



# 2nd International Electronic Conference on Medicinal Chemistry

1-30 November 2016

chaired by Dr. Jean Jacques Vanden Eynde

sponsored by



pharmaceuticals

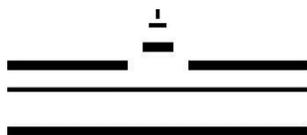
## TLR4-mediated activation of monocytes by human $\alpha_{S1}$ -casein

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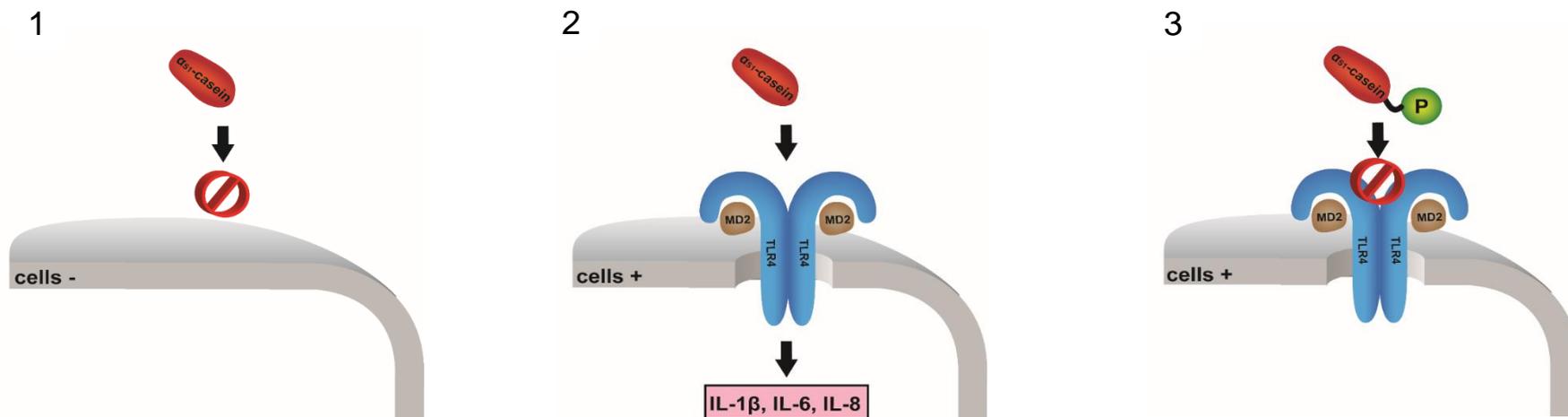
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## TLR4-mediated activation of monocytes by human $\alpha_{S1}$ -casein



**Human  $\alpha_{S1}$ -casein exerts proinflammatory effects *via* TLR4-signaling pathway in a Phosphorylation-dependent manner**

1.  $\alpha_{S1}$ -casein (CSN1S1) induces no expression of cytokines in cells without TLR4 receptor.
2. Recombinant CSN1S1 exerts expression of cytokine IL-1 $\beta$ , IL-6 and IL-8 concentration- and time-dependently.
3. Phosphorylation of recombinant CSN1S1 suppressed proinflammatory effects *via* TLR4-signaling.



## Abstract:

Human milk protein  $\alpha_{s1}$ -casein (CSN1S1) was shown to be overexpressed in autoimmune diseases (osteoarthritis, benign prostatic hyperplasia, multiple sclerosis) as well as in cancer. CSN1S1 displays opioid-like activity and modulates the innate immune response of intestinal cells. Recently, it was demonstrated, that CSN1S1 induces the expression of proinflammatory cytokines (IL-1 $\beta$  and IL-6) in monocytic cells *via* MAPK-p38 signaling [1, 2].

In this study the human TLR4 receptor, a receptor of the innate immune system, was identified as interaction partner of human CSN1S1 inducing expression of cytokines IL-1 $\beta$ , IL-6 and IL-8 in human monocytic cells concentration- and time-dependently [3]. In HEK293 cells cotransfected with TLR4 human CSN1S1 (purified from *Escherichia coli*) induced secretion of chemokine IL-8. *In vitro* flow cytometric assay confirmed CSN1S1 - TLR4 receptor interaction. Chemokine secretion as well as binding was not detected for CSN1S1 phosphorylated by protein kinase CK2 as well as denaturated CSN1S1. This supports the hypothesis, that CSN1S1 is a ligand of the TLR4 receptor exerting proinflammatory properties in phosphorylation-dependent manner. In conclusion CSN1S1 could contribute to the development of a potent immune system in breastfed offspring.

