Végjelentés / Final report

Azonosító / Identifier: KH 130376

Cím: A komplement lektin és alternatív útvonalát összekötő proteáz, a MASP-3 aktiválódásának mechanizmusa

Title: Activation mechanism of MASP-3, a key protease linking the alternative and lectin pathways of complement

Aim and outcome of the project:

During the project we aimed to decipher the activation mechanism of MASP-3. MASP-3 is a serine protease component of the lectin pathway of the complement system. Previously, we proved that the active form of MASP-3 is responsible for the activation of pro-factor D producing factor D. Factor D, another serine protease, is essential for the alternative pathway of complement. In this respect we identified a fundamental link between the two complement pathways. By now, this mechanism has become generally accepted among immunologists. We also showed earlier that MASP-3 is present in the blood predominantly in the active form. Originally we hypothesized three possible activation scenarios: (1) MASP-3 is activated by a lectin pathway protease; (2) another protease in the blood is responsible for MASP-3 activation; (3) activation occurs within the cells synthesizing MASP-3 (possibly by a pro-protein convertase).

Now we can state that we found the protease responsible for the activation MASP-3. We found that proprotein convertase subtilisin/kexin 6 (PCSK6, aka PACE4) is the activator MASP-3 in the blood. We have submitted our results to the Journal of Immunology last year and after revision the final publication has been published just recently (**see the list of publications item #2**). Our discovery reveled a very early activation step required for alternative pathway and also connects the proprotein convertase system to the complement system.

Technical details:

Continuously during the project, we expressed and purified full-length zymogen MASP-3 from the baculovirus insect cell expression system. Both the wild-type and the enzymatically inactive S664A variants were produced. We also expressed, refolded and purified truncated MASP-1, MASP-2, MASP-3 variants from E. coli. MASP-1- and MASP-2-specific small recombinant inhibitors were also from E. coli.

During the first year we found that both fluorescently labeled and unlabeled MASP-3 variants became cleaved (activated) in human hirudin plasma. We also established that neither MASP-1 nor MASP-2 nor any protease controlled by C1-inhibitor are responsible for the activation of MASP-3.

Later, we found that EDTA and the paired basic amino acid-specific proprotein convertase (**PBA-PC**) inhibitor, decanoyl-Arg-Val-Lys-Arg-chloromethylketone, completely inhibited the activation of MASP-3. There are only two secreted PBA-PCs, designated proprotein convertase subtilisin/kexin (PCSK) 6 and PCSK5. We expressed both PCSK6 and PCSK5 in CHO cells, and found that both could actually activate MASP-3 in vitro. We measured the levels of both proteases in human serum and hirudin plasma samples. While PCSK6 was present in human blood samples at a concentration of about 100 ng/mL, PCSK5 was undetectable. PCSK6 had been also shown by others to activate corin

in the blood. In all, PCSK6 emerges as the major MASP-3 activator in the blood, while PCSK5 might serve as an activator in other tissues, or not at all. Our results were firmly established during the second year of the project.

Conclusion:

We have uncovered the activation mechanism of MASP-3: it is activated by at least one PBA-PC. While further studies are required to determine its exclusivity, PCSK6 is very likely to be the major MASP-3 activator in the blood. Proprotein convertases had been known to process complement components intracellularly. Now, our discovery identifies a novel and centrally important function of this enzyme class: enabling the proper functioning of complement alternative pathway via the constitutive extracellular activation of MASP-3. This finding reveals a hitherto hidden essential link between these enzymes and the complement system.

Future plans:

We plan to study the activation of MASP-3 in the blood of PCSK6 knock out (KO) mice. We already established collaboration with researchers who maintain such KO mice. We plan to measure the activation level of endogenous MASP-3 and factor D in the PCSK KO mice and compare it to wild-type control. Also, we plan to measure the activity of the alternative pathway both in KO and wild-type mice. We have already acquired samples and reagents, however these ongoing experiments will probably extend beyond the closure of the current grant.

Published peer-reviewed articles during the time-frame of the project:

Since 2020-12-01:

(#1) (Support by KH 130376 is indicated.)

