The complement system is one of the most important part of the innate immune system. This network of more than 30 soluble and cell surface proteins plays an important role in innate defence as well as instructing and regulating adaptive responses. Complement activation can be triggered by a wide variety of substances, including immune complexes, foreign structures, and apoptotic bodies. The cascade involves regulated activation of complement components, leading to inflammation, clearance of pathogens and apoptotic cells. In the course of activation several biologically active fragments are generated, which exert immune regulatory functions by binding to various complement receptors and regulators expressed by various cell types. The complement cascade activated by either of the three pathways leads to the proteolytic cleveage of the central component, C3. The larger cleavage product C3b, can be further processed to generate iC3b, which remains covalently attached to the activating surface (eg. microbes, apoptotic cells, immune complexes) and binds to complement receptors CR3 (CD11b/CD18) and CR4 (CD11c/CD18).

Human monocytes (Mo), macrophages (M), neutrophil granulocytes (Neu) and (immature) dendritic cells (DC) are phagocytes. These cells are known as sentinels of the immune system that can be found all over the body. Pathogen microbes entering the body become opsonized by complement proteins, mainly by the larger fragments of C3, which help eliminate antigens by the phagocytes. Depending on the activation stimuli in the microenvironment they can mediate several functions. Phagocytes play a decisive role in immunity; they interact with various pathogens via several pattern recognition and different opsonophagocytotic receptors, including Fc- and complement-receptors. Moreover macrophages and dendritic cells are professional antigen presenting cells. The basic importance of mature DC in generating immunity is underlined by their unique ability to activate naive T lymphocytes, linking innate and adaptive immunity this way. For the efficient activation of T cells DC have to provide three separate signals, such as presentation of antigens, expression of costimulatory molecules and production of cytokines. In the absence of inflammation, DC in the peripheral tissues take up apoptotic cells, process self-antigens and migrate constitutively to the draining lymph node. Without danger signals however, they do not express costimulatory molecules, therefore fail to stimulate T cells. Maturation of DC can be induced by various stimuli including microbial products, immune complexes and inflammatory cytokines.

Integrins are heterodimeric transmembrane glycoproteins consisting of a non-covalently coupled alpha and beta chain. β_2-integrins, including complement receptors CR3 and CR4 participate in many immunological processes, especially those involving cell

migration, adherence, and phagocytosis. Both CR3 and CR4 are widely expressed on most of the myeloid cell types, although the level of their expression varies. In humans, CR3 and CR4 are simultaneously expressed in human phagocytes – Mo, M, DC, Neu -, and NK cells. Since the main ligand of CR3 and CR4; the iC3b is identical, the study of the individual function of these integrins is challenging. CR3 and CR4 are generally thought to mediate overlapping functions; however the *possible distinctive role of these receptors has not been investigated so far*. Our aim was to explore whether there is a difference between the binding and uptake of various complement-opsonized microorganisms, mediated by CR3 and CR4.

We found that CR3 expression is downregulated on human monocyte derived DC (MDDC) during maturation, in contrast to CR4 which is significantly enhanced in the same time. We demonstrated that both receptors were present in the cell membrane in an active conformation (Sándor,Immunobiol,2013). We showed that ligation of CD11b directs MDDCs to enhanced phagocytosis, while the maturation of the cells and their inflammatory cytokine production are not affected. Blocking CD11c alone did not change the uptake of opsonized yeast or bacteria by MDDCs. We confirmed these results using siRNA; namely downregulation of CD11b blocked the phagocytosis of microbes while silencing CD11c had no effect on their uptake. Our data clearly demonstrate that iC3b opsonized Staphylococcus aureus and Saccharomyces cerevisiae of MDDCs is mediated mainly by CR3.

Due to the known differences between various phagocytes regarding the process of internalization and killing, we set out to investigate human phagocytes (Lukácsi,Imm.Lett,2017). In order to distinguish surface bound and digested bacteria, pHrodo Green conjugated bacteria were also used in addition to Alexa488 labelled, complement opsonized S. aureus particles. The former one is a pH sensitive dye, which becomes highly fluorescent in the acidic milieu of phagolysosomes. The participation of CR3 and CR4 was compared in the process of binding, internalization and digestion of iC3b opsonized Staphylococcus aureus by Mo, monocyte derived macrophages (MDM), MDDC and Neu. We can strengthen our earlier findings by showing that only CR3 blocking decreases the digestion of S. aureus by phagocytes. Employing this method, in the case of MDMs we found an interesting collaboration between the two complement receptors. Namely, we observed that blocking CR4 decreased the amount of surface bound particles, while the digestion of iC3b opsonized S. aureus was dependent on functional CR3. The different mobility and interacting partners of CR3 and CR4 could influence the outcome of phagocytosis in a cell type specific manner.

