# Project closing report – NKFI K115623

## Results broken down to specific tasks

Task 1.1: We assessed the possible interactions between MASP-1 and several pro-inflammatory molecules that are frequently produced/secreted in the vicinity of injured or inflamed endothelial cells (ECs). We chose lipopolysaccharide (LPS) as a major bacterial pro-inflammatory compound, histamine (HA) as the main mediator of allergic reaction, bradykinin (BK) as a pro-inflammatory peptide deliberated during contact system activation, and interferon gamma (IFNγ) as a predominant anti-viral cytokine to modify the effects of MASP-1 on ECs. The experimental setup comprised three types of treatment: 1) MASP-1 and the other pro-inflammatory coactivator (together PICs) were given to the ECs simultaneously, 2) ECs were pretreated with MASP-1 followed by the PICs, and 3) ECs were pretreated with PICs followed by MASP-1. We utilized Ca-mobilization tests, permeability test (XperT, developed by us as a major output of the OTKA project), cellular ELISA for adhesion molecules, sandwich ELISA for cytokines and qPCR for receptor quantification at mRNA level in this task. We found several positive interactions between MASP-1 and PICs summarized in **table 1**. The most notable synergism was between MASP-1 and LPS, where permeability (**Figure 1**), E-selectin expression and IL-8 production were significantly higher than in the presence of single

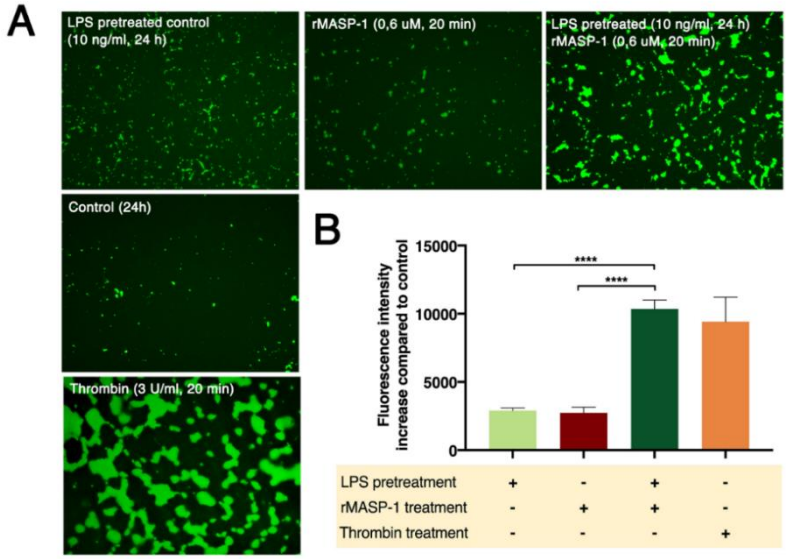


**Figure 1.** Interaction of MASP-1 and LPS in EC permeability measured by XperT test. A) Representative images, green areas are proportional to intercellular gaps. B) Statistical analysis using 3 parallels and calculated from values measured by fluorescence plate reader.

treatments. We also found, in some cases, how the interaction might work between MASP-1 and PICs. Using qPCR, we showed that MASP-1 pretreatment significantly elevated the expression of BK receptors BDKRB1 and BDKRB2. Moreover, LPS pretreatment significantly induced PAR2, a known receptor for MASP-1 (data not shown).

***Table 1. Interaction between MASP-1 and pro-inflammatory coactivators.*** *Orange color represents positive interaction, white color represents the lack of interaction.*

| | | | Response | | | | |
|---|---|---|---|---|---|---|---|
| | **Pretreatment** | **Treatment** | **$Ca^{2+}$-mobilization** | **Permeability** | **E-selectin** | **VCAM-1** | **IL-8** |
| **Cotreatment** | | rMASP-1 + LPS | | | | | |
| | | rMASP-1 + Histamine | | | | | |
| | | rMASP-1 + IFNγ | | | | | |
| | | rMASP-1 + Bradykinin | | | | | |
| **Pretreatment with rMASP-1** | rMASP-1 | LPS | | | | | |
| | rMASP-1 | Histamine | | | | | |
| | rMASP-1 | IFNγ | | | | | |
| | rMASP-1 | Bradykinin | | | | | |
| **Pretreatment with coactivator** | LPS | rMASP-1 | | | | | |
| | Histamine | rMASP-1 | | | | | |
| | IFNγ | rMASP-1 | | | | | |
| | Bradykinin | rMASP-1 | | | | | |

Our results further support the pro-inflammatory role of active MASP-1, since its effects are significantly enhanced if other stimuli act on ECs. Our results were presented at several Hungarian and international conferences [1-3], the manuscript has been written and, after approving by the coauthors, will be submitted in February, 2021.