Fasciola hepatica is refractory to complement killing by preventing attachment of mannose binding lectin (MBL) and inhibiting MBL-associated serine proteases (MASPs) with serpins.
De Marco Verissimo C, Jewhurst HL, **Dobó J**, Gál P, Dalton JP, Cwiklinski K.
PLoS Pathog. 2022 Jan 10;18(1):e1010226. doi: 10.1371/journal.ppat.1010226.

(#2) (This article describes the activation mechanism of MASP-3, which was the major goal of the project. Support by KH 130376 is indicated.)

Proprotein Convertase Is the Highest-Level Activator of the Alternative Complement Pathway in the Blood.
Oroszlán G, Dani R, Végh BM, Varga D, Ács AV, Pál G, Závodszky P, Farkas H, Gál P, **Dobó J.**
J Immunol. 2021 May 1;206(9):2198-2205. doi: 10.4049/jimmunol.2000636. Epub 2021 Apr 15.

(#3) (Support by KH 130376 is indicated.)

ITIH4 acts as a protease inhibitor by a novel inhibitory mechanism.
Pihl R, Jensen RK, Poulsen EC, Jensen L, Hansen AG, Thøgersen IB, **Dobó J**, Gál P, Andersen GR, Enghild JJ, Thiel S.
Sci Adv. 2021 Jan 8;7(2):eaba7381. doi: 10.1126/sciadv.aba7381.

2019-12-01 … 2020-11-30:

(#4) (Support by KH 130376 is indicated.)
Patterns of C1-Inhibitor/Plasma Serine Protease Complexes in Healthy Humans and in Hereditary Angioedema Patients.
Kajdácsi E, Jandrasics Z, Veszeli N, Makó V, Koncz A, Gulyás D, Köhalmi KV, Temesszentandrási G, Cervenak L, Gál P, **Dobó J**, de Maat S, Maas C, Farkas H, Varga L.
Front Immunol. 2020 May 5;11:794. doi: 10.3389/fimmu.2020.00794.

(#5)
Human primary endothelial label-free biochip assay reveals unpredicted functions of plasma serine proteases.
Debreczeni ML, Szekacs I, Kovacs B, Saftics A, Kurunczi S, Gál P, **Dobó J**, Cervenak L, Horvath R.
Sci Rep. 2020 Feb 24;10(1):3303. doi: 10.1038/s41598-020-60158-4.

(#6) (Support by KH 130376 is indicated.)
Key Components of the Complement Lectin Pathway Are Not Only Required for the Development of Inflammatory Arthritis but Also Regulate the Transcription of Factor D.
Holers VM, Borodovsky A, Scheinman RI, Ho N, Ramirez JR, **Dobó J**, Gál P, Lindenberger J, Hansen AG, Desai D, Pihl R, Thiel S, Banda NK.
Front Immunol. 2020 Feb 21;11:201. doi: 10.3389/fimmu.2020.00201.

2018-12-01 … 2019-11-30:

(#7) (Support by KH 130376 is indicated.)
Ecotin, a microbial inhibitor of serine proteases, blocks multiple complement dependent and independent microbicidal activities of human serum.
Nagy ZA, Szakács D, Boros E, Héja D, Vígh E, Sándor N, Józsi M, Oroszlán G, **Dobó J**, Gál P, Pál G.
PLoS Pathog. 2019 Dec 20;15(12):e1008232. doi: 10.1371/journal.ppat.1008232.

(#8) (Support by KH 130376 is indicated.)
MASP-1 of the complement system alters fibrinolytic behaviour of blood clots.
Jenny L, Noser D, Larsen JB, **Dobó J**, Gál P, Pál G, Schroeder V.
Mol Immunol. 2019 Oct;114:1-9. doi: 10.1016/j.molimm

(#9)
MASP-1 Increases Endothelial Permeability.
Debreczeni ML, Németh Z, Kajdácsi E, Schwaner E, Makó V, Masszi A, Doleschall Z, Rigó J, Walter FR, Deli MA, Pál G, **Dobó J**, Gál P, Cervenak L.
Front Immunol. 2019 May 3;10:991. doi: 10.3389/fimmu.2019.00991.