PROJECT SECTION

Project 1:
Name: Role of complement biding in the life cycle of the human astroviruses.

Funding Sources: NIH, EVMS startup funds, etc.

General Background. *Please provide a brief (4-8 sentences) background description of the project in your laboratory. Include the long-range goal(s) of your work as well as specific hypotheses to be tested.*

Human astroviruses (HAstVs) belong to a family of non-enveloped, icosahedral RNA viruses that cause gastroenteritis, predominantly in infants. The long-range goal of our research program is to decipher the molecular mechanisms of astrovirus pathogenesis as a prerequisite to the development of therapeutic methods to attenuate or prevent the disease process.

The specific hypothesis behind the proposed research is that human astrovirus coat protein (CP) directly binds components of the human complement system present in normal human serum to locally disable this innate immune response as well as gain access to specific complement receptors on the host cell for virus attachment and/or entry. Our laboratory has recently determined that authentic HAstV particles and specifically the CP of this virus, binds C1q, a component of the classical pathway of complement to inhibit downstream complement activation (e.g., C3a, C3b, C5a and C5b-9 formation). Based upon these findings, the experimental focus of this application is on astrovirus CP interactions with soluble and membrane-bound complement system factors and the consequence of these interactions on the life cycle and pathogenesis of this virus.

Specific Aims. *Please state your experimental specific aims and sub-aims. Include method/techniques utilized to achieve these aims.*

1. To characterize the mechanism of c′ inhibition at C1q by the viral CP. Experiments to test these hypotheses will consist of:

(1) Mapping the region of C1q that binds CP. Techniques utilized: negative stain EM, ligand overlay blots, competition ELISA as well as surface plasmon resonance (BIAcore) technology.
(2) Mapping the region of CP required for C1q interaction and activity by utilizing CP deletion mutants. Techniques utilized: cloning and recombinant baculovirus production of CP deletion mutants, ligand overlay blots, ELISAs and hemolytic (CH50) assays utilizing normal human serum.
(3) Determining if C1r and C1s activation occurs in the presence of CP. Techniques utilized: ELISA and immunoblot assays.
(4) Analysis of purified C1-CP complex to assess if CP inhibits C1 function by dissociating or stabilizing the C1 complex. Techniques utilized: sucrose density gradients.

2. To characterize the effect of CP-mediated c′ suppression on the pathogenesis of astrovirus infection. Experiments to test these hypotheses will consist of:

(1) Measuring the effects of CP on the generation of the inflammatory mediators C3a or C5a. Techniques utilized: ELISAs.
(2) Assess the effects of CP on leukocyte chemotaxis. Techniques utilized: under agarose chemotaxis assay.

(3) Evaluate the rate of serum c′ inhibition by CP in umbilical cord blood versus adult blood. Techniques utilized: hemolytic assays.
(4) Determine whether Fab fragments of antibody to CP can block CP interaction with C1, thus allowing c′ activation, the production of inflammatory mediators and cell damage. Techniques utilized: hemolytic assay and ELISAs.

3. To determine if HAstVs utilize C1q to bind c′ receptors on epithelial cells to facilitate infection. Human astroviruses are able to bind, enter and replicate in the human enterocyte-like epithelial cell line CaCo-2. Given that HAstV CP specifically binds C1q, we hypothesize that astrovirus binding to C1q allows it to interact with specific C1q receptors on the cellular surface to mediate attachment and possibly entry into CaCo-2 cells. This hypothesis will be tested by the following experiments:

(1) To determine whether CaCo-2 cells express and secrete soluble C1q. Techniques utilized: ELISA and immunoblot.
(2) Test the binding of CaCo-2 cells to C1q-coated CP, authentic virions or virus-like particles. Techniques utilized: flow cytometry.
(3) Test the binding of C1q-coated CP, authentic virions or virus-like particles to CaCo-2 membrane preparations and identify putative receptors. Techniques utilized: overlay blot assay, flow cytometry, mass spectrometry. Demonstration of an interaction between virus/CP and C1q receptor(s) will lead to experiments to determine putative co-receptors required for viral entry and intracellular signaling events.