Task 1.2: Our goal was to assess the effects of MASP-1 on ECs under flowing conditions. We put significant effort into setting up flow-cell-culture system applicable to our cells and detection assays. Although we successfully accomplished to set up the HUVEC culture under flowing conditions, and started to develop measurements that fit the flowing cell culture, several problems emerged. Our microscopy based flow-cell-culture system (iBidi GmbH, Germany) obtained by the support of a MedinProt Synergism Grant could not simultaneously reproduce shear-stress and flow velocity measured in capillaries. When the shear stress values reached those of capillaries or venules, the high velocity damaged the ECs, when the velocity was similar to *in vivo* values, the very low shear stress did not affect ECs. Although we gained experience with a delicate flow-cell-culture system, we could not reach our goals in Task 1.2.

Task 1.3: We aimed to study the effects of MASP-1 on vasodilation/vasorelaxation of *ex vivo* vessels. At the beginning of the project, we focused on human umbilical cord vein and artery as model systems. However, it became clear soon that the myographs applicable for human umbilical cord vessels would require extreme amount of recombinant MASP-1, which we could not produce. Moreover, the preliminary results with human umbilical cord arteries showed a fundamentally different response to adrenalin, histamine and serotonin from those of other typical arteries of the body. Therefore, since the difference between human and murine protease activated receptors (PARs), the known receptors of MASP-1, is minor, we decided to carry on the further experiments in mice. We demonstrated a strong vasodilatory effect of MASP-1 on murine aorta *ex vivo* system, which is dependent on PAR-2 receptor and eNOS activity using knock out animals. The effect of MASP-1 could also be blocked by C1-inhibitor or when endothelium-denudated vessels were used (**Figure 2**). By this finding, we could assign all 3 PARs, as substrate of MASP-1, to different functions.

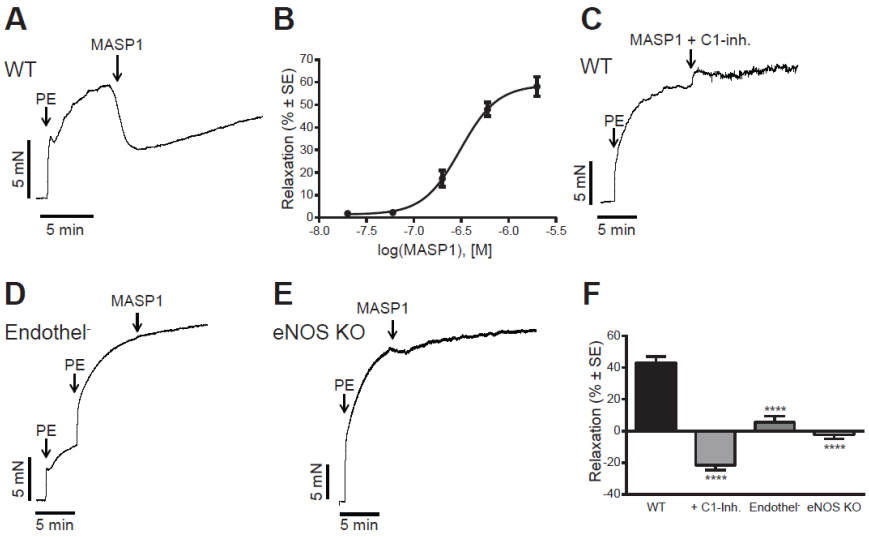


**Figure 2.** MASP-1 causes vasodilation (A) in murine aorta. Its half maximum is around 300 nM concentration (B). The effect of MASP-1 can be blocked with C1-INH (C), by the absence of ECs (D) or by the utilization of eNOS knock-out animals (E). Statistical analysis is shown on panel F.

Our results were presented at Hungarian conferences [4, 5], and the manuscript is now ready for submission.

Task 2.1: Here we studied whether MASP-1 is able to modify the permeability of ECs. This question is not only important in hereditary angioedema (HAE), our model disease, where C1-inhibitor deficiency may induce uncontrolled MASP-1 activation, but in sepsis and COVID-19, too. Both of the latter diseases can be characterized by complement activation and pulmonary edema possibly linked together by the permeability increasing effect of MASP-1. We successfully set up a robust permeability assay (a modified XperT method) measuring true mass transport through paracellular gaps between ECs. Utilizing the XperT method, we showed that activated MASP-1 is a strong permeability increasing agent. We published our results in Frontiers in Immunology [6]. Moreover, with the transcriptomic database created by the support of my previous OTKA project (K100684), we described that MASP-1 significantly modify expression pattern of the pro-inflammatory genes [7], as well as the permeability-related genes [6], therefore it also has a long-term effect on permeability regulation. To further study the effects of MASP-1 on EC permeability, we also set up a real-time, label-free, high-throughput biosensor-based biochip method called EnLaB (utilizing Corning Epic BT system) with Róbert Horváth (Nanobiosensorics Momentum Group, Institute of Technical Physics and Materials Science, Centre for Energy). By using EnLaB, we could verify the effects of MASP-1 observed previously by XperT. EnLaB may well be applied in drug research, since there is an increasing need for screening endothelial permeability. Besides MASP-1, several plasma serine proteases are activated in HAE and in sepsis, together affecting EC permeability. Therefore, we also

