Keynote Lecture V
How Do Coronaviruses Recognize and Infect Host Cells?

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Kathryn Holmes completed her undergraduate training at Harvard in 1962, and in 1968 received her PhD in Virology and Cell Biology from the Rockefeller University in New York where she worked on interactions of paramyxoviruses with host cells under the direction of Dr Purnell W Choppin. Her postdoctoral work at Harvard was done with Keith Porter from 1968-70. She has been a faculty member at Georgetown University Schools of Medicine and Dentistry in Washington, DC (1970-72), University of Texas Southwestern Medical School in Dallas, Texas (1972-76), Uniformed Services University of the Health Sciences in Bethesda, MD (1976-95), and has been at the University of Colorado Health Sciences Center in Denver since 1995.

She has studied coronaviruses since 1975, emphasizing the biochemistry and biology of the viruses and their interactions with host cells. Her laboratory identified the first receptor for a coronavirus in 1991, the murine CEACAM1a glycoprotein which is a receptor for murine coronavirus, MHV. They showed that MHV-resistant SJL mice express an allele called CEACAM1b, which is responsible for their resistance to the virus. Recent structural and mutational analysis of CEACAM1 revealed the molecular basis for MHV resistance and identified virus-binding residues on the receptor. In 1992, the lab identified a receptor for human respiratory coronavirus HCoV-229E as human aminopeptidase N, and in 1996 they showed that feline aminopeptidase N is a receptor for feline coronavirus FIPV. The species specificity of coronavirus infection is largely determined by interactions of the viral spike glycoprotein S with specific host cell receptors. Recent studies have explored the molecular basis for extended host range in MHV mutants from persistently infected murine cells, and the role of carbohydrate moieties on APN proteins of different host species in determining species specificity. Her laboratory recently discovered that CD209L (L-SIGN) can serve as an alternative receptor for SARS coronavirus.

She served as President of the Chesapeake Society for Electron Microscopy in 1983-1984 and as President of the American Society for Virology in 1993-1994. She was elected Fellow of the American Association for the Advancement of Science in 1997.

Coronavirus envelopes are studded with trimers of large spike (S) glycoproteins that bind to host cell receptors and mediate fusion of the viral envelope with the host cell membrane to initiate virus infection. The ~200 kDa S proteins are type 1 viral fusion proteins, with an N-terminal receptor-binding domain called S1 and a C-terminal membrane fusion domain called S2 that contains two long heptad repeats called HR-N and HR-C. The S1 domain determines the receptor specificity and the host range of the virus. Binding of S1 to a specific receptor at 37 °C induces a series of conformational changes in S that leads to membrane fusion. Fusion can be inhibited by heptad repeat peptides that inhibit the conformational changes in S.

Although S proteins of all coronaviruses mediate membrane fusion and entry in this way, unique properties of S proteins of different coronaviruses determine which host cells and tissues can be infected, whether the virus enters by fusion at the plasma membrane or in endosomal membranes, whether cell-to-cell fusion occurs during virus infection, and the host range of the viruses. During their evolution coronaviruses have adapted to growth in different host species, using several different cell membrane glycoproteins as receptors. All coronaviruses in group 1 utilize aminopeptidase N (APN) as receptors, but the viral S proteins recognize species-specific determinants on the APN proteins. Thus, human coronavirus 229E infects via human APN, but not by pig

APN; while porcine coronavirus TGEV infects via pig APN but not by human APN. Feline APN is a receptor for all group 1 coronaviruses. We are interested in learning how S proteins of group 1 coronaviruses have adapted to use the APN protein of their natural host, and what restricts them from using APN proteins of other species. How many mutations in S would be needed to allow a group 1 coronavirus to "jump" to a new host species?

Murine coronavirus MHV naturally infects only mice, using murine CEACAM1 and related proteins as receptors. However, MHV mutants with extended host range are readily obtained from persistently infected murine cells. Surprisingly few mutations in the receptor-binding domain of MHV S are needed to prevent binding to murine CEACAM1. Structural studies show that although all MHV strains use CEACAM1a as a receptor, virus strains differ in the way they recognize this receptor. MHV strains that have labile connections between S1 and S2 can mediate receptor-independent cell-to-cell fusion, spreading infection from murine cells to hamster cells or cells of other species. Thus, differences between spike proteins of MHV strains have profound consequences on the pathogenesis of MHV diseases.

SARS coronavirus (SARS-CoV) caused an explosive epidemic of human disease in the winter of 2002-3. After the epidemic was controlled, only a few sporadic cases of SARS have been identified, although animals such as the Himalayan palm civet and raccoon dog carry the virus without obvious signs of disease. Farzan's group identified human angiotensin converting enzyme 2 (hACE2) as the principal receptor for SARS-CoV from the human epidemic. The receptor-binding site on the S protein was identified, and there are several differences in amino acid sequence between human and animal isolates of SARS-CoV in this region. Many fascinating questions remain about how the virus "jumped" to humans and what factors facilitated human-to-human spread of the virus.

We recently discovered that a C-type lectin called CD209L (L-SIGN) can serve as an alternative receptor for SARS-CoV and several other coronaviruses. We are exploring the intriguing possibility that sequential interactions of the large S protein of coronaviruses with CD209L or related host cell lectins, followed by interaction with a specific receptor such as ACE2 may play a role in the pathogenesis of coronaviruses and in adaptation of the viruses to new host species.