**Keywords:** CSN1S1 / Inflammasome / Nursing / Toll-like receptor4

## Literature:

[1] S. Vordenbäumen, et al., *BMC Immunol*, **2013**, 14, 46.

[2] S. Vordenbäumen, et al., *J Immunol*, **2011**, 186, 592.

[3] S. Vordenbäumen, T. Saenger et al., *Mol Nutr Food Res*, **2016**, 60,1079.



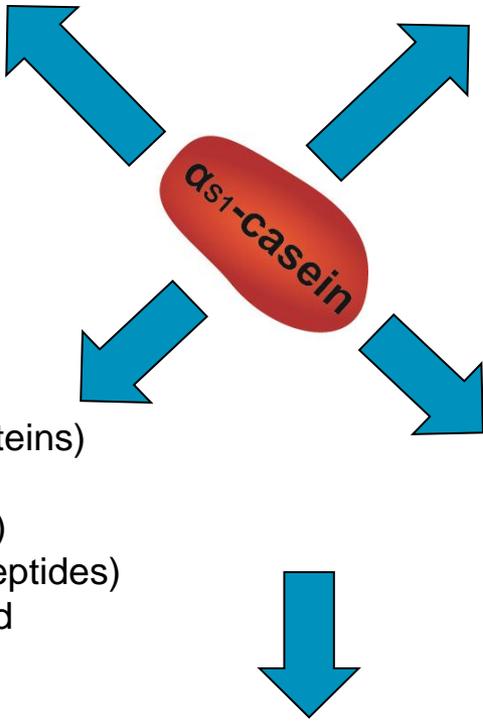
# Introduction

## Caseins are breast milk proteins ...

- Nutritional source for infants
- Forms multimers and micelles
- Transport of compounds (Ca<sup>2+</sup>, PO<sub>4</sub><sup>3-</sup>, ...)
- Calcium-sensitive
- Resource for amino acids

## CSN1S1 acts as ...

- Chaperone (similar to heat shock proteins)
- Tumor suppressor in breast cancer
- Potential tumor antigen (renal cancer)
- (Ant-)agonist of κ-opioid receptors (peptides)
- Orally determined autoantigen caused by breast feeding



## CSN1S1 is overexpressed in ...

- Mammary gland (trace amount in breast milk)
- Tissue of benign prostatic hyperplasia patients
- Multiples scleroses
- Rheumatiod arthritis

**CSN1S1 may posses immunomodulatory functions**

- Stimulates expression of pro-inflammatory cytokine GM-CSF<sup>2</sup>
- Induces upregulation of CD14
- Signaling is mediated by MAPK (ERK, JNK, p38)<sup>1</sup>

