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Ganoderma Lucidum Induces the Expression of CD40/CD86 on Peripheral Blood Monocytes

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ABSTRACT

Background: The major immuno-modulating effects of *Ganoderma lucidum* include mitogenicity and activation of immune effector cells such as T cells, macrophages and natural killer cells resulting in the production of cytokines. **Objective:** The purpose of this study was to evaluate the expression of CD40 and CD80 by *G. lucidum*-treated human peripheral blood mononuclear cells. **Methods:** Monocytes were isolated and incubated at 37°C and 5% CO_2 for 24 h and 48 h in the presence or absence of different concentrations of *G. lucidum*. Cells were then incubated with labelled monoclonal antibodies against CD14, CD40 and B7-1(CD80) molecules utilizing standard protocols, and analyzed by flow cytometry. **Results:** The results showed that incubation of monocytes with *G. lucidum* led to marked enhancement of CD40 and B7-1 expression in a dose- and time- dependent manner ($p<0.001$). *G. lucidum* was more effective in enhancing the expression of CD80 and CD40 molecules of cells obtained from females than male donors ($p<0.001$). **Conclusion:** *G. lucidum* enhanced the expression of CD40 and CD80 molecules on peripheral blood monocytic cells derived from both sexes in a dose-dependent manner, with a preferential higher effect on cells obtained from female donors.

Keywords: *Ganoderma lucidum*, Monocytes, CD14, CD40, CD80

INTRODUCTION

Ganoderma lucidum has been widely employed in China and other Asian countries and is effective in modulating immune function and promoting antitumor activity and it also has antiviral effects (1). Studies have demonstrated the antineoplastic action of *G. lucidum* and attributed this activity to its ability to activate host immune responses (2). A polysaccharide from *G. lucidum* has been reported to increase the cytotoxic activity of natural killer cells and to enhance the release of tumor necrosis factor and interferon (IFN) from macrophages and lymphocytes, respectively (3). The polysaccharide component from *G. lucidum* has also been reported to produce anti-apoptotic effects on neutrophils,

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primarily due to the activation of Akt-regulated signalling pathways (4). This component has also been reported to induce gene expression changes in human dendritic cells and promote Th1 immune response in Balb/c mice (5). It was also found that in cyclophosphamide immunosuppressed mice, low doses of *G. lucidum* polysaccharide accelerated the recovery of bone marrow cells, red blood cells, white blood cells, and splenic natural killer cells, and also enhanced T and B cell proliferation (6).
Cell-to-cell interaction through intercellular adhesion molecule-1 (ICAM-1), B7(1,2), and CD40 on monocytes and their ligands on T cells has been suggested to have a role in some inflammatory responses such as TNF-α and interleukin-10 production (7). Co-stimulatory molecules are necessary for T cell activation to be complete, and the differential expression of co-stimulatory molecules on the surface of antigen presenting cells (APC) is thought to influence the type of effector T cell response (Th1/Th2) (8).
Chan et al. demonstrated that *G. lucidum* enhanced the maturation of dendritic cells (DC) in terms of their ability to up regulate the expression of CD40, CD80, and CD86 molecules (9). Thus they concluded that *G. lucidum* has immunomodulating effects on human immune cells and therefore can be used as a natural adjuvant for cancer immunotherapy with DC. Therefore, the aim of the present study was to find out whether *G. lucidum* modulates the expression of CD40 and CD80 on $CD14^+$ human blood monocyte cells in vitro.

MATERIALS AND METHODS

All studies were conducted following approval from the Human Use Ethics Committee of the Baqiyatallah University of Medical Sciences. Human monocytes were isolated as described previously (10,11) with some modifications from peripheral blood cells (PBC) of 12 adult volunteers (age range 40 to 50 years, 6 from each sex). Briefly, PBC were obtained from healthy donors by Ficoll-Hypaque gradient centrifugation (Pharmacia, Norway). Cells were plated out at $1X10^4$ cells/well in RPMI 1640 containing 10% fetal calf serum (FCS) (Gibco, USA), 50 µg/ml streptomycin and 50 U/ml penicillin (Gibco) in 96-well plates (Nunclon DELTA, Denmark) in the presence or absence of different concentrations of *G. lucidum* (JHS Natural Products, USA) Reishi extract with 12% polysaccharide and 6% triterpenes ranging from 100 µg to 6400 µg/ml, and incubated in 5% CO_2 for 24 h and 48 h. The cells were then harvested and washed twice with ice-cold FACS buffer (PBS containing 2% FCS and 0.1% sodium diazide). Twenty percent bovine serum albumin was used to block nonspecific antibody binding. Cells were stained with PE conjugated anti-CD14 monoclonal antibody (IQ Products, Netherlands) plus anti-CD80 coupled to FITC (IQ products, Netherlands) or anti-CD40 mAb coupled to FITC (AbD Serotec, UK) for 30 min at 4°C in the dark. The stained cells were then washed twice, fixed with 1% paraformaldehyde in FACS buffer, and analyzed with a flow cytometer (Becton Dickinson, USA). Immunophenotypes were evaluated by determining the absolute number of $CD80^+$ and $CD40^+$ cells in *G. lucidum*-treated cells compared to control cells.
Statistical Analysis: The data were expressed as the mean ± S.E.M. Analysis of variance (ANOVA) was used to determine the differences between the control and the test wells. When statistically significant differences ($p<0.05$) were found between the groups, an unpaired t-test was used to determine the level of significance between the treated and non-treated cells.

