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Funding Source: Life Sciences Undergraduate Research Opportunity Program

Lectin labeling and morphological analysis of Hamster M cells

M (microfold) cells initiate mucosal immune events by preferentially binding and translocating soluble and particulate antigens across the surface epithelium and delivering the captured antigens to underlying antigen-presenting cells. The M cell is usually found within the follicle associated epithelium (FAE) above organized collections of mucosa-associated lymphoid tissue which are found within close proximity of the epithelial layer. This project is focused on characterizing the M cells present in the mucosal tissues of the hamster. Our initial studies are using FAE from intestinal Peyer's patches but our longer term goal is to use the markers we develop to look for M cells in the hamster conjunctiva. A panel of 10 biotinylated lectins was incubated with excised Peyer's patches in vitro for 10 min at 4°C to allow labeling of surface glycoconjugates. Following extensive rinsing, the samples were fixed in 2% paraformaldehyde and embedded in butyl-methyl-methacrylate resin. Semi-thin 0.5 µm cross-sections of follicles were prepared and stained with streptavidin-Alexa 488. Fluorescence microscopy was then used to identify lectins which selectively labeled cells within the FAE. The N-acetylgalactosamine binding lectin SBA and the sialyllactose binding lectin MAL-I were identified as labeling non-overlapping subsets of cells in the FAE and therefore chosen for further characterization. Since M cells have microvilli or microfolds distinct from those on neighboring enterocytes, Peyer's patch explants were incubated in vitro with each of the lectins at 4 C to label surface features. These tissues were then counterstained with streptavidin conjugated to colloidal gold and prepared for analysis using scanning electron microscopy. To look for uptake of the lectins, explants were incubated at 37°C for 10, 20 or 30 min. Studies using light and transmission electron microscopy to visualize uptake are currently being analyzed.