The absolute numbers and the conformational state of CR3 and CR4 expressed by the cells were assessed (Sándor,PlosOne,2016). We determined the exact numbers of CD11b and CD11c on these cell types by a bead-based technique, and found that the ratio of CD11b/CD11c is 1.2 for MDDCs, 1.7 for MDMs and 7.1 for monocytes, suggesting that the function of CD11c is preponderant in MDDCs and less pronounced in monocytes.

In contrast to the human system, murine CD11c/CD18 expression is mainly limited to dendritic cells, therefore CD11c can be used to identify this cell population. Results obtained in studies on mouse CD11b/CD18 however cannot be simply translated to the human system, due to the differences between the two species. Originally we also planned to analyse the role of CR3 and CR4 in CD11b KO mouse model. However new data were published showing profound differences regarding the expression and function of in the mouse and human systems. Moreover it turned out that CR3 or CR4 deficiency cause less severe consequences in animal models than in humans. Mice models with CD18 deficiencies have been developed and all animals deficient in these molecules are viable and fertile, and only two of the mutations to date have resulted in phenotypes with spontaneous infections. Thus – with permission of OTKA - instead of the very expensive animal model and test systems we concentrated on the adherence studies that look much more promising based on our data.

Monocytes, macrophages and dendritic cells are phagocytes, which are able to adhere to extracellular matrix components; like fibrinogen via different integrin molecules. These molecules mediate several functions that are associated with cytoskeleton rearrangements, including cell-to-cell and cell-ECM contacts, proliferation, phagocytosis and transendothelial migration of immune cells. The most abundant integrins expressed by cells of the monocytic linage are CR3 and CR4 β2-integrins. The main natural ligand of CR3 and CR4 is iC3b, however, they bind several othermolecules in common, like fibrinogen, ICAM-1, factor X, etc. Our next aim was to determine the participation of CR3 and CR4 in a different function linked to β_2 -integrins; namely *cellular adhesion*.

We analysed the adhesion on fibrinogen using classical immunological method also (Sándor,PlosOne,2016). We blocked either CD11b or CD11c prior to adhesion by ligand binding site specific monoclonal antibodies. Unspecific binding of the antibodies was prevented by treating all samples with FcR blocking reagent and the number of adhered cells was determined as percentage of untreated control samples. Blocking CD11b decreased the number of adhered monocytes slightly but had no effect in the case of MDM and MDDC. Blocking CD11c however decreased the number of adhering MDM, MDDCs and monocytes. The contact area of the differently treated MDM, MDDCs and monocytes by confocal

microscopy was also investigated. Blocking CD11b on MDMs results larger contact areas while blocking CD11c decreases the contact area compared to untreated samples. In the case of MDDCs blocking CD11b induced a more polarized and slightly larger contact surface and CD11c inhibited cells showed a bit more rounded shape but similar contact area to control cells. Blocking of CD11b had an increasing effect on contact area in the case of monocytes also, however blocking CD11c did not had any effect and these cells showed similar morphology as control. To quantify these observations, we established contact size categories and determined the proportion of the different categories in the samples; small, medium and large. By blocking CD11b, cells with small contact area almost completely disappeared from MDM and MDDC samples and their proportion decreased in monocyte samples also compared to control. Simultaneously the ratio of large cells increased in the case of all cell types, showing that CD11b acts against spreading. In the contrary, blocking CD11c elevated the ratio of small contact area cells in MDMs and the ratio of medium area cells in MDDCs and had no effect on monocytes.

To further dissect the mechanism of monocyte adhesion we started a collaboration with a group at the Department of Biological Physics (Róbert Horváth, Bálint Szabó) at Eötvös University. In these studies we wish to exploit the potentials of an optical biosensor, namely the Optical Waveguide Lightmode Spectroscopy (OWLS). OWLS beside the analysis of biomolecular interaction has been utilized in a wide spectrum of applications, including live cell studies. So far however OWLS has been applied to investigate the adhesion and spreading of cell lines only. We are the first to study primary cells by this instrument (Orgovan, Biosensors and Bioelectronics, 2014). The high sensitivity and the in-situ measurements offer a label-free technique and might contribute to a better understanding of adhesion. To perform kinetic studies on adherence to fibrinogen the EPIC label free optical biosensor was used. This method enables the real-time monitoring of a 100-200 nm width layer over the adhesive surface by analysing the refractive index alterations in this field. Cells can reach this area only by adhesion, thereby non adhering cells are excluded from the measurement. Signal is detected as shift in wavelength ($\Delta\lambda$) and the higher this shift is, the larger area of the sensor is covered. This means that using this method we detect a combined signal of the number of the adhered cells and the size of their contact area. These data strengthen the previous findings obtained by classical microscopy, namely that blocking CD11b on MDMs and MDDCs increases the size of the contact area while blocking CD11c decreases number of adhering cells and also the contact size.

