# ImmunoTools *special* Award 2014

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## The role of placental fractalkine on maternal monocyte activation

Fractalkine is synthesized as a transmembrane chemokine, comprising an extracellular N-terminal domain, a mucin-like stalk, a transmembrane α-helix and a short cytoplasmic tail. The extracellular domain represents the chemokine domain with two cysteins separated by three other amino acids. Membrane-bound fractalkine is constitutively cleaved by metalloproteases into a soluble isoform, consisting of the chemokine domain and the mucin-like stalk. Thus, fractalkine mediates different steps of leukocyte recruitment, depending on whether it is cleaved or not. While the membrane-bound form promotes flow resistant adhesion of leukocytes to endothelial or epithelial cells via its corresponding G protein-coupled, 7-transmembrane receptor CX3CR1, the soluble form has chemoattractive activity for most $CD16^+$ natural killer cells, $CD3^+$ T-cells and a majority of $CD14^+$ monocytes. From a pathophysiologic point of view, the chemokine/receptor duo fractalkine/CX3CR1 has been suggested to be involved in critical events including fibrogenesis, neuropathic pain sensation, the pathogenesis of certain cancers and neurodegenerative diseases.

The knowledge concerning a role of the fractalkine/CX3CR1 axis in human placenta development and physiology is very limited and based on a very small number of studies. We have recently shown that placental fractalkine is expressed at the apical microvillous plasma membrane of the syncytiotrophoblast, where it can be released into the maternal circulation by constitutive metalloprotease dependent shedding. In this study we demonstrated fractalkine expression and release of the soluble form in a trophoblast cell line, in primary trophoblasts and in placental

explants. However, a putative role of soluble fractalkine on activation of maternal peripheral blood mononuclear cells during pregnancy has not been considered so far.

The aim of this project is to test whether or not placenta derived soluble fractalkine can activate maternal monocytes. For this purpose trophoblasts and placental explants will be co-cultured with THP-1 monocytes in presence or absence of fractalkine neutralizing antibodies in a transwell system. After 24h co-culture, monocytes will be collected and analyzed for their activation status. For this purpose monocytes will be subjected to flow cytometry analysis using FITC - conjugated anti-human CD14, CD16, CD33, CD62L, HLA-DP, HLA-DR, IL-6 and PE - conjugated anti-human IFN-gamma, IL-6, IL-8, TNFa. In parallel, cytospins of monocytes will be immunohistochemically stained using APC - conjugated anti-human CD14, CD16, CD62L. Controls will be performed using respective Control-IgG isotypes.

In addition to fractalkine neutralizing antibodies, silencing of the fractalkine receptor CX3CR1 in monocytes will be performed as an alternative approach. After successful CX3CR1 silencing, monocytes will be subjected to co-culture with trophoblasts or placental explants and analyzed for their inflammatory profile as described above. CX3CR1 silencing in monocytes or the presence of fractalkine neutralizing antibodies in co-culture should show an impaired activation of monocytes. Results from this project may have implications on treatment of pregnancies complicated with inflammatory diseases.