Status of CIPKeBiP: Partner
Title of the project: **Role of legumain in infection and inflammation**
Coordinator: **Prof.dr. Marko Fonović**
ARRS code: J7-9435 (E)

General information on financing

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Participating research organizations

ARRS code	Research organization	Status
0106	Jožef Stefan Institute	Public research institution (coordinator)
2990	Centre of excellence for integrated approaches in chemistry and biology of proteins, Ljubljana	Private research institution

Membership of the project team

Name	ARRS code	Research area	Position
Dr. Vidmar Robert	33762	Biochemistry and molecular biology	Researcher
Dr. Vizovišek Matej	32171	Biochemistry and molecular biology	Researcher
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Dr. Fonović Marko	18801	Biochemistry and molecular biology	Coordinator

Abstract

Legumain or asparaginyl endopeptidase (AEP) is a member of the CD clan of cysteine proteases and cleaves protein substrates exclusively after asparagine or (to a minor extent) aspartic acid residues. It is a highly conserved protein which is present in a large variety of animal species. It was shown that legumain has possible roles in normal lysosomal functions, antigen presentation, immune response and immune signalling, but also in apoptosis and osteoclast remodelling.

The most reliable experimental evidence for major physiological roles of legumain was obtained from animal models, where legumain null mice exhibited hemophagocytic syndrome, impaired kidney function and accumulation of macromolecules in the lysosomes which is characteristic for lysosomal storage diseases. Recently, several reports linked legumain to various pathological conditions such as cancer and Alzheimer's disease. However, one of the most important physiological roles of mammalian legumain is in the immune response, where it was originally thought to participate solely in the processing of foreign proteins for presentation on the MHCII complex. In the last decade it was also shown to activate TLR receptors of the innate immune system and influence signalling pathways through the processing of other membrane receptors.

Contrary to many other proteases, physiological substrates of legumain were never studied on a system wide level and all known substrates were identified on a case-by-case basis. Although legumain is emerging as physiologically and clinically relevant target, due to the lack of experimental data on its substrates the majority of legumain physiological functions, especially the ones related to immune response, still remain largely unknown. This emphasizes the need to address its physiological functions by identification of its substrates, which has the potential for a major scientific breakthrough and could therefore significantly improve our understanding of legumain-dependent mechanisms behind immunity.

We recently performed a pilot proteomic analysis of macrophages from legumain null mice and our preliminary results showed that legumain ablation in macrophages caused extremely high upregulation of two peroxidases related to the antimicrobial immune response (myeloperoxidase and eosinophil peroxidase). Both peroxidases are known to produce hypochlorous acid which is a potent antimicrobial agent involved in elimination of infectous organisms. However, hypohalous acids were also reported to

damage the host tissue during the inflammatory conditions such as asthma and hypereosinophilic syndrome which means that legumain could be directly involved in regulation of those processes, but the molecular mechanisms remain unknown. The data obtained in our preliminary experiments demonstrated that the proposed research can provide an explanation of legumain-null phenotype at the molecular level. Moreover, this project enables a novel and unique insight into the role of legumain in immune response and inflammation, as well as on the regulation of immune response and inflammation in general.

In this proposal, we plan to expand our research by performing a systematic proteomic analysis of several tissue types of young and aged mice in order to obtain a detailed understanding of how legumain regulates physiological processes at the molecular level. The obtained results will be supplemented with quantitative PCR and antibody array data and will be used for biochemical and biological validations of the identified molecular processes and pathways. Finally, the *in vivo* role of legumain in defense against pathogen infection will be examined in animal models. Legumain dependant effect on antimicrobial action of immune cells through the generation of hypochlorous acid could open new venues for development of therapeutic approaches to fight infection.

Phase of the project and its realization

The project has just started and we are starting with the first phase of the work.

Bibliographical references

Publications will be prepared in the later stages of the project when sufficient experimental data will be obtained.