The Effect of Beta Interferon on Dendritic Cells and Cytokine Synthesis by $CD4^+$ T Cells

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ABSTRACT

Background: Dendritic cells (DC) are a key regulator of the immune response, and interferon-beta (IFN-β) is considered an immunomodulatory molecule for DC. **Objective:** The purpose of this study was to evaluate the ability of IFN-β treated DC to induce cytokine secretion by $CD4^+$ T cells. **Methods:** Dendritic cells were generated from blood monocytes with granulocyte-monocyte colony-stimulating factor and interleukin-4 with or without IFN-β. We analyzed the production of $CD4^+$ T helper cytokines (IL-17, IFN-γ and IL-10) in the supernatant of the dendritic cell-T cell co- cultures by ELISA. We also studied the effects of HLA-G and costimulatory molecules on immature and mature DC. **Results:** IFN-γ and IL-17 decreased significantly in the presence of HLA-G-bearing DC compared to control cultures ($p<0.05$). **Conclusion:** Using the mixed leukocyte reaction, we found that DC treated with IFN-β mediated the inhibition of T cell activation via cytokine production. We conclude that this is important for preventing overactivation of the immune system.

Keywords: Dendritic cells, Interferon-beta, Interleukin-17

INTRODUCTION

Dendritic cells (DC) are professional antigen-presenting cells with the unique capacity to prime naïve T-cell responses. Varieties of positive as well as negative regulatory signals are provided by DC that can modulate T-cell responses (1-3), and T-cell cytokines can modify immune reactions (4,5). Recently, a novel subset of $CD4^+$ T cells, designated Th17 cells, was identified which produce interleukin 17 (6,7). This cytokine together with IL-10 and IFN-γ, secreted by T-helper $CD4^+$ cells, have an important role in immune reactions. IFN-β, an immunomodulatory mediator normally induced by viruses or double-stranded RNA (8), is considered a regulatory molecule for DC functions (9-11).

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Another immunoregulatory molecule in the immune response is HLA-G, which plays an important role in cancer, pregnancy and transplantation (12,13). Functional studies have identified HLA-G as a key mediator in immune tolerance (14-17). Huang et al. showed that the production of IFN-γ and IL-2 by peripheral blood mononuclear cells decreases in DC treated with IFN-β (18). However, Giacomoni et al. suggested that IFN-β is a BCG adjuvant and acts in DC maturation. They showed that DC infected by BCG after treatment with IFN-β displayed a mature phenotype and secreted IL-12p70 (19). In the present study we investigated the effects of IFN-β on DC including HLA-G expression, cytokine secretion and metabolic activity of $CD4^+$ T cells in mixed leukocyte reactions (MLR).

MATERIALS AND METHODS

Generation of Dendritic Cells from Adherent Blood Mononuclear Cells. Whole blood was collected from healthy volunteers after obtaining informed consent. Blood mononuclear cells were isolated from heparinized blood by centrifugation on a Ficoll Histipaque 1.077 column (Sigma, USA). Cells from the interphase were collected and washed three times with RPMI 1640 medium (Sigma). Cell viability was determined by trypan blue exclusion. Monocytes (>90%) were isolated from MNC by plastic flask adherence Mononuclear cells (5-8 $\times 10^6$ /ml) were then cultured in 5 ml RPMI 1640 medium supplemented with 10% fetal calf serum, 50 units/ml penicillin and 50 μg/ml streptomycin. After 2 h of incubation at 37 °C in an atmosphere of 5% CO_2, the adherent cells were cultured in complete medium containing 1000 units/ml rhGM-CSF (Serotec, UK) and 500 units/ml rh IL-4 (Serotec, UK) with or without 1000 units/ml IFN-β (Avonex, Biogen, UK) to generate DC.
Nonadherent cells were removed for T-cell isolation. $CD4^+$ T cells (>95%) were purified using a $CD4^+$ T cell isolation kit (Miltenyi Biotec, Germany). These cells were counted and then frozen in 10% dimetylsulfoxide containing a mixture of 60% RPMI and 30% FCS.
For antigen maturation, DC were stimulated with 10μg/ml lipopolysaccharide (LPS) for 5 days and then processed for 48 h at 37^oC, in 5% CO_2.
Flow cytometry. Surface expression of immune cells was determined by flow cytometry in a fluorescence activated cell sorter model, (Becton Dickenson, USA).
Harvested cells were washed twice with PBS supplemented with 1% bovine serum albumin. Cells were stained for 30 min at 4 °C with the following FITC-conjugated antibodies: anti-CD83, anti HLA-G and PE-conjugated antibodies, i.e., anti-HLA-DR, anti-CD86 or isotype controls.
Co-culture Experiments with $CD4^+$ T Cells. Isolated T-cells and autologous DC were cocultured. In 96-well U-shaped plates, 10×10^4 T-cells were cultured in each well with 1×10^4 DC and a final volume of 200 μl per well. All samples were run in triplicate. Cultures were incubated at 37 °C in a humidified 5% CO_2 atmosphere for 5 days and then pulsed with 200 μl 3-[4,5-dimethylthiazolyl]-2,5-diphenyl-tetrazolium bromide (MTT) (Sigma, USA) as a color indicator of metabolic activity (20). Supernatants were harvested 4 h later, and then 200 μl DMSO was added. The color change was read in an ELISA reader at 575 nm.
ELISA. Cytokine concentrations were determined in duplicate in culture supernatants using commercially available human IL-10, IFN-γ and IL-17 ELISA kits (Bender

