~~CR1 INHIBITS THE TLR9-INDUCED ACTIVATION OF HUMAN B CELLS, WHILE IT DOES NOT INFLUENCE THE TLR1/2- and TLR7-DEPENDENT FUNCTIONS~~
INHIBITION OF TLR-DEPENDENT FUNCTIONS OF HUMAN B CELLS BY COMPLEMENT RECEPTOR TYPE 1 (CD35)

Bernadett Mácsik-Valent[1], Mariann Kremlitzka[2], Anna Erdei[1,2]
[1]Department of Immunology, Eötvös Loránd University, [2]Research Group of the Hungarian Academy of Sciences, Budapest, Hungary

Introduction/Background

The complement system and Toll-like receptors (TLRs) are involved in two effector arms of innate immunity, which provide an immediate reaction against invading pathogens. Although it is well accepted that separate activation of these two systems functions to initiate and shape the adaptive immune response, much less is known about the modulation of ~~-~~various B cell functions by the simultaneous~~way how coincidental~~ activation of these two systems~~ affect the final outcome of B cells' functions~~. Therefore we investigated how engagement of complement receptor type 1 (CR1, CD35) influences the TLR1/2-, TLR7- and TLR9-induced activation of human B cells in the absence and presence of the BCR-mediated stimulus.~~.~~

Methods

For the stimulation of ~~Resti~~resting tonsillar B cells ~~were stimulated~~ via BCR a~~using~~ suboptimal dose of F(ab')$_2$ anti-human IgG/M/A was used and activation via TLR1/2~~s~~, TLR7 and TLR9 was carried out employing ~~using~~ synthetic activators – such as ~~(~~Pam3CSK4 ~~for TLR1/2~~, R-837 ~~for TLR7 and~~ CpG ODN 2006, respectively. ~~for TLR9) eit~~The stimuli were applied either ~~her~~ separately or simultaneously in the presence or absence of the CR1 ligand. The complement receptor was crosslinked ~~Cross-linking of CR1 was assessed~~ by isolated, heat aggregated complement component C3, a ~~"~~multimeric "C3b-like C3". The e~~E~~ffect of CR1 clustering on the investigated stimuli was measured ~~on various B cells' functions was measured~~ by ^{3}H-thymidine incorporation (proliferation) ~~(proliferation)~~, ELISA (~~ELISA (~~cytokine production) ~~) a~~and by flow cytometry (activation marker expression and ~~—~~plasmablast differentiation)~~flow cytometry (plasmablast differentiation and activation marker expression)~~.

Results

We demonstrate~~have~~ ~~shown~~ that CR1 clustering ~~of CR1 by heat aggregated C3~~ significantly and dose dependently reduces the TLR9-induced activation, proliferation and cytokine (IL-6) production of resting human tonsillar B cells, but ~~it~~ has no effect on the TLR1/2- and TLR7-induced functions. Similiarly to earlier results, ~~As described earlier,~~ we have experienced a synergistic, enhanced ~~functional~~ response~~s~~ to the simultaneous engagement of the different TLRs and the BCR, which~~. Interestingly, this enhanced activation~~ was inhibited by ~~the cross-linking of~~ CR1 in the case of all the three TLR-stimuli, namely ~~both~~ TLR1/2, TLR7 and TLR9. ~~The effect of CR1 clustering on additional TLR-mediated B cell functions is in progress in our laboratory.~~

Conclusions

Our data demonstrate that in the absence of the BCR-mediated stimulus, engagement of CR1 downregulates~~ only~~ only the~~the~~ TLR9-induced B cell~~s'~~ functions but does not influence the TLR1/2 and TLR7 mediated processes. Interestingly however, when B cells are ~~directly, while it suppresses both~~simultaneously triggered via BCR+TLR1/2, ~~and~~ BCR+TLR7 and also via BCR+TLR9, CR1 clustering ~~mediated~~inhibits the B cell response. We assume that the complement receptor exerts its inhibitory effect by acting ~~synergistic responses probably by negative regulation~~ o~~f~~ the BCR-linked signaling molecules – a process which is currently under investigation. Our results give evidence that CR1

efficiently modulates~~influences~~ the TLR-induced functions of B lymphocytes~~cells~~, which reveals a so far undescribed interaction between complement and TLRs in the regulation of B cell responses~~humoral immunity~~.