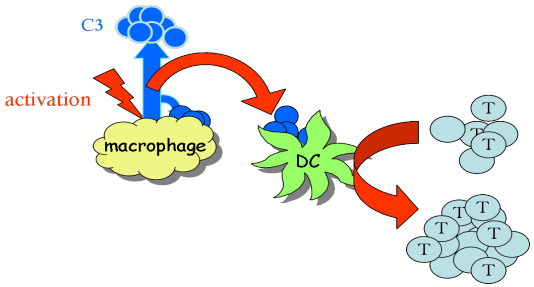
PhD thesis

The effect of complement C3 on the function of human monocyte derived dendritic cells



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Introduction

Antigen presentation has essential role in the initiation of the immune response. Dendritic cells (DCs) are the most important antigen presenting cell type (APC) due to their unique ability to activate naive T-lymphocytes. The properties that make them proper for this challange are the following: they are located at the site of the antigen entry, they take up and process the antigen and present it in the lymph nodes int he context of proper costimulatory molecules. A prominent property of dendritic cells is their cytokine production, thereby the shaping of the immune response. They have the ability to induce both humoral or cellular response, moreover, they have indispensable role in the generation of immunological tolerance.

Dendritic cells are mentioned as natural adjuvants, for bridging innate and adaptive immunity. The complement system has this bridging role also. Complement system is part of the innate immune system, consists of approximately 30 proteins, including the proteins of the cascade, their regulators and receptors. The complement system can be activated trough three different ways, these are the classical, the lectin dependent and the alternative pathways, those differ only in their initiating molecules. After the short initiating phase, all three leads to the activation of the central component C3, and continues on a common way to the generation of the membrane attack complex. C3 has also central role in the regulation of the immune response, both B and T cell activation are damaged in the absence of the protein. The identification of the underling mechanism is continous, but more clear in the case of B cells.

Our workgruop formerly demonstrated, that one possible explanation for how complement system affects T cell responses, is the modulation of dendritic cells. The first component of the classical pathway, C1q, is an effective activator of dendritic cells, it enhances the expression of the molecules that are important in antigen presentation, and it also enhances their T cell stimulatory capacity and their proinflammatory cytokine production. C3 is long known to be very important in proper quality and quantity T cell activation, so it may be hipothesized, that the background is the modulation of dendritic cells function via C3.

Objectives

- To elucidate the C3 production of human monocyte derived dendritic cells (MDCs).
- The investigation of C3 deposition in cocultures of MDCs and monocyte derived macrophages (MMs) in steady state and proinflammatory

conditions. We analyzed the deposition of C3 derived from MMs.

- The study of C3 binding of MDCs using purified C3 and normal human serum (NHS).
- To follow up the effects of covalently bound C3
    - We analyzed the fate of cell-bound C3, its intracellular and cell surface amount
    - We determined the effect of covalently bound C3 on the phenotype of MDCs
    - We studied the T cell activating capacity of native C3 treated MDCs
    - We investigated the cytokine production of native C3 treated MDCs
- The functional analization of CR3 and CR4 complement receptors on MDCs

Applied methods

- Culturing of primary human cells
- Fast Protein Liquid Chromatography
- SDS-PAGE
- Western-blot
- Flowcytometry
- Confocal laser scanning microscopy
- ELISA
- RNS silencing technique

Results

- We demonstrated that human MDCs in contrast to MMs do not produce C3, but they are able to fix the C3 that is secreted by activated macrophages.
- We proved, that MDCs fix native C3 covalently on their surface in a dose dependent manner.

- We demonstrated that the cell bound C3b enters the cells within 30 minutes, on the other hand it can be detected on the cell surface even 48 hours after the C3 treatment. The covalently bound and internalized C3 fragments are partially released on nanoparticles.
- The covalently bound C3b enhances the CD83, CD86 and MHCII expression of MDCs.
- MDCs treated with native C3 have elevated T cell stimulatory capacity. We proved, that the C3b fragments present on the surface of MDCs have direct role in the activation of T cells. This is supplemented by the modulation of dendritic cell phenotype and cytokine production.
- Native C3 treatment enhances MDCs IL-6, TNF-α and IL-8 production.

- Based on our results, we concluded that the covalently bound C3 doesn't act via CR3 and CR4.

Publications connected to the thesis

Noémi Sándor, Domonkos Pap, József Prechl, Anna Erdei, Zsuzsa Bajtay: „**A novel, complement-mediated way to enhance the interplay between macrophages, dendritic cells and T lymphocytes"** Molecular Immunology 2009. 47(2-3):438-48. (IF: 3,742)

Zsuzsa Bajtay, Eszter Csomor, Noémi Sándor, Anna Erdei: **„Expression and role of Fc- and complement-receptors on human dendritic cells"** Immunology Letters 2006. 104(1-2):46-52. (IF: 2,35)

Noémi Sándor, Katalin Kristóf, Katalin Paréj, Anna Erdei, Zsuzsa Bajtay: **„The different role of**

complement CR3 and CR4 in regulation of dendritic cell functions". Submitted for publication.

Other publications

Anna Erdei, Andrea Isaák, Katalin Török, Noémi Sándor, Mariann Kremlitzka, József Prechl, Zsuzsa Bajtay: **„Expression and role of CR1 and CR2 on B and T lymphocytes under physiological and autoimmune conditions".** Molecular Immunology 2009. 47(2-3):438-48. (IF:3,742)

Eszter Csomor, Zsuzsa Bajtay, Noémi Sándor, Katalin Kristóf, Gerard J Arlaud, Steffen Thiel, Anna Erdei: **„Complement protein C1q induces maturation of human dendritic cells".** Molecular Immmunology 2007. 44(13):3389-97. (IF:3,742)

Katalin Kristóf, Krisztina Madách, Noémi Sándor, Zsolt Iványi, Anna Erdei, János Gál, Zsuzsa Bajtay: „**Molecular mimicry between pathogens**

and self reduces severity but lengthens recovery of pneumonia induced sepsis". Submitted for publication.

Published conference abstracts

Eszter Csomor, Zsuzsa Bajtay, Noémi Sándor, Nicole Thielens, Steffen Thiel, Gerard Arlaud, Anna Erdei: „**Immobilized C1q induces maturation of human monocyte-derived dendritic cells".** FEBS Journal 2005 272(1): 288-289 (IF 3,03)

Oral conference presentations: 3
International conference abstracts: 8
Home conference abstracts: 4