Abstract

B cells are an essential component of the adaptive immune system, which secrete soluble antibody against pathogen (antigen). B cells recognize antigen through the B cell receptor (BCR), which triggers receptor clustering and intracellular signaling. Despite this key feature of B cell activation, the mechanisms that cluster antigen receptors are not well understood. Given that glycan-galectin lattices have been shown to regulate cell surface glycoprotein organization and signaling, it seems likely that these cell surface structures may regulate receptor clustering in B cells. One of these potential galectins regulating B cell signaling is Galectin-9 (Gal-9); a secreted lectin with two carbohydrate recognition domains linked by a flexible peptide. A recent report found that antibody production is increased in Gal-9 deficient mice; however the molecular mechanism for this observation has not been investigated. Here, we show that Gal-9 is expressed at the cell surface of primary naïve B cells, organized into discrete puncta. Given the potential of Gal-9 to regulate protein-protein interactions at the cell membrane, we examined the effect of Gal-9 deficiency on the formation of BCR signaling microclusters. Using artificial planar lipid bilayers
to mimic membrane-bound antigen, we found that deficiency in Gal-9 leads to enhanced BCR microcluster formation and increased proximal BCR signaling. This increased signaling in Gal-9 deficient B cells can be attenuated by treatment with exogenous Gal-9. Moreover, treatment of wild-type naïve B cells with exogenous Gal-9 is sufficient to nearly completely inhibit cell signaling upon BCR stimulation, suggesting that Gal-9 acts as a negative regulator of B cell activation. Using a pull-down assay and mass spectrometry, we identified CD45 and IgM as ligands for Gal-9. We show that treatment with exogenous Gal-9 induced relocalization of IgM and CD45 to Gal-9 lattices. Taken together, our data suggests that Gal-9-glycoprotein interactions at the surface of B cells plays an important role in regulating B cell signaling and consequently B cell activation.