TLR9 mediated signals significantly reduced apoptosis, and the rescuing effect of TLR9 was sensitive for inhibitors of protein kinase C and p38. TLR9 synergised with BCR not only in inhibiting apoptosis but also in inducing proliferation, indicating that TLR9 may support B cell growth in the presence and in absence of antigen. Antibody synthesis was also enhanced by the co-signals of TLR9 and BAFF-R.

To reveal the mechanism behind the collaboration between various signals in B cells, phosphorylation of crucial signalling molecules was tested. Proteome profiler microarray has shown that activities of Erk2, JNK, p38 and Rsk1 were synergistically enhanced by the co-stimulation via BCR and TLR9. These results were confirmed by Western blots. Additionally, the authors found that the mitogen-activated protein kinase kinase kinase, transforming growth factor β (TGFβ) activated kinase 1 (Tak1) plays a crucial role in co-signalling. Inhibitor of Tak1 blocked p38 and Rsk1 phosphorylation, and diminished proliferation induced by BCR and TLR9.

Conclusions These data suggest that BAFF may function independently of BCR, rescuing B cells from Fas-mediated death. It may enhance the risk of autoimmune diseases by promoting the survival of bystander B cells in the germinal centre. TLR9 mediates both antiapoptotic and proliferative signals, and it co-operates with BCR-induced signals at the level of Tak1-p38 pathway. Thus TLR9 may significantly contribute to the positive selection, activation and growth of B cells, and thus may help to break tolerance.

A35 COOPERATION BETWEEN SIGNALLING PATHWAYS LEADING TO SURVIVAL, PROLIFERATION OR DEATH OF B CELLS

Daniel Szili,[1] Anikó Hancz,[1] Judit Pozsgay,[1] Zoltán Hérincs,[1] Gábor Koncz,[2] Gabriella Sármay[1,2] *[1]Department of Immunology, Eötvös Loránd University, Budapest, Hungary; [2]Immunology Research Group of the Hungarian Academy of Science, Eötvös Loránd University, Budapest, Hungary*

10.1136/ard.2010.148965.5

Background and objectives Antigen driven positive selection of B cells depends on signals from B cell receptor (BCR) and a plethora of positive and negative regulating receptors determining B cells' fate. Fas/CD95 mediated signals kill bystander or autoreactive B cells, while the receptor for B cell activating factor of tumour necrosis factor family (BAFF-R) mediates survival signals for transitional/mature B cells. Toll-like receptor 9 (TLR9) induced signals stimulate B cells and may enhance the antigen-specific B cell response. The study aim was to reveal the collaboration between these signalling pathways in human B cells.

Materials and methods B cells were isolated from peripheral blood of normal blood donors by negative selection, and stimulated by various combinations of ligands binding to BCR, BAFFR, TLR9 and Fas. Kinase activities were tested by protein profiler microarray and Western blot. Proliferation of 5-(and 6)-Carboxyfluorescein diacetate succinimidyl ester (CFSE) loaded cells was tested by flow cytometry, antibody production was measured by ELISA. Apoptosis was evaluated by determining the ratio of subdiploid cells by flow cytometry.

Results The authors found that BAFF induced a small but significant reduction of Fas-mediated cell death; however, BAFF and BCR mediated rescue signals did not cooperate.