MedSystems, Austria). Supernatants of cocultures were harvested after 5 days of incubation and were frozen for subsequent cytokine analysis.

Statistical Analysis. Statistical analysis was performed using SPSS software version 11.5. We determined P-Values in all cases by ANOVA. Statistical significance was defined as a P-Value less than 0.05.

RESULTS

Phenotype Analysis of DC. Mature DC generated from blood mononuclear cells with GM-CSF and IL-4 in the presence of IFN-β (IFNbeteDC) or absence of IFN-β (DC) followed by LPS stimulation were analyzed for the expression of DC surface markers. The phenotypes of the mature DC were compared with immature DC not stimulated with LPS (Figure1). Analysis of the phenotypes showed the expression of HLA-DR and minimal levels of CD83 on their surface before stimulation with the antigen. Lipopolysaccharide stimulation significantly upregulated the surface expression of HLA-DR and CD83 on DC. The expression of CD83 and HLA-DR are indications of DC maturation (Figure1). HLA-G expression on DC after treatment with IFN-β was higher than in controls (Figure 2).

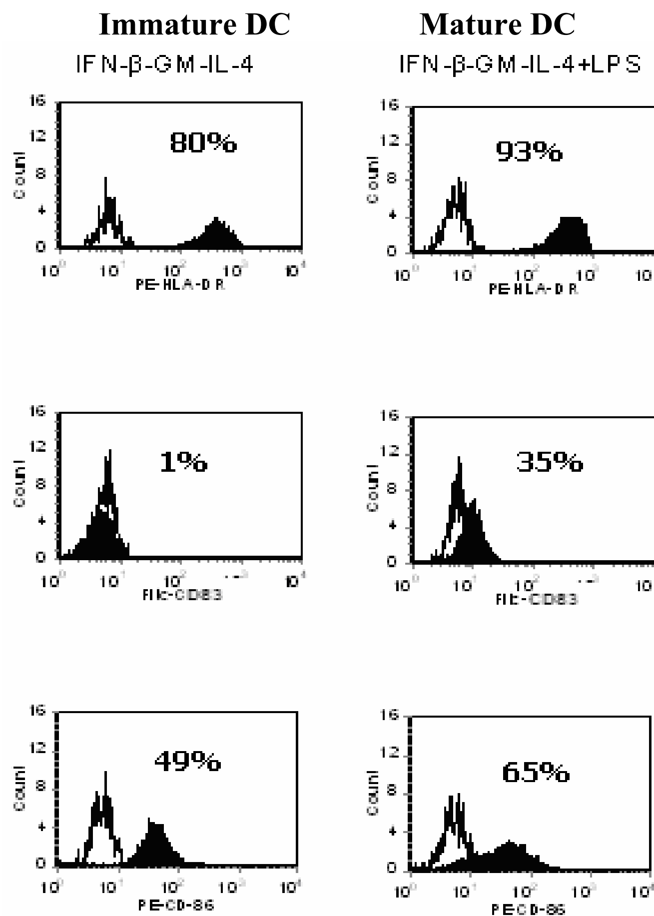

Figure 1. Phenotype analysis showing the expression of HLA-DR, CD86 and minimal level of CD83 on the DC surface before stimulation with LPS (Immature DC). LPS significantly upregulated the surface level of HLA-DR and CD83 on dendritic cells (Mature DC). Expression of the CD83 marker and HLA-DR is an indication of DC maturation.

