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Influenza A Virus PB1-F2: A Small Protein with a Big Punch

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Virulence factors, such as the recently discovered PB1-F2, contribute to the pathogenesis and comorbidity of influenza A virus. In this issue of *Cell Host & Microbe*, McAuley et al. characterize the role of PB1-F2, including in the pandemic 1918 virus, in causing increased lung pathology and fatal pneumococcus infection in mice. This work sheds light on the mechanisms of pathogenicity during influenza A virus infections.

Influenza A virus is an important pathogen worldwide. During years of pandemic influenza outbreaks there is significant morbidity and mortality that can influence entire countries' economies. The most deadly recorded pandemic, in 1918–1919, killed on the order of 50 million people worldwide, and many of those deaths are thought to have been caused by secondary bacterial pneumonia (Beveridge, 1991; Johnson and Mueller, 2002; Nguyen-Van-Tam and Hampson, 2003). Historically, secondary bacterial pneumonia is the leading cause of influenza-related deaths; however, most studies on influenza pathogenesis focus exclusively on virally induced pathology, morbidity, and mortality. In this issue of *Cell Host & Microbe*, McAuley et al. (2007) describe a new virulence factor (PB1-F2) contributing to secondary bacterial infection and comorbidity after influenza A virus infection.

In order to identify the proteins that contribute to pathogenesis during natural influenza infections, it is important to examine the effect of viral factors on the susceptibility of the host to secondary bacterial infections. Research into virulence factors of influenza has been extensive over recent years. Focus on this topic has increased, in some part, due to the threat of an impending pandemic by H5N1 avian viruses. Many researchers believe that a new pandemic is imminent, and the more we know about the pathogenicity of the influenza A virus, the better prepared we will be to control and treat pandemic infections.

PB1-F2 is the most recently discovered influenza A virus protein and contributes to viral pathogenesis (Figure 1). PB1-F2 was discovered through a search for novel peptides presented to CD8^{+} T cells during influenza A virus infection (Chen et al., 2004). PB1-F2 induces apoptosis by localizing to the inner and outer mitochondrial membranes through a nontraditional mitochondrial targeting sequence in the C-terminal region of the protein (Gibbs et al., 2003). In the mitochondrial membrane, PB1-F2 interacts with ANT3 and VDAC1, punching holes into the mitochondrial membrane and causing cytochrome *c* release (Zamarin et al.,

2005). The C-terminal region has also been found through crystallization studies to have an α-helical structure, which might be important for the proapoptotic function (Bruns et al., 2007). The mitochondrial function of PB1-F2 in vitro has been investigated by multiple groups, but until recently little research has been done to investigate the function of PB1-F2 in vivo. Work by Zamarin et al. (2006) showed the impacts of PB1-F2 during influenza A virus infection in the mouse model. In these studies Zamarin and colleagues showed that a mildly pathogenic virus in mice had decreased pathogenicity when PB1-F2 expression was knocked out. These results demonstrated the ability of this small protein to impact the outcome of influenza A virus infections in mice.

McAuley et al. (2007) have characterized the effects of PB1-F2 on secondary bacterial infections in addition to analyzing the contribution of the PB1-F2 from the 1918 pandemic virus to virulence. The paper defines the role of PB1-F2 in the establishment of fatal secondary bacterial pneumonia.
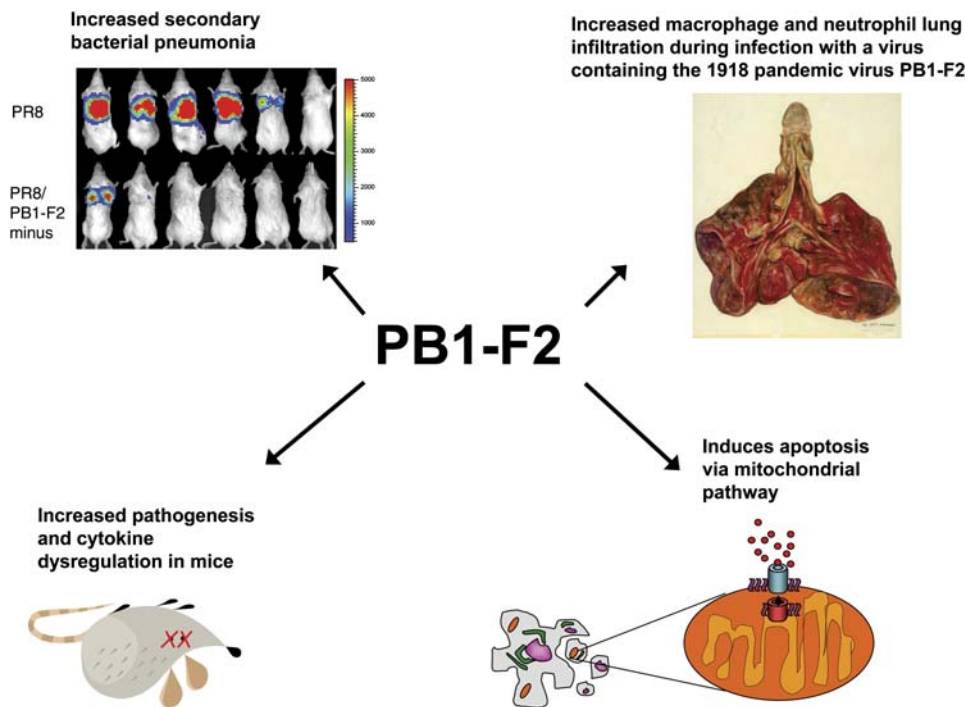
Figure 1. The Function and Effects of Influenza A Virus PB1-F2
(Top left) The severity of secondary bacterial pneumonia caused by *Streptococcus pneumoniae* is increased when the infecting influenza A virus contains full-length PB1-F2 (photograph depicts mice with pneumonia that are coinfected with influenza A virus and *S. pneumoniae* expressing luciferase; McAuley et al., 2007). (Top right) The presence of PB1-F2 from the 1918 pandemic virus increases lung pathology and causes increased neutrophil and lung infiltration (drawing depicts lung infected with 1918 pandemic influenza A virus; courtesy of the National Museum of Health and Medicine, Armed Forces Institute of Pathology, Washington, D.C. [Schwarz1919_RedLungPneumonia]; http://www.nmhm.washingtondc.museum/collections/archives/agalleries/1918flu/Red_lung_pneumonia_Schwarz_1919_front.jpg). (Bottom right) Apoptosis has been demonstrated via the interaction of PB1-F2 (purple) with ANT3 (red cylinder) and VDAC1 (blue cylinder) in the mitochondria through cytochrome *c* (red dots) release. (Bottom left) PB1-F2 increases pathogenesis and causes cytokine dysregulation in the mouse model.

