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Neutrophil serine proteases: roles in cell death and infectious diseases

Biosketch. I am a veterinarian and immunologist with an interest for molecular mechanisms of immune cell homeostasis and responses to infection and inflammation. I obtained my veterinary degree (DVM) from the University of Liège, Belgium (1996), where I worked on bovine herpesviruses in my undergraduate project. I obtained my PhD in immunology at Royal Veterinary College, University of London (2001), where I characterized the first horse chemokines and their role in equine allergic skin disease. I completed my post-doctoral training at the Center for Blood Research, Harvard Medical School, Boston, working on the serpin family of serine protease inhibitors, neutrophil biology and lung diseases. I was a recipient of the **Parker B. Francis Fellowship for respiratory research**. I first established my lab at the Theodor Kocher Institute, University of Bern, Switzerland (2009-17). In Bern, I further developed my interest in cell death, lung inflammation and infection. Since 2017, I am group head of the Molecular Immunology group and deputy head of the Immunology Division at the Institute of Virology and Immunology (IVI), which is a research institute of the Federal Food Safety and Veterinary Office in collaboration with the Vetsuisse faculty of the University of Bern. I am also Associate Professor of Immunology at the Vetsuisse Faculty, University of Bern and a member of the Multidisciplinary Center for Infectious Diseases (MCID).

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Current research. My laboratory broadly investigates molecular, cellular and in vivo pathways of inflammation and innate immune responses to infection. At the gene and molecular level, our work has focused on the multiple functions of **serpins**, the largest family of inhibitors of serine and cysteine proteases. At the cellular level, our expertise lies with the biology of innate immune cells and particularly **neutrophils**. At the level of the organism, we are interested in **lung diseases and in vivo infection models**. In particular, we have investigated the role of genetic and environmental cues (microbiome, cigarette smoke) on pathogenesis of acute lung infections, chronic obstructive pulmonary disease, as well as autoimmune diseases. I have a strong track record in the generation and characterization of **mouse models** including CRISPR/Cas9. Since joining the IVI, I have taken advantage of the unique BSL3 animal facilities to investigate **highly pathogenic viruses** of animals such as African Swine Fever and zoonotic viruses such as SARS-CoV-2 and Wesselsbron virus. Our research uses innovative unbiased approaches such as proteomics and targeted genetic models to investigate pathways amenable to therapeutic intervention in infectious, inflammatory and autoimmune diseases.

Abstract

Serine proteases TMPRSS2 and furin, or cathepsins L and B are critical for SARS-CoV-2 entry by proteolytic processing of the spike protein (S). Severe COVID-19 is associated with a massive influx of neutrophils in the lungs and these inflammatory cells are known to release potent serine proteases (neutrophil elastase (NE), cathepsin G (CatG), and proteinase-3 (PR3)) as a host defense mechanism against the virus. Little is known about the role of neutrophil serine proteases (NSPs) in SARS-CoV-2 replication and pathogenicity.

Using purified human NSPs, we found that all three NSPs degrade the S protein of the original pandemic virus protein S(D614G) or the mouse-adapted S(MA10) protein. Pre-incubation of chimeric vesicular stomatitis virus (VSV) expressing S (VSV*dG-S) with each NSP significantly reduced virus entry and replication in vitro. Pretreatment of Vero/TMPRSS2 cells with CatG modestly reduced VSV*dG-S entry, whereas NE and PR3 had no effect. NSPs had no effect on VSV*dG-S previously adsorbed to cells. Pre-incubation of SARS-CoV-2 with each NSP significantly reduced virus entry and replication in vitro. In NSPs knock-out mice infected with SARS-CoV-2 MA10, we demonstrate that deletion of CatG, but not of NE nor PR3, is associated with higher virus titers in the lung. Importantly, we show that lung cytokine and chemokine expression, and pulmonary pathology were particularly increased in *NE.CatG*$^{-/-}$ double-deficient mice compared to wild-type mice.

These findings demonstrate that NSPs contribute to the early anti-viral defenses against SARS-CoV-2 infection via proteolytic inactivation of the S protein and by limiting pulmonary inflammation. Therefore, therapeutic inhibition of NSPs or CatC in COVID-19 patients should be evaluated carefully during the acute phases of infection.