Using the commonly used adherence measurement techniques we can assess the adhering capacity of a certain population of cells quickly, but cannot characterize the adherence of a single cell. To exploit the versatility of the computer controlled micropipette we can measure the adhesion force of human Mo, MDM and MDDC on the extracellular protein fibrinogen. Using this method single cell data can be generated of hundreds of cells (Salánki,PlosOne,2014). Automated single cell sorting might be a useful method for studying single cell interactions with specific macromolecules, moreover using this technique the probed single cells potentially could be easily picked up and further investigated by other techniques (Salánki,ApplPhysLett,2014). Automated single cell sorting and deposition in submicroliter drops are challenging, when intact cells are needed for further investigations. This new technique may provide excellent opportunity for single cell biology. Using this method, human cells were let to adhere on fibrinogen coat and after that their adhesion force was probed by trying to pick them up with a computer controlled micropipette using fluid flow that was established using vacuum. The pick-up process was repeated several times with increasing vacuum and the measurement is followed up by continuous microscopic analysis of the number of remaining cells after each round. Blocking CD11b elevated the force of adhesion in the case of MDM and MDDC, however, it decreased the strength of adhesion in monoctyes. Blocking CD11c decreased the adhesion force in each celltype, supporting the importance of this receptor in the process. Our results provide further evidence that human CR3 and CR4 are involved in different cellular functions, despite their capacity to bind the same ligands. Applying state-of-the-art biophysical techniques, we proved that cellular adherence to fibrinogen is dominated by CD11c.

In addition to being a phagocytic receptor, CR3 and CR4 are known to mediate cell adhesion, spreading and migration through the establishment of cell-cell and cell-extracellular matrix connections. Podosomes are known to mediate short-lived adhesion spots that are formed and quickly remodelled during migration. These structures have an F-actin core surrounded by an adhesion ring and can be arranged in clusters, rings or belts. Podosomes are characteristic for monocytic cells, such as monocytes, macrophages, osteoclasts and dendritic cells. Here we studied the podosomes of human phagocytes adhered to fibrinogen coated surface (Lukácsi,ImmLett,2017. Monocytes and MDMs mostly showed an even distribution of podosomes scattered across the contact surface, however a belt like pattern could also be observed for MDMs. The podosomes of dendritic cells were found to be arranged in clusters, or condensed in the leading edge of migrating cells. Whether human neutrophils have podosomes is still debated. In this study we detected the formation of actin rich dots evenly

spread on the contact surface of human neutrophils adhered to fibrinogen. The differences in podosome formation by various cell types could be explained by the different milieu and motility of the investigated cells. Monocytes and neutrophils circulate in the bloodstream and adhere to vessel walls when they reach the site of infection. They migrate through the endothelium into the injured tissue, whereas DCs move from tissues to lymph nodes through lymphatic vessels after antigen uptake. During this journey DCs go through maturation and lose their capacity for phagocytosis as well as podosome formation, because of changes in cytoskeletal reorganization. Thus, podosome formation might be restricted to cells with an actively migrating phenotype. The importance of β2-integrins in podosome formation and podosome mediated adhesion has been proven, but the individual role of these receptors had not been studied so far. Here we show that both CR3 and CR4 are located in the adhesion ring of podosomes in human MDMs and MDDCs attached to fibrinogen. Additionally, a strong staining for the receptors can be observed inside the cells, around the nucleus. Integrins are known to rapidly recycle between the cell membrane and endosomes, which can occur via distinct routes. In the long loop of recycling integrins go through the perinuclear recycling compartment before returning to the cell surface. No data is available yet on the exact recycling route used by β2-integrins, but the staining pattern we found around the nucleus might implicate a passage through the perinuclear recycling compartment.

In summary our data highlight differences in the function of human CR3 and CR4 in the process of uptake, digestion and cellular adherence, while in the process of podosome formation both receptors equally take part.