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Signaling Events through CD40 in B Cell Activation and Maturation

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CD40, a B cell surface receptor, delivers key activation signals to the B cells. This leads to the proliferation, differentiation, isotype switching of immunoglobulin (Ig) gene, secretion of cytokines, and up-regulation of many surface molecules. CD40 is expressed in B cells of all stages except those of early pro- and pre-stages. After activation by antigen, in the presence of T cell help through CD40 naïve B cells rapidly develop into memory B cells and plasma cells that are active participants in humoral immune responses. However, deregulated and cancerous plasma cells, as well as, secretion of inappropriate antibodies that recognize self proteins lead to certain human diseases, such as, multiple myeloma and autoimmune disorders. Therefore, understanding the molecular mechanisms of B cell activation through CD40 that ultimately leads to the B cell maturation and differentiation is believed to help understand related disease processes and find strategies for cure. Here, our recent findings in signaling events through CD40 will be discussed: (i) role of p190RhoGEF in which the expression is induced following CD40 stimulation in B cell activation and maturation, (ii) mechanisms for CD40 signaling by reactive oxygen species (ROS) in B cells.

Stimulation of the B cell surface receptor CD40 induces transcriptional activation and protein expression. To determine which proteins are required for the CD40-mediated B cell activation, a two-dimensional gel electrophoresis of the WEHI 231 B cell lysates was performed. One protein in which the expression was remarkably induced following CD40 stimulation was identified as the p190 Rho guanine nucleotide exchange factor (GEF), p190RhoGEF, a recently identified GEF that is specific for RhoA. Overexpression of either p190RhoGEF or RhoA (Q63L), a constitutively active form of RhoA, mimics the effects of CD40 stimulation, such as changes in cellular structures and NF-κB activation. These p190RhoGEF overexpression effects are abrogated when coexpressed with a dominant negative form of RhoA (T19N). The CD40-mediated cellular changes were abrogated evidently in cells that are overexpressed with the dominant negative form of either p190RhoGEF (Y1003A) or RhoA (T19N). The p190RhoGEF expression, being low in an immature stage of B

cells (WEHI 231) or in resting splenic B cells, was increased following activation to the similar level as in a mature stage of B cells (CH12.LX). Moreover, the expression of syndecan-1, a marker molecule on plasma cells, was also increased in the WEHI 231 cells that p190RhoGEF or RhoA (Q63L) was overexpressed. Collectively, these data demonstrate that the p190RhoGEF expression is induced following B cell activation, which may result in the maturation of B cells.

As in several studies after the ligation of tumor necrosis factor receptor (TNFR) superfamily members, the reactive oxygen species (ROS) that are produced by CD40 ligation in B cells were shown important in the downstream signaling events that lead to the activation of B cells. Studies in the WEHI 231 B cell line to examine the possible mechanisms for CD40-induced ROS production and consequent activation of the p38 mitogen-activated protein kinase (MAPK) showed that the CD40-induced ROS production requires the activity of NADPH oxidase, but not the activity of 5-lipoxigenase (5-LO). Also, phosphatidylinositol 3-kinase (PI3-K), Rac1, and TNFR-associated factor 3 (TRAF3) play roles that are upstream of the NADPH oxidase. Studies in human B cells isolated from peripheral blood, however, revealed that both pathways of the NADPH oxidase and the 5-LO play roles in the CD40-induced ROS production and p38 MAPK activation. CD40-induced ROS production by the 5-LO also required activities of the PI3-K and Rac1, but not TRAF molecules. These data collectively suggest that CD40 stimulation generates ROS by the TRAF3-dependent activation of NADPH oxidase or TRAF-independent activation of 5-LO, and that ROS link the CD40 ligation to further downstream signaling events, such as the p38 activation in the distinct stages of B cells.