tested MASP-2, MASP3, kallikrein, FXIIa, C1r, C1s and thrombin in EnLaB system. We described for the first time that C1r, MASP-2 and kallikrein are also able to elevate EC permeability in a dose-dependent manner. Although this project had not been planned in the research proposal, the permeability research was certainly the most successful part of our OTKA. We published our results in Scientific Reports [8]. Based on our published results, we were invited to review the permeability regulation of endothelial cells in hereditary angioedema. The review article is accepted for publication in a special issue of Clinical Review in Allergy and Immunology [9].

Task 2.2: Our goal was to investigate whether MASP-1 acts on ischaemia/reperfusion injured ECs. Since our hypoxia system only works under flowing conditions, and we failed to reproducibly set up such a system (see Task 1.2), we used an alternative approach. It is well established that $CoCl_2$ treatment can induce HIF1α stabilization and model hypoxia. We successfully set up such a $CoCl_2$ treatment-based model, and verified it with HIF1α nuclear translocation. By this model, we showed that hypoxia pre-treatment significantly increased the intracellular $Ca^{2+}$-mobilization (data not shown), the E-selectin expression (**Figure 3**) in response to MASP-1 when compared to non-pretreated, MASP-1 activated ECs. Meanwhile both MASP-1 and hypoxia increased the permeability of ECs, we did not find interaction between the two treatments in this function. We will repeat the most important interactions between hypoxia and MASP-1 on ECs in a recently obtained hypoxia chamber. Our results were presented at a Hungarian conference [10], and the manuscript is under preparation.

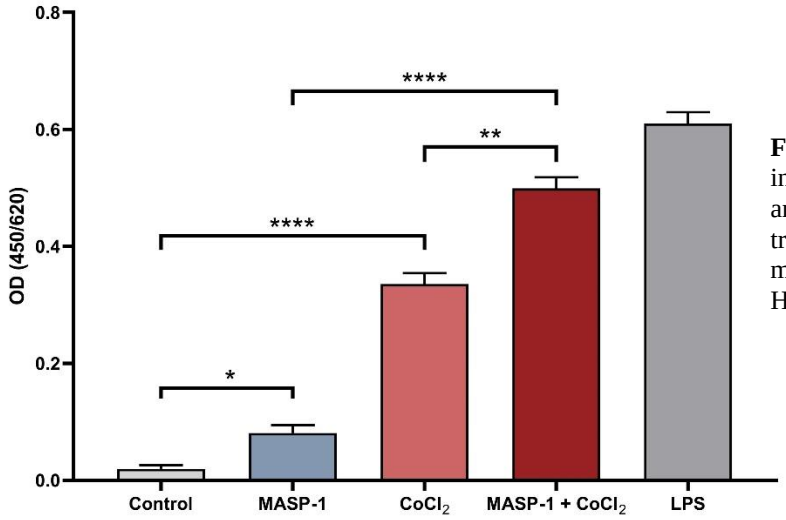


**Figure 3.** E-selectin expression was increased in response to both MASP-1 and CoCl2, however, the simultaneous treatment was even more effective measured by cellular ELISA on HUVECs.

Task 3.1: We assessed whether the novel MASP-1 inhibitor produced by Gábor Pál and Péter Gál (previous OTKA project NK 100769/NK 100834) can be utilized in cell culture without any cytotoxic effects. We applied the MASP-1 inhibitor SGMI-1 in different doses in EC culture and tested the time-dependent cytotoxicity. We observed that even in a 10-fold molar excess than the effective dose in previous *in vitro* enzyme assays, SGMI-1 showed no cytotoxic effects (data not shown). Therefore, we could proceed and applied it in several further EC-based experiments.

Task 3.2: Our goal was to investigate whether MASP-1 function can be effectively blocked by our novel MASP-1 inhibitor SGMI-1 in EC culture. With 20 μM SGMI-1, we could completely block the MASP-1 induced $Ca^{2+}$-mobilization, myosin light chain di-phosphorylation and permeability increase [6]. Our results show that SGMI-1 can be a useful tool in *in vitro* MASP-1

research, and SGMI-1 or its modified version, adapted better to human application, may be utilized as potential drug targets.

