Doctoral thesis

Signaling pathway interactions and their inhibition in healthy and RA B cells

Daniel Szili

Supervisor:
Prof. Gabriella Sarmay, DSc

Doctoral School of Biology
Immunology PhD Program
Head: Prof. Anna Erdei, DSc

Department of Immunology, Institute of Biology
Eötvös Loránd University, Budapest, Hungary

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Introduction

B lymphocytes are central players in humoral immunity. Their hyperactivity may result in autoimmunity and their hypoactivity leads to immunodeficiency. A variety of activatory and inhibitory signals modulate B cells function and the sum of these effects defines B cell response: survival, proliferation, anergy or apoptosis. Antigen-binding to its cognate B cell receptor (BCR) triggers antigen-specific humoral immune response, however numerous non antigen-specific stimuli influence B cells activation: Toll-like receptors (TLRs) recognize various pathogen-associated molecular patterns, while B cell activating factor of the tumor necrosis factor family (BAFF) is an endogenous soluble factor playing a role in B cell survival. BAFF-R, BCR and TLR9 have a prominent role in the regulation of B cells survival, proliferation, antibody and cytokine production [1, 2]. According to the literature, cross-talk between receptors can greatly affect the immune response of B lymphocytes [3, 4], therefore the examination of the interaction between the signaling pathways is necessary to better understand the fine tuning of B cell activation and the development of autoimmune diseases.

The death receptor Fas (CD95) serves to eliminate low affinity, potentially dangerous, self-reactive B cells. Additional survival signals can rescue these cells from Fas-mediated cell death, increasing the risk of autoimmunity [5].

Autoantibodies are highly specific markers of rheumatoid arthritis (RA). An effective treatment of RA is anti-CD20 (Rituximab) therapy, which depletes the peripheral B lymphocytes. Xencor Inc. has produced an anti-CD19 monoclonal antibody with modified Fc part (XmAb5871) which has ~400X higher affinity to the FcγRIIb. Through the cross-linking of CD19 and FcγRIIb, XmAb5871 can inhibit B cell activation without depletion [6].

The main goal of my thesis is to examine the signaling and functional consequences of the co-activation of B cells through BAFF-R, BCR and TLR9, to elucidate their rescuing effect on Fas-mediated apoptosis and to investigate the effect XmAb5871 on BCR- and TLR9-induced B cell functions.

Aims / I.

Effect of BAFF-R, BCR and TLR9 co-stimulation on B lymphocytes

In order to gain a better insight into the activation of B cells via multiple signals, we investigated:

- the cooperation between BAFF-R-, BCR- and TLR9-triggered signals in the phosphorylation of various signaling molecules, cell proliferation, cytokine and IgG production.
- the role of TAK1 in the above mentioned functions.
- the possible difference a) in the basal phosphorylation level of B cells from active RA patients and healthy donors; and b) in their responsiveness for BCR and TLR9 stimuli.

Aims / II.

Role of BAFF-R, BCR and TLR9 in the Fas mediated cell death

The death receptor family member Fas plays an essential role in the elimination of the low affinity, autoreactive B cells. It is well known that defective Fas signaling or excess of survival factors can rescue B cells from Fas mediated apoptosis, thus our objective was to study:

- the survival promoting effect of BAFF-R-, BCR- and TLR9-mediated signals on the Fas-induced cell death of A20 cells.

- The effect of BCR- and TLR9-mediated signals on the Fas-induced pan-caspase and caspase 8 activation.

Aims / III.

The inhibitory potential of XmAb5871

Anti-CD20 is effectively used in RA treatment to eliminate autoreactive B cells, however it depletes the whole circulating B cell population. The anti-CD19 XmAb5871 has an enhanced binding capacity to the inhibitory FcγRIIb thus it can inhibit B cell functions without depletion. Our aim was to examine the effect of XmAb5871 on:

- the BCR- and TLR9-induced FcγRIIb, Erk and Akt phosphorylation and the anti-Ig mediated Ca^{2+} flux.
- B cell proliferation and on the production of IL-6, IL-10 and TNFα cytokines and total IgG.
- the in vitro secretion of citrullinated filaggrin peptide-specific IgG by B cells of RA patients .

Methods

- Isolation of human peripheral and tonsillar B cells
- Proliferation assay (CFSE)
- Cytokine measurement assay (FlowCytomix technology)
- Plasmablast differentiation (Flow cytometry)
- ELISPOT (Detection of Ig-producing B cells)
- ELISA (Measurement of Ig-secretion)
- Flow cytometry (FCM)
- Western Blot

Results and conclusions / I.

BCR and TLR9 synergistically activates human B cells in a TAK1-dependent way

To get a better understanding of the interplay between signaling pathways, we studied the effect of BAFF-R-, BCR- and TLR9-mediated signals on various B cell functions.

- We have shown that BCR and TLR9 synergistically activate MAPKs and the NFκB pathway through TAK1, while AKT and FOXO1 phosphorylation is only BCR dependent. BAFF treatment induced IκB and p38 phosphorylation, but alone it was a weak activator. BCR and TLR9 co-stimulation induce synergistic B cell proliferation, a significant synergistic increase in IL-6, IL-10 and TNFα production and plasma cell generation.
- We have proven that TAK1 plays a central role in BCR- and TLR9-induced synergistic B cell activation, since TAK1 inhibitor diminished the enhanced phosphorylation of IκB and MAPKs as well as proliferation and cytokine production in dual stimulated samples.
- In context with previous results, we have verified that peripheral B cells from active RA patients have elevated basal activation status and we have demonstrated for the first time that this higher basal activation affects the responsiveness for BCR and TLR9 stimuli.