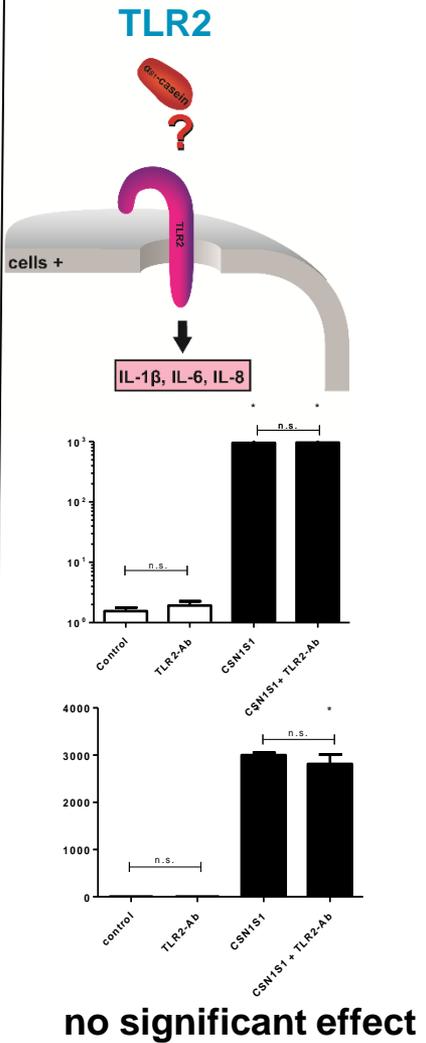
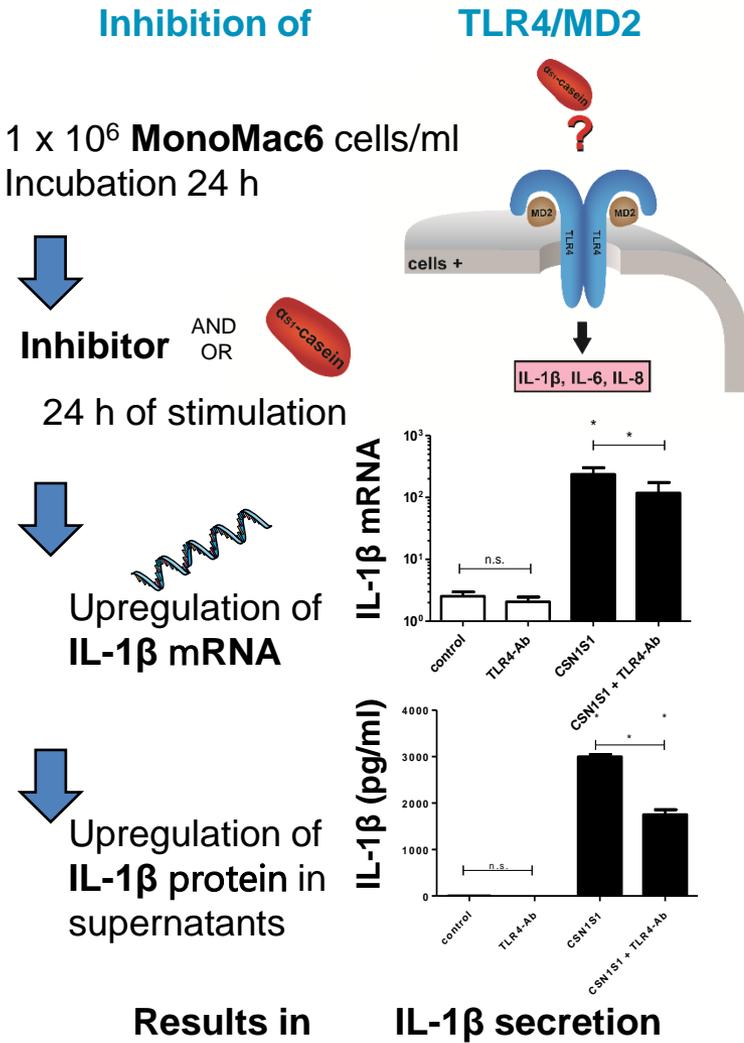
**CSN1S1 binds to surface of monocytes**  
→ Potentiell receptor could be on cell surface



