ImmunoTools *special* Award 2013

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Evaluating the immunological function of the Amyloid Precursor Protein (APP) and Aβ-peptides in macrophages

The major pathological hallmarks of Alzheimer´s disease (AD) are depositions of amyloid-β (Aβ)-peptides and neurofibrillary tangles. Aβ-peptides are generated through sequential cleavage of the amyloid precursor protein (APP). Assuming that the β-amyloid deposits are causative for the development of Alzheimer´s dementia, several substances were developed to reduce Aβ-peptide deposition. Unfortunately, coming to clinical trials, none of these substances succeeded in slowing AD-progression although plaque deposition was effectively reduced. Interestingly, patients treated with these medications suffered an increased incidence of infections and in mouse models deficient of APP processing an increased neonatal mortality was observed when bread under non pathogen free conditions. These observations suggest a function of APP and the Aβ-peptides in the immune defence. APP is constitutively expressed on mononuclear phagocytes. Its cleavage to generate Aβ-peptides can be induced by lipopolysaccharide (LPS). However, the physiological function of APP and Aβ-peptides in mononuclear phagocytes is widely unknown.

The AIM of this study is, to provide evidence for a function of APP and Aβ-peptides in the immune defence as an immunreceptor and a soluble messenger, respectively.

In order to explore the immunological functions of the APP metabolism in the various types of differentiated macrophages, the APP knock-down will be performed in three models: THP-1 macrophages as well as primary human M1- and M2-macrophages. THP-1 cells are differentiated into macrophages with phorbol-12-myristate-13-acetate (PMA) over 3 days. To generate primary macrophages, peripheral blood

mononuclear cells (PBMC) are isolated by ficoll density centrifugation and differentiated with GM-CSF or M-CSF to induce an M1- or M2-phenotype, respectively.

The knock down of APP expression in peripheral mononuclear cells (PBMC) and differentiated THP-1 macrophages is performed via lipofection with APP siRNA. Successful transfection is validated by missing APP immunofluorescence in flow cytometry or SDS-PAGE of the cell lysates.

In order to explore the immune competence of the APP knock-down, macrophages, IL-1β, IL-6, IL-8, IL-10 and TNFα will be quantified in cell culture supernatants after a challenge with LPS, zymosan particles or polysterene beads.

Also, the phagocytic function of primary human M1 and M2-macrophages as well as differentiated THP-1 macrophages will be assessed after the APP knock-down. Therefore the differentiated macrophages will be challenged with fluorescent polysterene beads or fluorescent, heat inactivated bacteria. This way, the amount of internalized particles can be quantified by flow cytometry.

To investigate the differentiation of APP-deficient monocytes, freshly isolated human monocytes will be transfected with APP specific siRNA and cultivated in the presence of M-CSF, GM-CSF or LPS. After three days, the macrophage differentiation markers (CD11c, CD14, CD16, CD36, CD40, CD68, CD206, MSRI) will be determined by flow cytometry. Furthermore secretion of IL-1, IL-6, IL-8, IL-10 and TNFα will be measured by ELISA. Expression of iNOS will be shown by immunocytochemistry.

Given, that the APP knock down leads to an immunodeficient phenotype, it will be very interesting to explore, if the immune-competence can be restored by supplementing the cell culture medium with recombinant Aβ-peptides.

Taken together, our studies will help to elucidate the physiological function of APP and Aβ-peptides in the immune system. Knowing, that Aβ-lowering therapies are currently tested in several clinical trials, more information on the physiological functions of Aβ-peptides are essentially necessary.