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# Hericium Erinaceus Polysaccharide Induced Maturation of Murine Bone Marrow Deprived Dendritic Cells

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**Abstract** Hericium erinaceus polysaccharide (HEP) is a Chinese herb and was reported to regulate the immune response effectively in different aspects. This study was aimed to investigate the influence of HEP on murine dendritic cells (DC). In this study, we found that HEP treatment induced DC maturation by the morphology and the up-regulation of co-stimulative molecules. The endocytosis activity was also reduced, which is also an important feature of maturation. HEP also increased the allogenic T cell proliferation in a MLR assay. Furthermore, it was found that HEP increase the expression level of IL-12. Finally, we concluded that HEP induced maturation of bone marrow DC.

***Keywords:*** *Hericium erinaceus polysaccharide, Chinese herb, immunity, Dendritic cells*

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## 1. Introduction

Dendritic cells (DC) are the most potent antigen presenting cells in immune system, they also control the whole immune response as well as immune tolerance in different conditions [1]. Once stimulated by antigen or other inflammatory agents, DC moved into the maturation process, and began to express high level co-stimulative molecules, migrated to the draining lymph node and activate T cells, thus the adaptive immune response was initiated [1].

As a traditional Chinese medicine, hericium erinaceus polysaccharide (HEP) has been report to enhance the immune response in vivo and in vitro [2]. HEP induced the proliferation of conA-treated thymus T cells, and showed an elevation of proliferation of spleenocytes. In immune suppressive mice, HEP administration also resulted in upregulation of pro-inflammatory cytokines, such as TNF, IL-6 and IL-1 etc. The cytotoxicity of natural killer cells in these kind mice was also greatly increased [2,3,4]. While there is no data covering the effect of HEP on DC. This study was aimed to investigate the influence of HEP on the maturation of murine bone marrow dendritic cells.

## 2. Materials and Methods

### 2.1. Preparation of HEP

HEP was prepared using the methods as previously described [3]. Briefly, H. erinaceus (300g) was heated with 6L deionized water for 2h. After cooled to room temperature, the mixture was filtered through Whatman No.4 filter paper. the retentate was concentrated and re-suspended by 70% ethanol. The precipitate was then lyophilized and ground to obtain a coarse powder of hot water extracted polysaccharides (80g). Based on our previous experiments, 100, 500 and 1000ug/ml was used in this study.

### 2.2. Culture of Murine Bone Marrow Deprived Dendritic Cells (BMDC)

BMDC was prepared by culture in presence of GM-CSF (10ng/ml) and IL-4 (1ng/ml) as previously reported [5,6]. Briefly, single cell suspension was obtained from bone marrow in femurs and tibias. After lysing red blood cells, bone marrow cells were cultured in RPMI1640 medium in six well plates (Corning, USA). The medium was supplemented with 10% fetal calf serum, GM-CSF and IL-4. After 48h culture, the floating cells was removed together with the medium, after which fresh medium was added and cultured anther three days. On day 5, immature DC (iDC) was collected and used in next experiments.

### 2.3. Morphology and Flow cytometric (FCM) Analysis

On day 5, iDC was first incubated in different concentration of HEP for 24h. The phenotype of DC was evaluated using murine PE-CD11c, PE-CD80, PE-CD86, PE-CD40 antibodies. DC was then analyzed by flow cytomertry (Becton Dickinson, San Jose, CA, USA). In different groups, the images of DC were obtained using an Olympus camera.

## 2.4. Endocytosis Assay

After different treatments, $10^5$ cells were incubated with 1mg/ml FITC-dextran (Sigma, MO, USA) for 1h. After then, cells were washed with cold PBS and analyzed by FCM.

## 2.5. Cytokines

Levels of IL-12 in the supernatants were measured using enzyme-lined immunosorbent assay (ELISA) kits according to the manufacturer's innstructions (Beyotime, Beijing, China).

## 2.6. Mixed Lymphocyte Reaction (MLR)

MLR assay was conducted as described in previous study [7,8]. CD3+ T cells were isolated using MACS assay (Miltenyi Biotec, Germany). iDC were treated with 25 mg/mL mitomycin C (AppliChem, Darmstadt, Germany) for 45min, and then washed twice with PBS. Isolated T cells was added into each well and cultured for another 120h at 37°C. T cell proliferation was measured by using a CCK8 kit (Beyotime, Beijing, China) according to the manufacturer's instructions.

## 2.7. Statistical Analysis

Data are expressed as the mean±SEM. We used an analysis of variance (ANOVA) followed by a Student-Newman-Keuls post-hoc test for statistical analysis. Experiments for quantification were conducted in a blinded fashion. $P<0.05$ was considered to be statistically significant.

# 3. Results

## 3.1. HEP Induced Phenotypic Maturation of BMDC

After co-cultured with 1000ug/ml HEP for 24h, most of DC showed long dendrites in morphology (Figure 1), which is a direct evidence of maturation.

