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# Effects of human plasma proteins on maturation of monocyte-derived dendritic cells

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**Abstract**

Dendritic cells (DC) are a promising tool for vaccine therapy due to their unique properties as antigen presenting cells and their ability to prime naïve T cells. Increasing evidence suggests that maturation stage of DC critically influences the fate of the immune response. Generation of monocyte-derived DC for clinically applicable immunotherapy requires the use of well-defined components and stringent culture conditions. An alternative strategy is to use human autologous serum. However, its constituents are not stable and reflect the inflammatory condition of the donor. In order to investigate whether DC properties are influenced by proteins present in the plasma, we matured human monocyte-derived DC with four main plasma components: fibrinogen, fibronectin, plasminogen or C-reactive protein. These purified proteins were added at various concentrations on day 6 after the initial differentiation induced by IL-4 and GM-CSF. The maturation was assessed by phenotyping of maturation-associated marker (CD83) and co-stimulatory molecule CD86 as well as IL-12 production. Functional properties of DC were assessed by endocytic activity and mixed leukocyte culture. Our results indicate that fibrinogen had DC-maturation effect comparable to poly-I:C, TNF-α and $PGE_2$ as a positive control, but it failed to induce IL-12 production. The other plasma proteins had no effect on DC maturation. CRP at high concentration had rather inhibitory effect on DC induced lymphocyte function. We conclude that none of the tested plasma components and acute phase proteins sufficiently induce fully competent mature DC. This finding is important for the preparation of human DC-based vaccines supplemented by autologous sera.
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*Keywords:* Dendritic cells; Maturation; Plasma proteins

## 1. Introduction

Immune response in vivo is associated with the production of multiple cytokines, chemokines and inflammatory proteins with systemic effects, which belong to the innate arm of the immune response. The current belief is that dendritic cells (DC) translate such activation as a maturation signal, and in that way function as the link between the innate and adaptive immune response. Therefore, the characterization of inflammatory signals influencing DC maturation has a profound therapeutic implication for vaccination strategies as well as suppression in case of autoimmunity.

Recently it has been appreciated that DC exist in at least two functionally and phenotypically distinct stages, immature and mature DC. Immature DC (imDC) reside in peripheral tissue where they capture antigens such as bacteria, viruses and damaged tissue [1]. Upon exposure to the innate pro-inflammatory signals or pathogen-derived products, DC loose their phagocytic capacity and migrate to draining lymph nodes while becoming mature DC (mDC) [2,3]. Human mDC have high antigen presenting capability

*Abbreviations:* DC, dendritic cells; imDC, immature DC; mDC, mature DC; CRP, C-reactive protein; LPS, lipopolysaccharide; MLR, mixed leukocyte reaction; PBMC, peripheral blood mononuclear cells; $PGE_2$, prostaglandin E2; poly I:C, double-stranded RNA polyinosinic:polycytidylic acid; TLR, toll-like receptor; MFI, mean fluorescence intensity

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and T-cell stimulatory capacity due to their high levels of antigen presenting molecules (HLA class I and class II), adhesive and co-stimulatory molecules CD80, CD86, CD40, CD54 as well as other DC-specific markers, such as CD83 and DC-LAMP [4,5].

Thus, maturation is a critical process in DC development that determines the fate of the immune response–tolerance versus immunity. Several publications report that imDC induce T-cell tolerance by induction of T regulatory cells [6,7]. More recently, Menges et al. described that stimulation by TNFα results in incompletely matured murine DC, which induce peptide-specific IL-10-producing T cells in vivo and prevent experimental autoimmune encephalomyelitis [8]. Lutz and Schuler propose to title such tolerogenic MHC (high), costimulation (high), cytokines (low) DC as "semi-mature", and the immunogenic MHC (high), costimulation (low), cytokines (low) DC as "fully mature" [6].

Plasma is a major source of inflammatory proteins, containing 490 identified proteins many of which exhibit systemic effects [9]. Little is yet known however, how many of them influence DC maturation. Many protocols [10–13] describe the requirement for the presence of plasma during DC maturation ex vivo. The complement system that is composed of more than 20 serum proteins and cell surface receptors has been implicated in enhancement of such process. On the other hand, immunoglobulins, are used clinically to suppress autoimmune and systemic inflammatory disorders and were shown to inhibit DC maturation [14]. Therefore, not only for our understanding of DC function in vivo, but also for purposes of ex vivo generation of DC-based vaccines, it is desirable to identify the essential plasma proteins that induce DC maturation. This would contribute to the improvement of standardized good manufacturing practice (GMP) protocols for large-scale production of DC-based immunotherapeutics.

