who were infected by other virus and diagnosed with autoimmune disease were excluded.

Treatment procedures and reagents

Patients in IFN-αgroup were treated with 5.0 million units IFN-α daily for 15 d and then every other day for an additional (85) d. The control group was not treated with IFN-α or any other anti-viral or immune modulation drug except routine therapy for protecting liver. Two milliliter blood each time was taken from the enrolled patients with informed consent pre- and post-therapy. The samples were sent for CD1 α DCs assay and the expression of the immune molecules on the cells. Fluorochrome-labeled antibodies were used for flow cytometric analysis such as anti-CD1α-PE, anti-CD80-FITC, anti-ICAM-1-FITC and anti-HLA-DR-FITC (Immunotech, Marseille, France).

Flow cytometric analysis of CD1αDC

CD1 α DCs of peripheral blood was analyzed by flow cytometry, and the expressions of ICAM-1, CD80, and HLA-DR was examined. The ratio of CD1 α DCs/peripheral blood mononuclear cells (PBMC) was counted. One hundred microliter peripheral blood with heparin was mixed with 10 μL homogenic antibody Ig-PE, then were hemolyzed by optilyse C, which served as negative control. One hundred microliter peripheral blood with heparin was mixed with 10 μL anti-CD1α-PE, then hemolyzed with optilyse C. After that, they were analyzed by flow cytometry. Lymphocyte or mononuclear cell subpopulations were distinguished by Forward Scatter (FSC) and Side Scatter (SSC). The percentage of CD1 α DCs in PBMC was investigated.

Analysis of ICAM-1, HLA-DR, and CD80 on surface of CD1αDC

The expression of immune associated molecules(ICAM-1, HLA-DR, CD80) on surface of CD1 α DC was evaluated by flow cytometry by analyzing the percentage in double-stained PBMCs. One hundred microliter peripheral blood with heparin was mixed with 10 ul homogenic antibody Ig-PE, then hemolyzed by formic acid, which served as negative control. One hundred microliter peripheral blood with heparin was hemolyzed by formic acid in each group, next mixed with 10 μL anti-CD1α-PE, and then blended respectively with anti-ICAM-1-FITC, anti-HLA-DR-FITC, and anti-CD80- FITC. Lymphocyte or mononuclear cell subpopulations were distinguished by FSC and SSC. The percentage of $CD1\alpha^+$ $ICAM\text{-}1^+$, $CD1\alpha^+$ $CD80^+$ or $CD1\alpha^+$ HLA-DR in PBMCs was investigated.

Statistical analysis

The results were expressed as mean ± SD and analyzed using the Student's *t* test. $P<0.05$ was taken as statistically significant.

RESULTS

CD1αDC in peripheral blood with chronic hepatitis B and effect of IFN-αon DCs

The results showed that CD1 α DCs existed in peripheral blood of patients with active chronic hepatitis B, and most of them was less than 1% of PBMCs. In IFN-αgroup, after treatment for three months, the percentage of CD1αDCs in PBMC in some patients was increased. There were significant differences between pre- and post-therapy with IFN-α ($P<0.05$). In control group, the percentage of CD1 α DCs pre-therapy was close to that post-therapy ($p>0.05$).In IFN-αgroup, the percentage of CD1 α DCs in the group with decreased HBV-DNA was more than that in the group with HBV-DNA not decreased ($p<0.05$) (Table 1).

Effects of IFN-α on the expression of ICAM-1,HLA-DR,and CD-80 on surface of CD1αDC

It showed that ICAM-1, HLA-DR, and CD-80 on surface of CD1 α DC in peripheral blood of patients with active chronic hepatitis B also existed to some extent. Among them, the expression of ICAM-1, HLA-DR was stronger than that of CD80. After treatment with IFN-α for three months, the expression of ICAM-1, HLA-DR, and CD80

Table 2 Effects of IFN-α on immune related molecules on CD1 α DC in peripheral blood of patients pre- and post-therapy

Group	*n*	ICAM-1		HLA-DR		CD80	
		Pre-	Post-	Pre-	Post-	Pre-	Post-
IFN-α	22	54.97±5.95%	70.61±5.72%a	44.84±9.14%	57.86±7.78%[a]	33.97±8.37%	43.41±8.13%[a]
Control	16	60.17±4.83%	59.90±4.83%	45.01±9.08%	40.50±9.47%	33.42±11.58%	34.80±6.01%

[a]$P<0.05$ *vs* pre-therapy.

on surface of CD1 α DC was increased to some extent. In IFN-α group, the percentage of ICAM-1, CD80, and HLA-DR on CD1αin peripheral blood after treatment with IFN-α for three months was more than that before treatment. There were significant differences between pre- and post-therapy with IFN-α ($P<0.05$). In control group, there were no significant differences between pre- and post-therapy ($P>0.05$) (Table 2).

DISCUSSION

Currently, IFN-α is frequently chosen in treatment of chronic hepatitis B[10], but only 30%-40% of treated patients show response to it. How to improve the efficacy of IFN-α is a challenge. IFN-α is involved in immune modulation besides direct anti-HBV effect. The defect in specific T cell immunity, especially HBV-specific cytotoxic T lymphocyte (CTL) dysfunction has long been assumed to be a central mechanism for hepatitis B virus persistent infection. The cause that effective specific T cell immunity is not induced in patients with chronic hepatitis B is not completely clear. DCs are the most potent antigen-presenting cells that initiate protective T-cell immunity. Recent studies in transgenic mice have suggested, however, that functional deficit of DCs is an underlying cause for T cell dysfunction. Studies showed that HBsAg presentation by cytokine-activated DCs can break tolerance and trigger an anti-viral CTL response in HBV transgenic mice[11-12].In chronic hepatitis B, DCs are present to some degree in peripheral blood and in the liver tissue and might be involved in the immunopathogenesis of chronic liver diseases[13-15]. The current results showed that CD1 α DCs existed in peripheral blood in patients with active chronic hepatitis B, and most of them was less than 1% of PBMC. Whether the immune function of these DCs is effective in patients with chronic hepatitis B needs to be studied.