These results revealed that BCR and TLR9 co-stimulate B cells through TAK1, while BAFF-R has marginal effect on B cell activation. Besides this, we have shown that peripheral B cells from active RA patients have decreased responsiveness for BCR and TLR9 ligand due to the higher basal phosphorylation status.

Results and conclusions / II.

BAFF-R-, BCR- and TLR9-mediated signals rescue A20 B cells from Fas-induced cell death through the downregulation of caspase 8 activity

Survival signals can rescue B cells from apoptosis. We have tested the effect of BAFF-R, BCR and TLR9 on Fas-induced cell death and caspase activation.

- We have proven that anti-Ig, BAFF and the TLR9 ligand CpG inhibit the Fas-mediated cell death, and the rescue effect was more pronounced in the co-stimulated samples.
- Our data prove that the BCR and TLR9 rescue A20 B cells through the inhibition of the caspase cascade.
- We have revealed that reduced caspase activity is due to the decreased activity of the initiator caspase 8 in the BCR and TLR9 ligand preincubated cells.

These results provide evidence that BAFF-R-, BCR- and TLR9-mediated signals rescue B cells from Fas-induced cell death - at least partially - through the inactivation of the initiator caspase 8. In addition, there is a positive cooperation between the anti-apoptotic effect of BAFF, anti-Ig and CpG, suggesting their receptors can contribute to the survival of the low-affinity, potentially autoreactive B cells.

Results and conclusions / III.

XmAb5871 inhibits human B cell activation through the co-ligation of CD19 and FcγRIIb

Studying the role of XmAb5871 on anti-Ig and CpG stimulated human B cells, we obtained the following results:

- We have demonstrated that the co-ligation of CD19 and FcγRIIb is sufficient to provoke FcγRIIb phosphorylation.
- We have shown that BCR- and TLR9-induced AKT and ERK phosphorylation and also Ca^{2+} mobilization was diminished in XmAb5871 pretreated samples.
- XmAb5871 also inhibits B cell proliferation, cytokine production and plasma cell differentiation.
- Finally, XmAb5871 also inhibited the IgG response of RA B cells to the RA-specific autoantigen epitope citrullinated filaggrin peptide.

We have demonstrated for the first time that XmAb5871 is capable to inhibit BCR- and also TLR9-induced and dual signal triggered B cell activation. It blocked B cell proliferation, cytokine production and plasma cell differentiation, suggesting that XmAb5871 can diminish B cell responses in multiple ways. Moreover, XmAb5871 inhibited the in vitro differentiation of citrullinated filaggrin peptide-specific antibody secreting cells from RA patients, suggesting that XmAb5871 has a potential as an alternative B cell suppressive therapy in RA.

Summary

In conclusion, our results suggest that BAFF-R, BCR and TLR9 can synergistically activate MAPKs and NFκB pathway, proliferation, cytokine production and plasma cell generation in a TAK1-dependent manner.

They also promote anti-apoptotic signals through caspase 8 inactivation, thus rescue B cells from Fas induced apoptosis.

We have proven that XmAb5871 has a potential to inhibit BCR- and TLR9-mediated B cell activation through the co-ligation of CD19 and FcγRIIb. It also diminished the citrullinated filaggrin peptide specific antibody production of B cells from RA patients.

In conclusion, we can say that BAFF-R-, BCR- and TLR9-mediated signals collaborate upon the activation and in promoting survival of B cells. In addition, our data indicate that inhibition of TAK1 or the XmAb5871 antibody may be effective tools to develop novel therapies for diseases with pathological B cell activation.

References

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Publications connected to the PhD thesis

1. Szili D, Bankó Z, Tóth EA, Nagy G, Rojkovich B, Gáti T, Simon M, Hérincs Z, Sármay G.
   TGFβ Activated Kinase 1 (TAK1) at the Crossroad of B Cell Receptor and Toll-Like Receptor 9 Signaling Pathways in Human B Cells
   PLoS One, 9(5):e96381., (2014), IF: 3.73

2. Szili D, Cserhalmi M, Bankó Z, Nagy G, Szymkowski DE, Sarmay G.
   Suppression of innate and adaptive B cell activation pathways by antibody coengagement of FcγRIIb and CD19
   mAbs, [Epub ahead of print], (2014), IF: 5.275

3. Hancz A, Koncz G, Szili D, Sármay G.

TLR9-mediated signals rescue B-cells from Fas-induced apoptosis via inactivation of caspases

Immunol Lett., 143(1):77-84., (2012), IF: 2.33

Other publications

1. Kövesdi D, Angyal A, Huber K, Szili D, Sármay G.
   T-bet is a new synergistic meeting point for the BCR and TLR9 signaling cascades
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2. Vajna B, Szili D, Nagy A, Márialiget K.
   An improved sequence-aided T-RFLP analysis of bacterial succession during oyster mushroom substrate preparation
   Microb Ecol., 64(3):702-12., (2012), IF: 3.27

Published abstracts

1. Szili D, Hancz A, Pozsgay J, Hérincs Z, Koncz G, Sármay G
   Cooperation between signalling pathways leading to survival, proliferation or death of B cells
   ANNALS OF THE RHEUMATIC DISEASES 70: *Paper 2 A15.* (2011)