The aim of this study was to investigate the effects, of a selected group of purified plasma proteins with documented immunological functions, on the phenotypical and functional properties of human monocyte-derived DC. We found that only fibrinogen reproducibly delivered potent maturation stimulus to DC with the exception of the induction of IL-12.

## 2. Materials and methods

### 2.1. Patients and normal donors

All patient and blood donor samples were acquired after informed consent according to the guidelines of the Institutional Review Board and the Helsinki protocol.

### 2.2. Monocyte isolation

Monocytes were obtained from peripheral blood mononuclear cells (PBMC) of healthy volunteers and leukapheresis products of three melanoma patients, as a principal candidates of DC-based immunotherapy (all from Blood Bank, Karolinska Hospital, Stockholm, Sweden).

Monocytes from buffy-coats were isolated by Ficoll density separation (Amersham Biosciences, Uppsala, Sweden), plated at $20 \times 10^6$/ml in a 75 $cm^2$ flask (Costar, Corning, USA) and incubated for 2 h in 5% $CO_2$ at 37 °C. Non-adherent cells were removed by gentle washing. Adherent cells were directly used in experiments.

In the case of melanoma patients, peripheral monocytes were obtained from leukapheresis products using elutriation in a Beckman Coulter Avanti J20XPI centrifuge as described [15]. Briefly, at constant flow rate 53 ml/min using a peristaltic pump Masterflex L/S (Cole-Parmer Instrument Company, Vernon Hills, IL) and at 1700 rpm ($278 \times g$) >85% purity of CD14+/CD45+ cells was obtained in all patients. Cells were directly used in experiments.

### 2.3. DC generation and maturation

DC were generated from monocytes in X-VIVO 15 medium (Bio-Whittaker, Cambrex, Belgium) serum-free supplemented with GM-CSF (50 ng/ml) (Leucomax), and IL-4 (40 ng/ml) (both Schering Plough, Sweden), in 75 $cm^2$ culture flask (Costar).

Fresh medium was added every 2–3 days. ImDC were harvested on day 6 and transferred to a 48-well tissue culture plate (Costar) at a final concentration $5 \times 10^5$ cells/ml ($2 \times 10^5$/400 μl). Maturation was induced by the addition of following proteins: fibrinogen, (Octapharma), fibronectin, plasminogen (Sigma–Aldrich, St. Louis, MO) and CRP (kindly provided by Holly Raeside, Scipac, UK) in presence of fresh GM-CSF and IL-4 for 48 h. All plasma proteins were tested at the wide range of concentrations: fibrinogen (4–5000 μg/ml); fibronectin (0.5–450 μg/ml); plasminogen (6.25–310 μg/ml); and C-reactive protein (CRP) (5–500 μg/ml) (5–10 tested concentrations). The physiological range of concentrations are: fibrinogen, 2.55 mg/ml; fibronectin, 300 μg/ml; plasminogen, 155 μg/ml; and CRP, 5 μg/ml.

In further experiments, plasma proteins were tested in concentrations resulting in highest CD83 expression: fibrinogen, 1250 μg/ml; fibronectin, 100 μg/ml; plasminogen, 75 μg/ml; and CRP 500 μg/ml (concentration of inflammatory state), 5 μg/ml.

Immature cells cultured with IL-4 and GM-CSF, without addition of any maturation stimuli, were used as negative control. DC matured with the combination of TNF-α (50 ng/ml) (Chiron, Emmeryville, CA), double-stranded RNA polyinosinic:polycytidylic acid (poly I:C) (50 μg/ml) Sigma), prostaglandin E2 ($PGE_2$) (1 μg/ml) (Sigma), served as positive control in our experiments.

### 2.4. Phenotypic and functional analysis

#### 2.4.1. Flow cytometric analysis

The generated DC were characterized by flow cytometry using a FACS Calibur cytometer (Becton Dickinson, San José, CA) on day 8. The following mAbs were used: Phy-

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