

MSc DEFENCE Tuesday, April 17th, 2018

Student: Stephanie Santacruz

Title: GRANZYME B DISRUPTS CELL-CELL ADHESION AND EPITHELIAL BARRIER FUNCTION

Time and location: 1:00pm PDT; Room 4280, iCORD Building, 818 West 10th Avenue, Vancouver, BC V5Z 1M9

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Instructions: Please email Matthew Sahl at sahl@icord.org and Laurelee Magtoto at magtoto@icord.org to arrange for access if you plan to attend.

ABSTRACT

The skin is comprised of multiple layers of keratinocytes which together form a barrier to the external environment, regulating temperature, water loss, and pathogen exposure. As such the skin barrier is vital for health as well as disease prevention. Disruption of the epithelial barrier can result in infection, allergen exposure, and inflammation, culminating into severe conditions. Many autoimmune conditions, such as pemphigus, involve a dysregulation and accumulation of immune cells, this results in a disruption in skin barrier causing a loss of function. Granzyme B (GzmB) is a serine protease that is expressed and secreted by a variety of immune and non-immune cell types. It can accumulate in the extracellular milieu and retain its proteolytic functions resulting in chronic inflammation and impaired tissue repair due to extracellular matrix (ECM) remodeling. As such, I hypothesized that GzmB disrupts epithelial barrier function through the proteolytic cleavage of cell junction proteins. The present study investigated the impact of GzmB on epithelial barrier dysfunction using Electric Cell-substrate Impedance Sensing (ECIS) and western blot analyses of intercellular junction cleavage fragments. Human formalin fixed, paraffin embedded blistered skin tissue was assessed for the presence of GzmB. GzmB treatment resulted in a loss of E-cadherin staining on the cell membrane which was supported by western blot analysis of the cell supernatants. Additionally, we observed a dose-dependent increase in E-cadherin fragmentation in GzmB-treated cells compared to controls. HaCaT cells exhibited a significant decrease in barrier function when treated with GzmB while cells treated with GzmB in the presence of a specific GzmB inhibitor remained unaffected. While absent in normal skin, GzmB was observed in abundance within the intra-epidermal blister in addition to the surrounding epithelium. In summary, GzmB contributes to a decline in epithelial barrier function in part through the proteolytic cleavage of cell-cell junctions.