RESULTS

The results showed that *G. lucidum* increased the frequency of CD14+ cells that express CD80 and CD40 in a time- and dose- dependent manner (Table 1). Minimal enhancement for both CD40 and CD80 was induced following incubation with as little as 50µg/ml and a maximal increase was noted with the use of 1600 µg/ml of *G. lucidum*. In most cases with the use of > 1600µg/ml no significant increase was noted in CD40 and CD80 expression. On the other hand, there was a difference in the over expression of CD40/80 response to *G. lucidum* between men and women.

Table 1. Effects of different concentrations of *G. lucidum* on immunephenotypes of $CD14^+$ human blood mononuclear cells [a]

G. lucidum **µg/ml**	**Men**				**Women**			
	$CD14^+/CD80^+$ %Cells		**$CD14^+/CD40^+$ %Cells**		**$CD14^+/CD80^+$ %Cells**		**$CD14^+/CD40^+$ %Cells**	
Incubation time	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Control	5	5	3	3	7	7	8	8
50	6	8***	6**	9*	9***	14*	10***	10***
100	8***	10*	8**	11*	11**	16*	11***	12**
200	10**	12*	11*	14*	13*	18*	15*	16*
400	11*	15*	13*	17*	17*	21*	18*	21*
800	18*	21*	16*	20*	22*	26*	24*	25*
1600	23*	26*	18*	23*	27*	33*	26*	28*
3200	23*	26*	21*	23*	27*	34*	27*	29*
6400	24*	26*	22*	24*	28*	34*	27*	30*

[a] Results were derived from the mean of expression on peripheral monocytes from 6 men and 6 women donors. The P-values represent the differences between treated and non-treated cells. * $p<0.001$, ** $p<0.005$, *** $p<0.01$.

DISCUSSION

We have previously demonstrated the immunomodulatory effects of *G. lucidum* and T-2 toxin on cytokine production by lymph node T cells and peritoneal macrophages (12-14). In this study, we report that *G. lucidum* enhanced the expression of CD40 and CD80 on $CD14^+$ human blood mononuclear cells. The enhancement appeared to be higher for the expression of CD80 compared to CD40 molecules. It is clear that antigen recognition by T cells on APC is not sufficient for full physiological T cell activation. Full T cell activation not only requires interaction between the T cell receptor and its cognate peptide-bearing MHC molecule, but also needs interaction between the costimulatory molecules, i.e., CD80 (B7-1) and CD86 (B7-2) molecules on APC and their ligand, CD28, on T cells. Therefore, increased expression of these molecules on human $CD14^+$ PBC by *G. lucidum* demonstrates the potential mechanisms of their positive effects on immune response. Supporting our view, Lin et al. (15) demonstrated that treatment of DC with *G. lucidum* results in enhanced cell-surface expression of CD80, CD86, CD83, CD40, CD54, and human leukocyte antigen (HLA)-DR, as well as enhanced production of interleukin (IL)-12p70, p40, and IL-10, and also IL-12p35, p40, and IL-10 mRNA expression. They also reported a suppressed capacity for endocytosis in DC. Regarding these results, Zhu et al. (6) demonstrated the effects of treatment with low-dose *G. lucidum* polysacharids in enhancing the activity of immunological effector cells in immunosuppressed mice. They also suggested that *G. lucidum* may provide a basis for using this herb as an efficacious adjunct in immunopotentiating therapy against

cancer chemotherapy-induced immunosuppression.
Our results are also in agreement with those of Chan et al. (9), who reported that *G. lucidum* enhanced maturation of DC by upregulating CD40, CD80, and CD86. These results are also in agreement with those of Lin et al. (16), who demonstrated that costimulatory molecules (CD40, CD54, CD80, and CD86) of human dendritic cells increase in response to *G. lucidum*.
On the other hand, Jing et al. (17), recently reported that *G. lucidum* inhibited the proliferation of human breast cancer cells by downregulating estrogen receptor and NF-kB signaling, demonstrating the possibility of sex differences in the immunomodulating effects of *G. lucidum*. In a recent study, Zaidman et al. (18) also demonstrated that *G. lucidum* decreased transcriptional activity of the androgen and glucocorticoid receptors in breast cancer MDA kb2 cells in a dose-dependent manner, and suppressed androgen receptor protein level in LNCaP and MDA-kb2 cells. Furthermore, *G. lucidum* crude extract and its GLF4 fraction interfered with androgen receptor function through competition with the natural ligand, dihydrotestosterone, and the suppression of androgen receptor/androgen response element complex formation (19).
It has also been reported that *G. lucidum* has a positive modulatory effect on autoimmune diseases such as rheumatoid arthritis (20,21). Therefore, based on our previous studies regarding the effects of sex steroid hormones on immune function (22,23) and a recent report by Cutolo et al. (24), who demonstrated the influence of sex steroid hormones on autoimmune diseases, it seems that androgen- and nonandrogen- mediated mechanisms might be responsible for the effects of *G. lucidum* on immune cells.
These findings provide evidence that *G. lucidum* has immunomodulating effects on human immune cells and therefore might be used as a natural adjuvant for immunotherapy

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