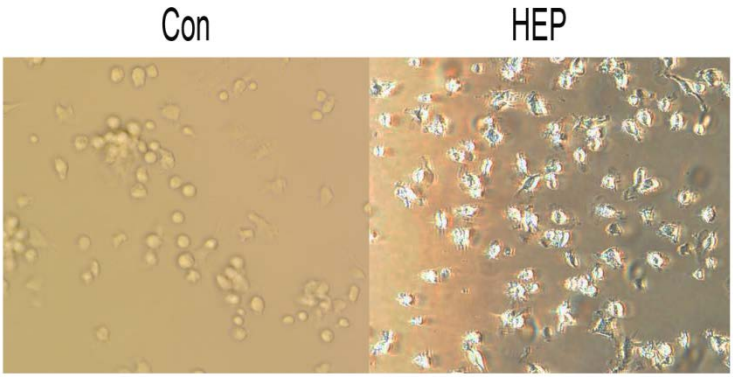


**Figure 1.** HEP induced phenotypic maturation of BMDC. Representative images of the immature DC and HEP-treated DC.

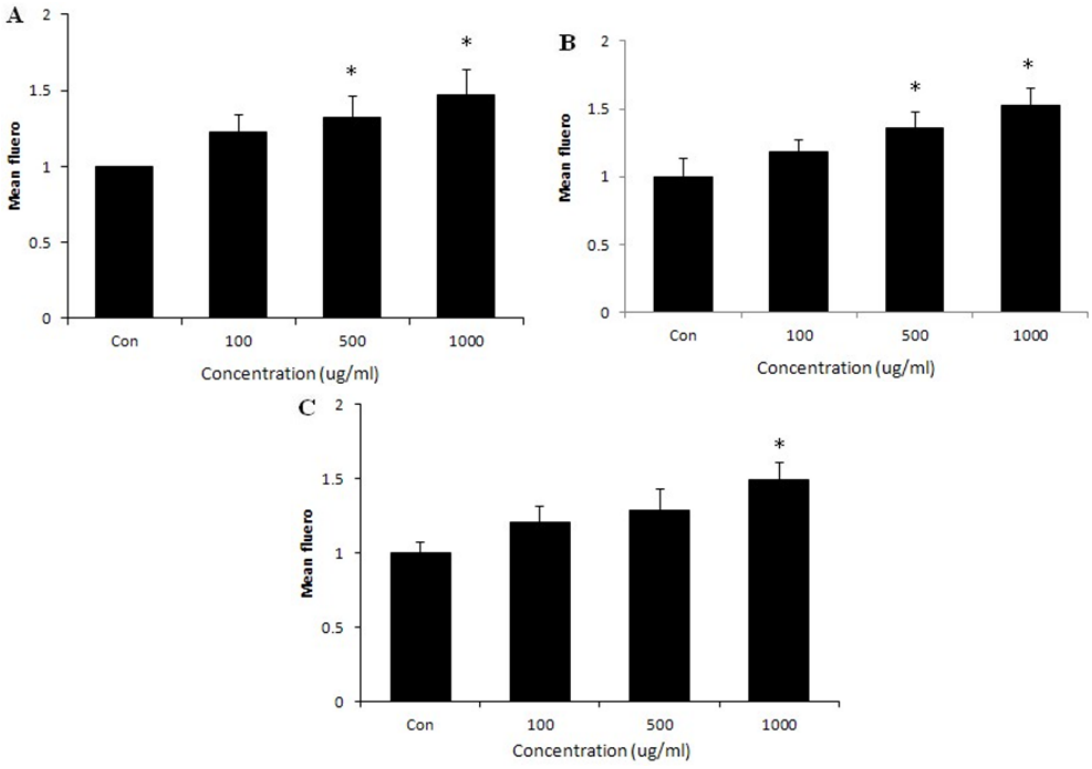


**Figure 2. HEP increased the expression of co-stimulatory molecules on DC.** Bar graphs of CD80 (A), CD86 (B), CD40 (C) mean fluorescence intensity of different concentrations of HEP-treated DC (n=5). *P<0.05 Vs the control group.

### 3.2. HEP Up-regulated the Expression of Co-stimulatory Molecules on DC

We measured the expression level of co-stimulatory molecules by FCM also after 24h co-culture with 100, 500 and 1000ug/ml HEP. The fluorescence intensity of CD80, CD86 were significantly enhanced in the 500 and 1000ug/ml HEP-treated groups compared with the control groups (Figure 2AB). While CD40 expression only increased in the 1000ug/ml HEP-treated groups (Figure 2C).

### 3.3. HEP Inhibited the Endocytosis Activity of BMDC

After 500 and 1000ug/ml HEP treatment, BMDC showed a reduced activity of endocytosis of FITC-dextran, a fluorescence labeled dextran (Figure 3). However, no significant difference was found in the 100ug/ml HEP-treated groups.