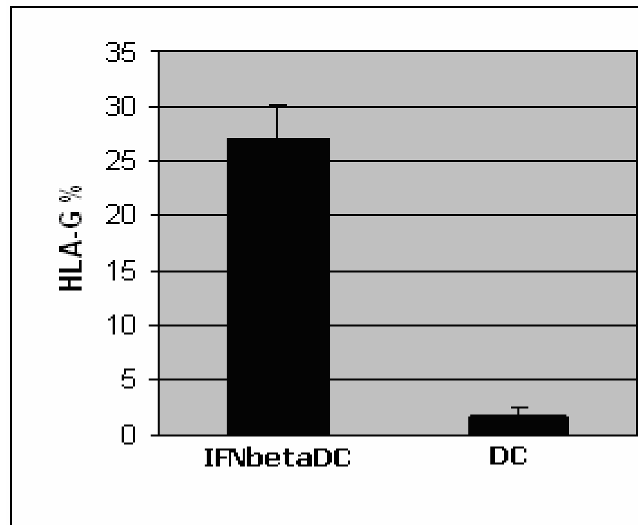

Figure 2. Percentage of DC-bearing HLA-G (IFNbetaDC) in the culture after 7 days of stimulation with or without IFN-β. The combination of IFN-β and GM-CSF+ IL-4 induced the development of HLA-G on dendritic cells. $CD14^+$ cells were cultured with GM-CSF and IL-4 with or without IFN-β. After 5 days, cells were stimulated with LPS for 2 days before staining for HLA-G analysis by flow cytometry. Data are the mean ± SD of five independent experiments.

Concentration of Cytokines in the Supernatant. IL-17, IL-10, and IFN-γ cytokine concentrations were significantly different between the two groups ($p<0.05$). The results are summarized in Table 1.

Table1. IL-17, IL-10, and IFN-γ concentrations (pg/ml) in the supernatant of mixed leukocyte reactions (MLR) between dendritic cells and T cells after 5 days. Values are the mean ± SD.

MLR	IFN-γ	IL-10	IL-17	P-value
IFNbeteDC + T	1.34±0.31	4.3±1.22	1.76±0.54	<0.05
DC + T	2.44±0.32	1.46±1.00	4.2±0.81	

Mixed Leukocyte Reaction between $CD4^+$ T Cells and Dendritic Cells. $CD4^+$ T-cell proliferation was inhibited in the coculture system containing DC bearing HLA-G compared to controls ($p<0.05$) (Figure 3).

DISCUSSION

The interaction between T lymphocytes and DC is a bidirectional process.We examined IL-17, IFN-γ, IL-10 cytokines in MLR supernatants in the presence of IFNbetaDC and DC. IFN-β has been shown to promote the differentiation of blood monocytes from DC, and contributes to DC maturation (21, 22). In this study T-cell proliferation was inhibited when $CD4^+$ cells were cultured with dendritic cells treated with IFN-β. Thus IFN-β and HLA-G molecules on DC are two components contributing to this immune reaction. Consistent with our results, Bartholome et al. found that IFN-β did not change DC morphology but resulted in significant upregulation of costimulatory molecules (23).

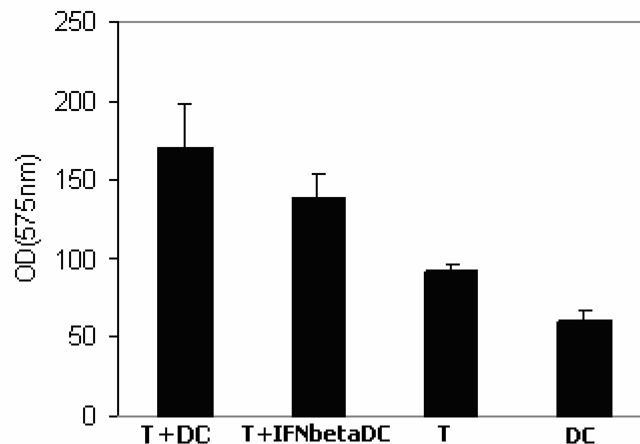

Figure 3. Effect of DC-bearing HLA-G (IFNbetaDC) on $CD4^+$ T cell proliferation with controls (T and DC) in mixed leukocyte reactions, evaluated by MTT assay ($p<0.05$). The data are OD at 575 nm read in an ELISA reader. Dendritic cells were cultured with T cells (1 to 10). Values are the mean ± SD.

Cytokine balance is known as the regulator of the immune system under normal conditions, and is impaired in many autoimmune diseases (24). Our results show that DC bearing HLA-G in MLR result in higher IL-10 production and a lower IL-17 and IFNγ production in comparison to control cultures ($p<0.05$). IL-10 may directly prevent T-cell activation via a decrease in IL-17 and IFN-γ production. IL-10 functions by inhibiting the capacity of peripheral blood DC to stimulate alloreactive T-cells (25). Our data suggest that DC receiving an IFN-β signal cannot induce the potential to develop into the Th17 lineage. Therefore, these results have important implications for maintaining peripheral tolerance in the presence of potentially pro-inflammatory cytokines.
On the other hand, our results show that some desirable immune modulatory effects of IFN-β may be accompanied by upregulation of HLA-G and IL-10. IFN-β, by increasing HLA-G expression on DC, can also downregulate the inflammatory response mediate by $CD4^+$ T cells in vivo. We propose that IFN-β and HLA-G are two new components of potential use in the treatment and control of pro-inflammatory cytokines during the pathogenesis of autoimmune diseases.

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