Other related results that was not planned in the proposal: With the support of my OTKA grant K100684, we had previously found that MASP-1 increases the expression of E-selectin, an adhesion molecule indispensable in EC/neutrophil granulocyte interaction. Since this result further supports that active MASP-1 enhances the antibacterial/antifungal immune response via ECs and neutrophils, we decided to directly measure the adhesion forces between neutrophils and MASP-1 treated ECs. Therefore, we applied for the first time a computer controlled micropipette mounted onto an inverted fluorescence microscope (cooperation with Bálint Szabó, Department of Biological Physics, Eötvös Loránd University) to measure this force. We found that MASP-1 increased not only the expression of E-selectin in ECs, but the adhesion forces to neutrophil model cells, PLB-985. We published our results in Molecular Immunology [11].

Our research concerning MASP-1 supported by the present OTKA project has been internationally acknowledged by an invitation for a review article in Immunological Reviews [12], and it resulted in a PhD degree defended in 2020 (Endre Schwaner), and two further PhD degrees that will be defended in 2021 (Márta Lídia Debreczeni) and in 2022 (Zsuzsanna Németh).

## Publications

(The peer-reviewed articles with impact factors are in bold type-face)

1. Németh Z, Debreczeni ML, Dobó J, Gál P, Cervenak L. Bradykinin, LPS and MASP-1 synergistically regulate endothelial permeability. 11th C1-inhibitor Deficiency & Angioedema Workshop, May 23th-26th. Budapest, Hungary, 2019.
2. Németh Z, Debreczeni ML, Dobó J, Gál P, Cervenak L. The synergistic effects of inflammatory factors and complement MASP-1 in endothelial cells. Magyar Immunológiai Társaság 46. Vándorgyűlése. Velence, 2017.
3. Németh Z, Debreczeni ML, Cervenak L. Gyulladási faktorok és a komplement MASP-1 szinergizmusa endotélsejteken. 47. Membrán-Transzport Konferencia, 2017. május 16-19. Sümeg, 2017.
4. Kerkovits NM. The role of PAR2 receptor in endothelium-dependent vascular responses. . VIII. International Scientific Conference 2017, Peoples' Friendship University of Russia. Moscow, 2017.
5. Dancs P, Ruisanchez É, Kerkovits N, Iring A, Offermanns S, Cervenak L, et al. Endotheliális PAR2 receptorok szerepe az értónus szabályozásában. Magyar Élettani Társaság és a Magyar Kísérletes és Farmakológiai Társaság és a Magyar Mikrocirkulációs és Vaszkuláris Biológiai Társaság közös vándorgyűlése. Debrecen, 2017.
6. **Debreczeni ML, Nemeth Z, Kajdacsi E, Schwaner E, Mako V, Masszi A, et al. MASP-1 Increases Endothelial Permeability. Frontiers in Immunology 2019; 10.**
7. **Schwaner E, Nemeth Z, Jani PK, Kajdacsi E, Debreczeni ML, Doleschall Z, et al. Transcriptome analysis of inflammation-related gene expression in endothelial cells activated by complement MASP-1. Sci Rep 2017; 7:10462**.
8. **Debreczeni ML, Szekacs I, Kovacs B, Saftics A, Kurunczi S, Gal P, et al. Human primary endothelial label-free biochip assay reveals unpredicted functions of plasma serine proteases. Scientific Reports 2020; 10.**
9. **Debreczeni ML, Németh Z, Kajdacsi E, Farkas H, Cervenak L. Molecular Dambusters: What's Behind Hyperpermeability in Bradykinin-mediated Angioedema? Clin Rev Allergy Immunol 2021; Accepted for publication.**
10. Demeter F, Németh Z, Dobó J, Gál P, Cervenak L. The synergistic effect of hypoxia and complement MASP-1 in E-selectin expression on endothelial cells. 48th Annual Meeting of the Hungarian Society of Immunology, October 16th-18th, 2019, Bükfürdő, Hungary, 2019.
11. **Jani PK, Schwaner E, Kajdacsi E, Debreczeni ML, Ungai-Salanki R, Dobo J, et al. Complement MASP-1 enhances adhesion between endothelial cells and neutrophils by up-regulating E-selectin expression. Mol Immunol 2016; 75:38-47.**
12. **Dobo J, Pal G, Cervenak L, Gal P. The emerging roles of mannose-binding lectin-associated serine proteases (MASPs) in the lectin pathway of complement and beyond. Immunological Reviews 2016; 274:98-111.**