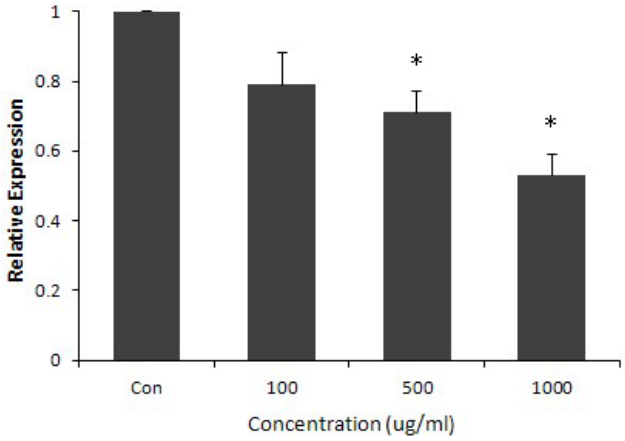


**Figure 3.** HEP inhibited the endocytosis activity of BMDC. A bar graph of the FITC relative fluorescence intensity of different concentration of HEP-treated groups (n=5). *P<0.05 Vs the control group

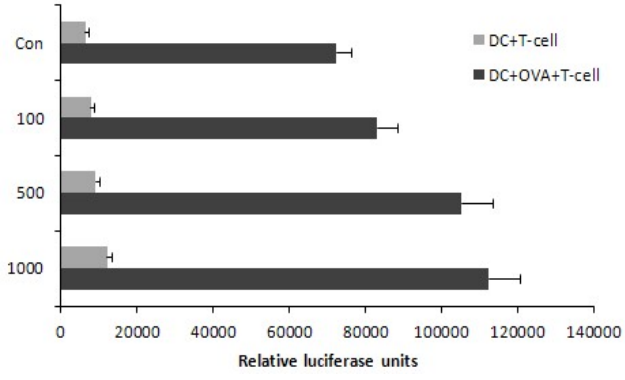


**Figure 4. HEP augmented the allogenic T cell proliferation.** A bar graph of T cell stimulating effects of HEP-treated DC (n=5). *P<0.05 Vs the control group.

### 3.4. HEP Augmented the Allogenic T cell Proliferation

In the MLR assay, the T cell proliferation showed the immune stimulatory ability of DC. And in the 500 and 1000ug/ml HEP group, the T cell proliferation was significant higher than the control group (Figure 4).

### 3.5. HEP Enhanced the Expression of IL-12

IL-12 is a function maturation maker of DC and exerts the immune stimulatory effects [9]. As shown in Figure 5, the concentration of IL-12 in supernatants of DC was significantly increased at 48h after incubation in a concentration dependent manner.

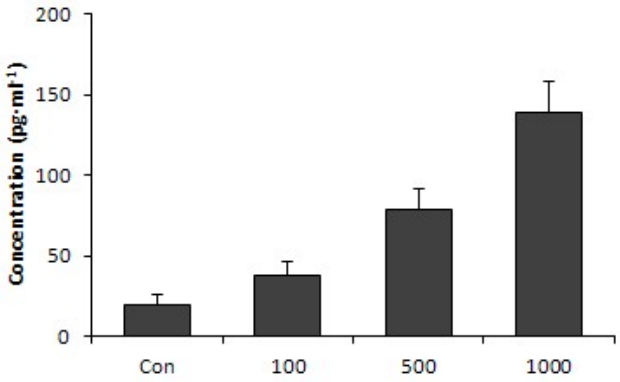


**Figure 5. HEP enhanced the expression of IL-12.** A bar graph of the concentrations of IL-12 in the surpernant in each groups (n=6). *P<0.05 Vs the control group.

## 4. Discussion

Hericium erinaceus polysaccharide (HEP) is a Chinese herb and was reported to regulate the immune response effectively in different aspects [2,10,11]. In this study, we focused on the influence on the most important antigen presenting cells, DC, which controls the entire immunity. We found that HEP treatment induced DC maturation by the morphology and the up-regulation of co-stimulative molecules. The endocytosis activity was also reduced, which is also an important feature of maturation. HEP also increased the allogenic T cell proliferation in a MLR assay. Finally, it was found that HEP increase the expression level of IL-12.

After stimulated by antigen or other inflammatory regents, iDC began to differentiate into mature DC (mDC) [1,12]. During this process, cells began to express long dendrites, express high level of co-stimulative molecules, such CD80, CD86, CD83 etc [1]. Our data showed that HEP administration induced longer dendrites, and increase the level of these three makers, which indicated that HEP induced the phenotypic maturation of BMDC. iDC exhibits strong endocytosis capacity, while mDC showed potent antigen presenting activity. We found HEP reduced the endocytosis activity of BMDC, which also proved that HEP promoted the transition from iDC to mDC. MLR assay is a classical method to examine the T cell stimulating ability of antigen presenting cells [13,14,15]. After HEP treatment, DC induced the proliferation of T cells more effectively. Finally, we examined the IL-12 level in the supernatants of BMDC, because IL-12 is a function maturation maker of DC. Our data showed that HEP increased the concentration in the supernatants, indicating a role of IL-12 in the enhancement of T cell proliferation.

In conclusion, HEP induced that maturation of murine BMDC, reduced the endocytosis activity, and enhanced

the T cell stimulatory effect. The increased level of IL-12 was also an evidence of maturation. The influence of HEP on toll like receptors and other pattern recognition receptors is to be investigated in further study.

## Declare of Interests

The authors have no interest to declare.

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