Comparison of Several Maturation Inducing Factors in Dendritic Cell Differentiation

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ABSTRACT

Background: Dendritic cells (DCs) are professional antigen presenting cells that have an important role in the initiation of immune response. The use of maturation factors in dendritic cell differentiation provides a promising approach in immunotherapy. **Objective:** In this study, we compared tumor necrosis factor-α, polyribocytidylic acid, lipopolysacharide and CpG oligonucleotides in inducing dendritic cell maturation. **Methods:** We generated immature dendritic cells with GM-CSF in combination with IL-4 from peripheral blood mononuclear adherent cells and used tumor necrosis factor-α, polyribocytidylic acid, lipopolysacharide and CpG for the induction of dendritic cell maturation. CD83 maturation marker on the dendritic cells was analyzed by flowcytometry after 7 days. In addition, mixed leukocyte reaction between dendritic cells and T cells was performed by MTT proliferation assay. **Results:** Flow cytometry results demonstrated a comparable high level of CD83 expression on the mature dendritic cells generated by TNF-α, CpG, Poly I:C, and LPS treatment of the immature dendritic cells. However, a significantly poorer proliferation of lymphocytes cocultured with the Poly I:C-treated DCs was observed compared to the CpG-treated DCs in mixed leukocyte reaction (p=0.026). Conversely, a significantly stronger proliferation of lymphocytes was observed when cocultured with TNF-α-treated DCs compared to the LPS-treated DCs (p=0.025). **Conclusion:** Our results indicated that all of studied maturation inducing factors can be used in DC maturation but TNF-α and CpG were the preferred in vitro maturation factors. It is concluded that maturation of dendritic cells by CpG motif and TNF-α can be used to regulate immune responses.

Keywords: CpG, Dendritic Cells, LPS, TNF-α

INTRODUCTION

Mature dendritic cells (DCs) are professional antigen presenting cells that have an important role in tolerance and autoimmunity (1,2). These cells can mature with the uptake of antigens and their presentation to T cells in the lymph nodes (3,4). Some microbial and synthetic agents can also cause DC maturation (5).

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The use of various maturation factors in dendritic cell differentiation is important in cancer immunotherapy, dendritic cell vaccination and autoimmunity. Several studies have demonstrated that tumor necrosis factor-α (TNF-α), lipopolysacharide (LPS), polyribocytidylic acid (Poly I:C)and CpG oligonucleotides can cause dendritic cell maturation in vitro (6-9). Poly I:C, a synthetic double stranded RNA (dsRNA) has been found to induce stable maturationof DCs (10). Lipopolysacharide, a gram negative endotoxin, has also been known as a dendritic cell maturation inducer (7). Furthermore, TNF-α is an inflammatory cytokine with an important role in the induction of dendritic cell maturation. On the other hand, CpG motif reacts with TLR9 on DCs and results in the generation of mature DCs (9). The purpose of this study was to evaluate the role of maturing factors in dendritic cell generation and their comparison with T cell proliferation in vitro.

MATERIALS AND METHODS

Isolation of Monocytes and $CD4^+$ T Cells from Peripheral Blood. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinzed blood, donated by 5 healthy volunteers, by centrifugation on a Ficoll histipaque 1.077 (Lymphoprep, Norway). Cells from interphase were collected and washed three times with RPMI-1640 medium (Sigma, USA). Cells were counted and their viability was determined by trypan blue exclusion. Monocytes and $CD4^+$ T cells were isolated from PBMCs using flask adherent cells and $CD4^+$ T cell isolation kit (Miltenyi Biotec, USA), respectively. Purity of $CD4^+$ T cells was >95% as analyzed by flowcytometry using monoclonal antibody specific to CD4.

Generation of Dendritic Cells. Monocytes (5×10^5) were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum, 100 units/ml penicillin and 100μg/ml streptomycin for 5 days with different combinations of purified recombinant human cytokines: 10 ng/ml interleukin-4 (IL-4, Serotec, UK), and 10 ng/ml granulocyte macrophage colony stimulating factor (GM-CSF, Serotec, UK) to generate immature DCs. To induce maturation of dendritic cells, the immature DCs were further incubated with either1μg/ml bacterial LPS, 1 μg/ml TNF-α, 1 μg/ml CPG or 1 μg/ml poly I:C (Sigma, USA) for an additional 48 h before harvesting the cells.