In literature, the reports on whether IFN-α is involved in immune modulation through DCs in treatment of chronic hepatitis B are few. DCs are most powerful to induce immune response among antigen presenting cells *in vivo*[16]. In recent years, more attentions have been paid to the relation between DCs and hepatitis B. DCs sensitized with HBsAg *in vitro* enhanced the proliferation response of T cell from chronic hepatitis B patients, and successfully induced MHC-I restricted HBV-specific CTLs in mice[4]. These suggest that DCs have powerful ability to present HBsAg. HBV-specific CTLs could be induced in HBV transgenic mice treated with sensitized DCs, therefore,the immune tolerance state of HBV transgenic mice would be broken[11]. It was reported that degree of activation of DC following vaccination would possibly help to predict the outcome of vaccine therapy in HBV carriers[17,18]. All above suggest that DCs play a role in inducing effective immune response to HBV.

However, recent studies have suggested that functional deficit of DCs is an underlying cause for T cell dysfunction. In hepatitis B, not only the numbers of DC subsets were decreased, but also the function of these DCs was impaired in peripheral blood[19-24]. DCs in liver from murine hepatitis B carriers also showed impaired functional capacities[25]. Therefore, to increase the number of and improve the function of DCs is important in patients with chronic HBV infection. DCs could be induced by many kinds of cytokines such as GM-CSF, IL-4 *in vitro*, and the expression of immune costimulatory molecules can be also up-regulated[7,9]. Most of studies on DCs in hepatitis B were done through incubation of PBMCs *in vitro*. In the process, some cytokines were added in order that DCs were induced. So the results observed *in vitro* could not reflect truly the condition of DCs *in vivo*. Whether DCs can be induced *in vivo* in patients with chronic hepatitis B treated with IFN-α was investigated in the current study in an effort to explore the mechanism of enhanced immune response through DCs of IFN-α treatment. In order to observe the changes of peripheral circulating CD1α+DCs and the expression of ICAM-1, HLA-DR, and CD80 on CD1 α DCs *in vivo* by IFN-α application, flow cytometry technique was employed to detect directly CD1α+DCs.

In our study, the effects of IFN-α on CD1α+DCs were investigated. The results showed IFN-α could up-regulate the percentage of CD1α+ DCs in PBMCs besides its direct anti-HBV action. Our findings that the number of CD1α+DCs rose in peripheral blood of patients treated with IFN-α suggested that IFN-α can induce DCs *in vivo*. Previous studies argued that DCs were lack in patients with chronic hepatitis B, and that immune activity was weak. IFN-α could enhance specific immune response to HBV through inducing DCs *in vivo*, consequently, facilitating antigen presentation of HBV and specific T cell triggering.

Previous studies suggested that DCs from patients with chronic hepatitis B showed significantly lower expression of costimulatory molecules B7-1 (CD80), B7-2 (CD86) and impaired allostimulatory mixed lymphocyte reaction, as well as decreased number compared with normal group[3,18]. It indicates that the immune response to HBV is enhanced by up-regulating the immune associated molecules on DCs in patients with chronic hepatitis B. Recent studies showed that IFN-α had similar action *in vitro*. IFN-α is partially involved in immune modulation by inducing DCs *in vitro*[9]. In our study, the expression

of ICAM-1, HLA-DR, and CD80 on surface of $CD1\alpha^+$ DCs differently increased in IFN-α group, but there was no significant change in control group. It suggests that IFN-α played some role in up-regulating the expression of ICAM-1, HLA-DR, and CD80. Many immune molecules are involved in triggering T lymphocyte cells by DCs, especially MHC-II, B7-1 (CD80), B7-2 (CD86), ICAM-1, LFA-3, etc. MHC molecules combined with antigen peptides provide first signal for the activation of T lymphocyte cells. The costimulatory molecules (B7-1, B7-2) are important to trigger T lymphocyte cells. The lowered expression of those immune molecules impaires DCs to trigger T lymphocyte cells. During the treatment of IFN-α for patients with chronic hepatitis B, the ability of DCs presenting HBV antigen was differently improved. Our results suggest that IFN-α improves the presenting ability of DCs to some degree, and strengthens the interaction of DCs and T lymphocyte cells through up-regulating the expression of ICAM-1, HLA-DR, and CD80. IFN-α up-regulating the expression of immune molecules on DCs might be an important mechanism of immune modulation in anti-HBV treatment for patients with chronic hepatitis B.

However, the correlation between the efficacy of IFN-α and DCs is still unclear. Although in patients with chronic hepatitis B, peripheral circulating DCs and their expression of ICAM-1, HLA-DR, CD80 are increased by IFN-αapplication, only a minority of treated patients show response to IFN-α. It indicates that the effects of anti-HBV treatment of IFN-α are affected by many other factors[26,27], for example, CD80-B7 interaction promotes immune response, and CD80-CTLA4 interaction down-regulates the response. The function of DC might be affected by these factors, as well[28,29].

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