The Role of Rac1 and Rac2 in Antigen Presentation and Lymphocyte Activation by Dendritic Cells

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The small RhoGTPases Rac1 and Rac2 mediate intracellular signal transduction pathways that stimulate actin polymerization, cytokinesis, gene transcription, and NADPH oxidase activity. Rac1 is ubiquitously expressed while Rac2 appears specific for cells of hematopoeitic origin. Dendritic cells are hematopoietic cells important for antigen presentation and lymphocyte activation. The process of antigen presentation involves many aspects of cellular function including actin polymerization, cytokinesis, and gene transcription, all activities that could potentially involve Rac GTPases. Previous experiments performed in our laboratory have shown that dendritic cells in Rac2 -/- mice show decreased capacity for antigen presentation and lymphocyte activation. To further investigate the role of both Rac1 and Rac2 GTPases in dendritic cells antigen presentation we are developing a transfectable model of antigen presentation by dendritic cells that will enable us to use cells derived from the Flox conditional Rac1 -/- and Rac1-/-Rac2-/- mice. Hematopoietic stem cells are derived from the bone marrows of Rac1-/-, Rac2-/-, and Rac1-/- Rac2-/- mice and are induced in the presence of hematopoietic stem cell preserving cytokines SCF, G-CSF, and MGDF with a vector containing Cre to knock out the rac1 gene in these cells. They are then differentiated into dendritic cells using a cytokine cocktail containing SCF, GM-CSF, and TNFα. The resulting dendritic cells are then incubated with T-hybridoma cells in the presence of the antigen ovalbumin and the hybridoma cells were subsequently analyzed for IL-2 expression as a measure of activation by flow cytometry. To address the initial failure to see IL-2 expressing cells by this assay, various control experiments have been added, including the stimulation of T-hybridoma cells with PMA and ionomycin and performing our procedure on IL-2 expressing cells. The establishment of a transducable model of antigen presentation by dendritic cells will provide an investigational tool that can be used to elucidate the molecular mechanisms behind antigen presentation. Knowledge of these mechanisms will increase our understanding of the immune system and provide potential targets for immune modulation.