Flowcytometry. After generation of dendritic cells, they were stained with FITC-anti-CD83 (eBiosciences, USA) or isotype control antibodies (eBiosciences, USA) for 30 min at 4°C. Surface expressions of antigens were measured by flowcytometry (Becton Dickenson, USA).

Co-culture of DCs with $CD4^+$ T Cells. 1×10^5 $CD4^+$ T cells and 1×10^4 DCs from five different donors were cultured separately in 96 U-shaped bottom plates (200 μl per well). All samples were run in triplicates. Cells were incubated at 37°C in a humidified 5% CO2 atmosphere for 5 days. T cell proliferation responses to dendritic cells were measured by pulsing the cells with 200 μl 3-[4,5-dimethylthiazolyl]-2,5-diphenyl-tetrazolium bromide (MTT, Sigma) for 4 h and reading the absorbance color at 575 nm which is an index of cell proliferation (11).

Statistical Analysis. Data are presented as mean ± SD. For statistical analysis, Wilkoxon Rank test was used. P value of less than 0.05 was considered statistically significant.

RESULTS

Generation of Mature Dendritic Cells by CpG, LPS, TNF and Poly I:C. DCs generated with GM-CSF and IL-4 cytokines were matured by adding either TNF-α, LPS, Poly I:C or CpG in their culture media. All DCs showed CD83 maturation marker as observed by flowcytometry (Figure 1).

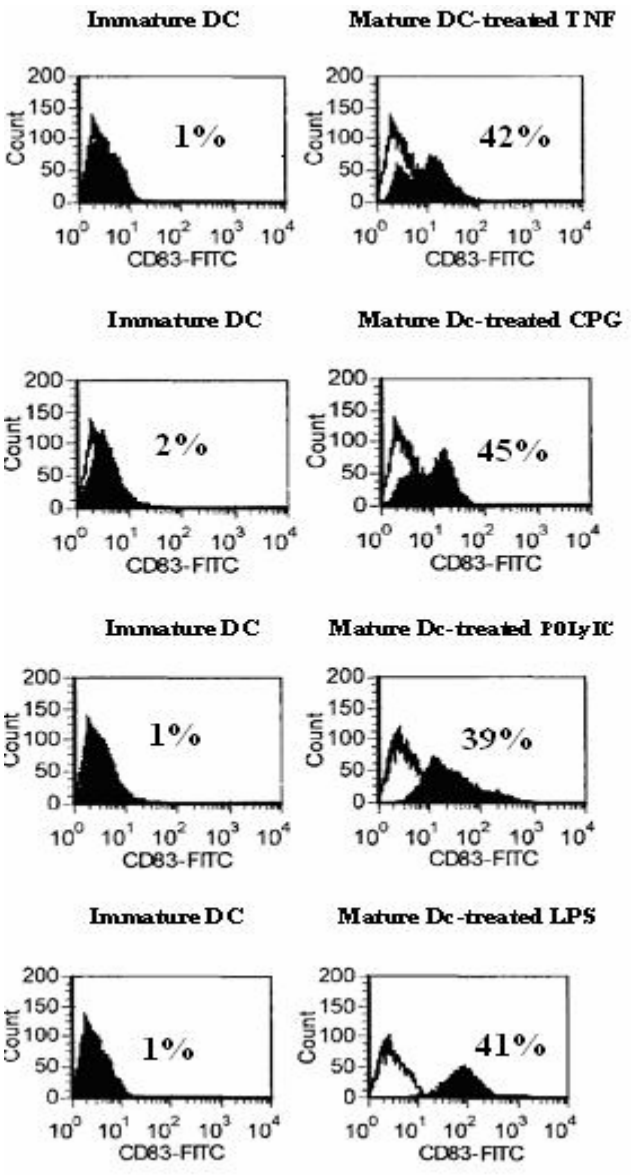

Figure 1. Analysis of phenotypes showing the expression of a minimal level of CD83 on the DC cell surface before stimulation with maturation agents (Immature DCs). Maturation factors significantly acted by upregulating the surface level of CD83 on dendritic cells (Mature DCs). Expression of CD83 marker indicates DC maturation.