# Results and discussion

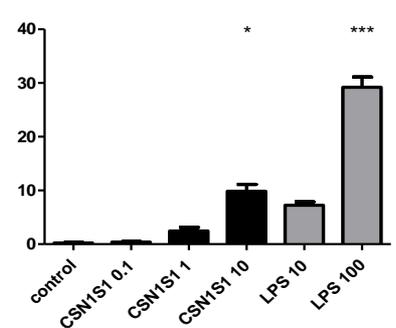
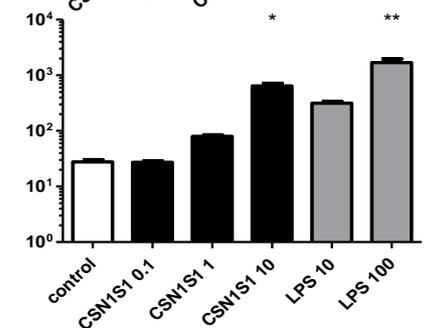
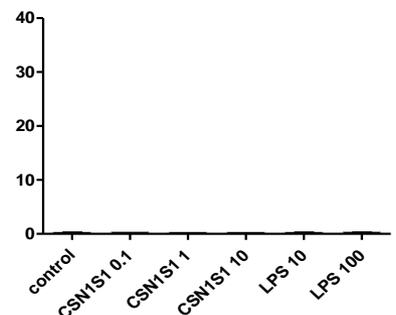
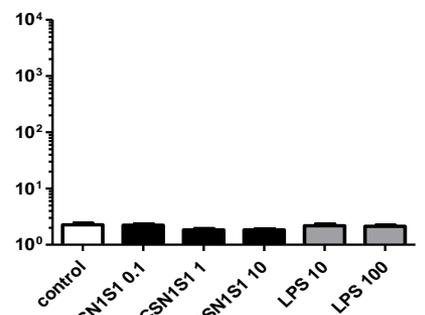
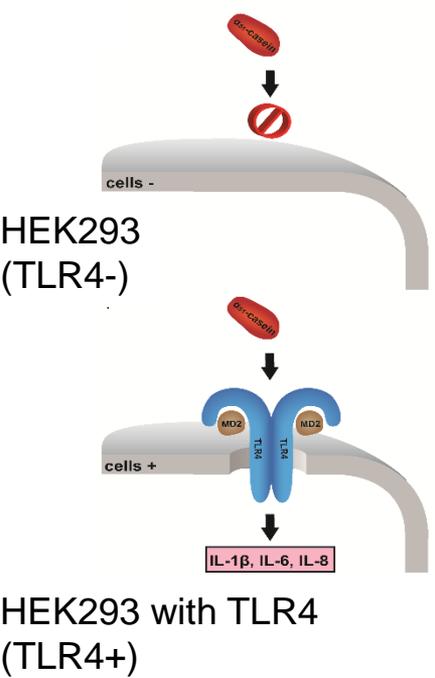
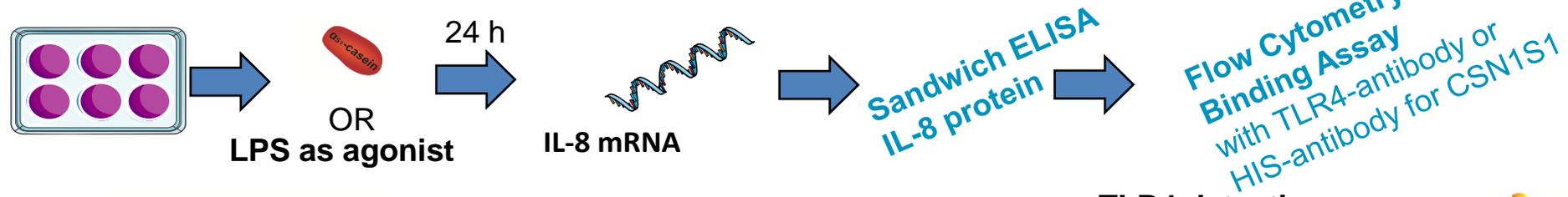
## 1. Inhibition of candidate receptors

CSN1S1 with His-Tag from *E. coli* cells induced secretion proinflammatory cytokine IL-1 $\beta$ . Stimulation of extracellular Toll-like receptors of the innate immune response TLR2 and TLR4 is known for the same action as described for CSN1S1. Therefore, TLR2 and TLR4 signaling pathways were inhibited by anti-TLR2, anti-TLR4 neutralizing antibodies.

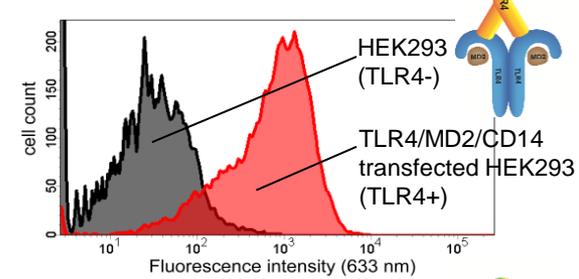


# Results and discussion

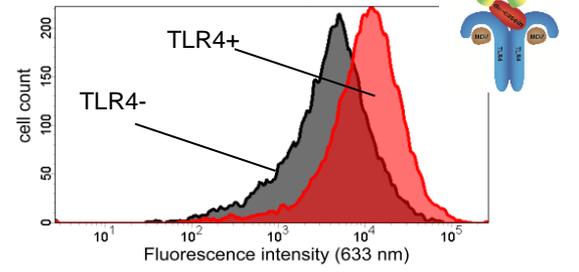
## 2. Human TLR4 prerequisite for CSN1S1-induced effects?



