MS6-P4 Porcine CD21 molecule is expressed on mature B cells in two different forms CD21^a and CD21^b

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Monoclonal antibodies IAH-CC51, BB6-11C9.6 and B-Ly4 are routinely used to detect CD21 orthologue on the surface of porcine B lymphocytes. Cross-reactive studies show that IAH-CC51 and B-Ly4 recognize only a portion of B cells that are positive for pan-specific BB6-11C9.6. This indicates that CD21 is always present on all mature B cells but can be expressed in at least two differential forms, and these were assigned as CD21^a and CD21^b. Detection of CD21^b by IAH-CC51 or B-Ly4 together with anti-CD2 antibodies can be used to define four subpopulations of B cells. Ontogenetic and in vitro culture studies, analysis of cell size, expression of CD11b and class-switched phenotype together with measurement of proliferation and cell death, revealed that these subsets represent distinct populations. Phenotypic and functional features collectively suggest that CD21^{b+} B cells are less mature than CD21^{b-}. Moreover, different CD21 forms are expressed differentially during B cell development in the bone marrow. Recent results indicate that CD21^a is expressed earlier in development than in humans and mice whereas expression of porcine CD21^b follows the established paradigm of humans. Unfortunately, western-blot and mass spectrometry studies that would show that all three antibodies recognize proteins with the same molecular weight and sequence could not be done. Specifically, we were unable to precipitate antigen from cell lysates, purify antigen from cell lysates by affinity chromatography or isolate antigen from reversible cross-linked antigen directly on cell surface. For that reason, a new approaches for isolation of porcine CD21 forms have to be introduced to characterize different forms that should have also different function and perhaps binding capacity for C3 and/or IFN-α. By our knowledge, this is the first indication that end-stage B lymphocytes can express differential forms of CD21, which can be significant not only for swine but also for other species including man. This work was supported by Czech Science Foundation grant P502/12/0110 and 15-02274S.

Keywords: B lymphocytes, Development of immune system

MS6-P5 Towards understanding phosphoinositide 3-kinase γ (PI3Kγ)-dependent signaling network

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Phosphoinositide 3-kinases (PI3K) play a crucial role in PI3K/Akt signaling pathway, involved in cell proliferation, differentiation, survival and migration. The PI3K/Akt signaling is one of the most commonly deregulated pathways in cancer. PI3Ks are lipid kinases activated downstream of receptor tyrosine kinases, G protein-coupled receptors and small GTPases of the Ras superfamily. They phosphorylate the 3'-hydroxyl group of the inositol ring of phosphatidylinositol lipid substrates, which act as second messenger molecules by recruiting and activating effector proteins to cellular membranes, *e.g.* Akt kinase (1). The aim of our work is to understand the role and mechanism of action of phosphoinositide 3-kinase γ (PI3Kγ) by its crystal structure elucidation and functional characterization (both *in vitro* and *in vivo* assays). PI3Kγ is a PI3K isoform expressed mostly in hematopoietic cells and in the heart. It has been linked to tumor formation and metastasis, chronic inflammation, autoimmune and heart disease. It is a heterodimer, which consists of a p110γ catalytic subunit that associates with either p87 or p101 regulatory subunit (1-3). Here we present overproduction in insect cells using the MultiBac expression system (4) purification and crystallization strategy for the wild type p110 γ/p101 complex as well as p110γ in complex with p101 deletion mutants. The p101 deletion mutants were designed based on hydrogen-deuterium exchange mass spectrometry experiments (5).

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