$CD4^+$ T Cell Proliferation. We also compared the ability of DCs in inducing allogenic $CD4^+$ T cell proliferation using MTT proliferation assay. $CD4^+$ T cells co-cultured with

DC-treated poly I:C proliferated at a significantly lower degree compared to those treated with CpG (P=0.02, Table 1). Furthermore, $CD4^+$ T cells co-cultured with DC-treated LPS proliferated at a significantly lower degree compared to those treated with TNF-α (P= 0.025, Table 1).

Table 1. Comparison of mean ± SD of proliferation assay by MTT in the co-culture media using either TNF-DC, LPS-DC, Poly I:C-DC, or CpG-DC with $CD4^+$ T cells.

Mixed leukocyte reaction	No	Mean ± SD	P value
T+DC (CpG)	5	358.00 ± 52.45	0.113
T+DC (LPS)	5	260.66 ± 14.01	
T+DC (CpG)	5	358.00 ± 52.45	0.026
T+DC (Poly I:C)	5	241.33 ± 45.60	
T+DC (CpG)	5	358.00 ± 52.45	0.425
T+DC (TNF-α)	5	329.66 ± 22.50	
T+DC (LPS)	5	260.66 ± 14.01	0.025
T+DC (TNF-α)	5	329.66 ± 22.50	
T+DC (LPS)	5	260.66 ± 14.01	0.629
T+DC (Poly I:C)	5	241.33 ± 45.60	
T+DC (Poly I:C)	5	241.33 ± 45.60	0.115
T+DC (TNF-α)	5	329.66 ± 22.50	
T (Control)	5	97.11 ± 12.01	

DISCUSSION:

In this study, we compared the effects of maturation agents on dendritic cell generation and evaluated the role of these cells in the proliferation of $CD4^+$T cells. The results showed that both TNF-α and CpG oligonucleotide were stronger stimulators in T cell proliferation. Mature dendritic cells have a central regulatory role in T cell response to different antigens (12,13). The data revealed that all factors used caused dendritic cell maturation, but TNF-α and CpG motifs were preffered maturation markers in the culture media. CpG reacts with Toll like receptor 9 on dendritic cells that associates with the activation of programmed death enzymes and the reduction of tumor progression (14).
Furthermore, TNF-α bind to its ligand on dendritic cells and markedly elevates the maturation marker on these cells.
Although several groups have showed the role of TNF-α in dendritic cell maturation, in this study we indirectly compared the capability of four different agents in $CD4^+$ T cell proliferation. These results indicate that the use of maturating agents during DC generation not only induces CD83 maturation marker but also activates allogenic $CD4^+$ T cell response in MLR (Figure 1). These results are consistent with the data of Verdi et al., who demonstrated that poly I:C can induce a stable maturation of dendritic cells (15). In the present study, poly I:C, CpG, LPS and TNF-α all could induce dendritic cell matura-

tion, but CpG motif and TNF-α were stronger maturating agents in T cell proliferation assays. The results showed that TNF-α and CpG motif probably have important roles in the regulation of DC maturation and function.

There are high levels of co-stimulatory molecules such as CD86, CD80 and HLA-DR on DCs after maturation (16). In this study, we only detected CD83 as a co-stimulatory molecule on DCs.

Our data indicate a significantly elevated $CD4^{+}$T cell proliferation in MLR with DC-treated CpG or TNF-α in comparison with the DC-treated LPS or Poly I:C. This study demonstrates the need of considering other stimulatory factors, such as CpG oligonucleotides in DC maturation and interaction with T cells after MLR for controlling the unwanted immune reactions. The present study suggests that although TNF-α is always used in dendritic cell maturation, but CpG motif has perhaps a stronger role in generating matured dendritic cells.

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