### TLR4 detection



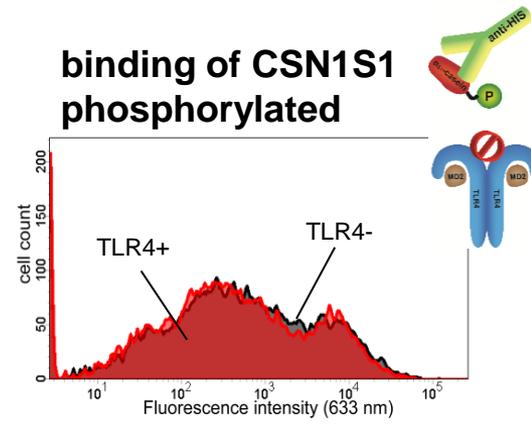
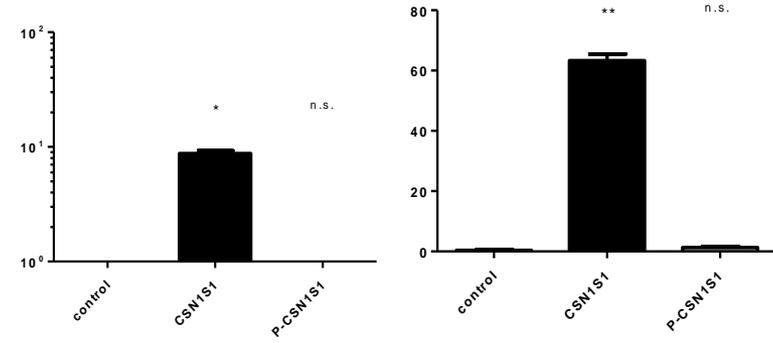
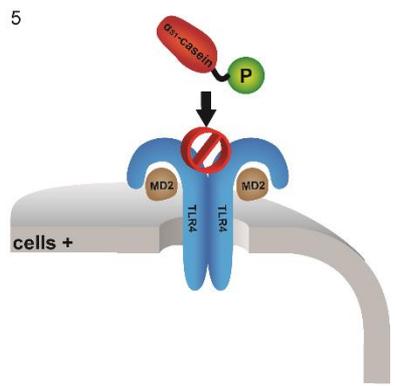
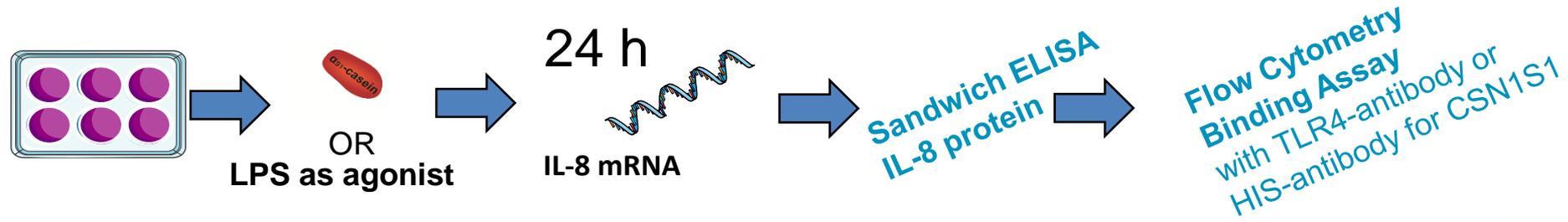
### CSN1S1 binding



TLR4 was suggested as receptor, because CSN1S1-induced effects were inhibited by neutralizing anti-TLR4. To support this results, a cellular model of HEK293 cells (TLR4-) without receptor and TLR4/MD2 cotransfected HEK293 cells (TLR4+) was used. This genetically modified cells could not be monitored by IL-1 $\beta$ , but showed a dose dependent secretion of IL-8.

# Results and discussion

## 3. Phosphorylation of CSN1S1



CSN1S1 is known to be partial phosphorylated in breast milk. Therefore, phosphorylation of CSN1S1 could be a mechanism for inactivation of the proinflammatory properties. To verify this hypothesis, CSN1S1 from *E. coli* was phosphorylated by human protein kinase CK2.

# Conclusions

- CSN1S1-induced expression of proinflammatory cytokines requires translocation of TLR4 (not dependent on pathogen LPS, which is a common agonist of TLR4).
- Posttranslational modification by phosphorylation of CSN1S1 inhibits proinflammatory properties as well as binding to TLR4.
- CSN1S1 may be a bioactive component possibly influencing development of the immune system of the new born (breast milk) as well as triggering to potential pathogens in diseases.



# Acknowledgements

Thanks to

Dr. Dagmar Aichele,  
Sandra Kohaus and the  
group of Joachim Jose



The authors greatly acknowledge financial support of this study by a grant from the Hiller Rheumatology Research Foundation, Erkrath, Germany and Hiller Research Center Rheumatology of Heinrich-Heine-University Düsseldorf, Germany.

