

ImmunoTools IT-Box-Cy55M-Award 2013



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Regulation of B cell function by Ly9 immunoreceptor

Immune responses are controlled by interactions between cell surface molecules expressed on leucocytes. The signaling lymphocytic activation molecule (SLAM) family of receptors is composed by nine transmembrane proteins, and most of them interact homophilically acting as costimulatory molecules. Engagement of these receptors results in downstream signaling pathways that regulate different lymphocyte functions, such as activation, differentiation, apoptosis or proliferation. Our group has shown that Ly9 (CD229) differs from the other SLAMF members by acting as an inhibitory regulator of iNKT cell development and activation. Ly9 is present on all T and B lymphocytes, and is highly expressed on splenic marginal zone (MZ) B cells. MZ cells are innate-like lymphocytes that are able to carry out rapid and intense antibody responses to blood-borne pathogens.

Although it has been reported that signaling by SLAM receptors regulates multiple aspects of the immune response, the role of Ly9 in B cells remains unknown. Our goal is to elucidate whether Ly9 modulates the development and activation of different B cell subsets.

Preliminary data using Ly9-deficient ($Ly9^{-/-}$) mice, in a BALB/c genetic background, indicate that this receptor may act regulating B cell function. A hallmark of B cell-mediated immunity is their ability to undergo class switch recombination (CSR), as the different isotypes generated possess distinct effector functions. Recombinant murine cytokines from the ImmunoTools IT-Box Cy55M will be used to assess the regulatory function of Ly9 in a set of *in vitro* studies in which B cells from wildtype and $Ly9^{-/-}$ mice will be sorted and stimulated with different isotype switching cocktails:

<u>Desired Isotype</u>	<u>Stimulation Cocktail</u> ¹
IgG1 and IgE	LPS (25 µg/mL) and IL-4 (10 ng/mL)
IgG1 and IgE	CD40L (10 ng/mL) and IL-4 (10 ng/mL)
IgG2a	LPS (25 µg/mL) and IFN-γ (10 ng/mL)
IgG2b and IgG3	LPS (25 µg/mL)
IgA	LPS (25 µg/mL), TGF-β (2 ng/mL) and IL-5 (1.5 ng/mL)

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After a 72h stimulation, we will check the ability of *Ly9^{-/-}* B cells to survive, proliferate, and switch. In another set of experiments, other cytokines from the *IT-Box Cy55M* that may affect B cell function will be also used.

¹Zaheen A., Martin A. (2010). *Induction and Assessment of Class Switch Recombination in Purified Murine B Cells. JoVE. 42. doi: 10.3791/2130.*

ImmunoTools *IT-Box-Cy55M* for Marta Cuenca
includes 55 recombinant mouse cytokines

rm EGF, rm Eotaxin / CCL11, rm FGF-a / FGF-1, rm FGF-b / FGF-2, rm FGF-8, rm Flt3L / CD135, rm G-CSF, rm GM-CSF, rm GRO-a / CXCL1, rm GRO-b / CXCL2, rm IFNgamma, rm IL-1alpha, rm IL-1beta, rm IL-2, rmIL-3, rm IL-4, rm IL-5, rm IL-6, rm IL-7, rm IL-9, rm IL-10, rm IL-11, rm IL-13, rm IL-15, rm IL-16, rm IL-17A, rm IL-17C, rm IL-17F, rm IL-19, rm IL-20, rm IL-21, rm IL-22, rm IL-25 / IL-17E, rm IL-27, rm IL-31, rm IL-33, rm IP-10 / CXCL10, rm LIF, rm MCP1 / CCL2, rm M-CSF, rm MIP-1 α / CCL3, rm MIP-1 β / CCL4, rm MIP3 α / CCL20, rm MIP3 β / CCL19, rm NGF-beta, rm PDGF-AA, rm PDGF-BB, rm RANTES / CCL5, rm sCD40L / CD154, rm SCF, rm SDF-1 α / CXCL12a, rm SDF-1 β / CXCL12b, rm TNF α , rm TPO, rm VEGF

[DETAILS](#)