The presence of PB1-F2 in the mouse-adapted PR8 influenza A virus enhances the development of secondary bacterial pneumonia from *Streptococcus pneumoniae* in mice and decreases survival. Mice challenged with *S. pneumoniae* had severe lung damage and hypercytokinemia in addition to increased bacterial load in the lungs compared to mice infected with PR8 virus lacking PB1-F2. This difference in pathogenicity can be linked to the increased number of neutrophils, macrophages, and T cells in the bronchoalveolar lavage fluid (BALF) from infected mice on day 7 post-viral infection (McAuley et al., 2007). The findings presented are important because they show that, while PB1-F2 might not impact the pathogenicity of all strains of influenza A viruses, there is an impact on comorbidity during secondary bacterial infection. These studies highlight the importance of modeling all aspects of human influenza in mice.

To further elucidate the role of PB1-F2 in enhancing secondary bacterial infections, McAuley et al. (2007) synthesized a C-terminal PB1-F2 peptide and administered it to mice intranasally. Unexpectedly, the peptide caused an increase in macrophages and neutrophils in the lungs similar to that induced by wild-type PR8 infection. When infection with *S. pneumoniae* was performed, all of the mice given the C-terminal peptide succumbed to infection, whereas all mice given a control peptide of the N-terminal portion of PB1-F2 survived without any signs of morbidity. This striking difference in morbidity and survival implicates PB1-F2 as a major contributor to complicating secondary bacterial infections through increasing immune cell infiltration of the lungs.

Based on their data showing that the PB1-F2 of influenza A virus contributes to pathogenesis and the outcome of secondary bacterial infections, McAuley et al. (2007) hypothesized that the introduction of the PB1-F2 of a highly pathogenic virus into the background of a low-pathogenicity virus could lead to an increase in pathogenicity. To test their hypothesis, the PB1-F2 of the 1918 virus was engineered into the PR8 virus by making eight amino acid changes that do not alter the PB1 protein, and the resulting virus was subsequently inoculated into mice. PB1-F2 from the 1918 virus makes the PR8 virus more deadly both with and without secondary bacterial infection. These data suggest that the PB1-F2 from the 1918 pandemic virus could have contributed to its high virulence and mortality rate by causing a more severe viral infection and exacerbating any resulting secondary bacterial pneumonia.

New work by Conenello et al. (2007) has identified a specific amino acid that contributes to increased pathogenicity. The single amino acid change was evaluated in a fully reconstructed

1918 virus, and the resultant data further support the conclusions made by McAuley et al. that the PB1-F2 of 1918 virus contributes to the extreme virulence of the virus in mice. However, none of the recent work on PB1-F2 and more specifically on the PB1-F2 of the 1918 pandemic virus provides a molecular mechanism for the ability of the 1918 PB1-F2 to cause macrophage and neutrophil infiltration into the lung or increase morbidity. Furthermore, there have been no studies connecting the known apoptotic function of PB1-F2 in vitro to the increased in vivo pathogenesis, or to the ability of PB1-F2 to bind PB1 (S. Ludwig, personal communication).

It seems the more we know about PB1-F2, the more puzzling questions there are to ponder. How could we prevent the detrimental effects of PB1-F2? Would it be possible to inhibit the activity of PB1-F2 through smart design of small-molecule drugs? What role does cytokine dysregulation have in increasing lung pathology, and can we prevent it by abrogating the function of PB1-F2? Exactly how is the C-terminal PB1-F2 peptide getting into cells to impact cell recruitment, or alternatively, is C-terminal PB1-F2 even entering cells at all? Lastly, could this peptide or full-length PB1-F2 be found extracellularly in a natural infection? Truly exciting work is now being done with PB1-F2, but there are still many holes in the knowledge we have about influenza's smallest protein.

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Orchestration of Dysregulated Epithelial Turnover by a Manipulative Pathogen

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Chronic *Helicobacter pylori* infection is the strongest known risk factor for the development of gastric cancer. In order to survive and propagate under the harsh conditions of the gastric niche, this pathogen has evolved numerous means to interact with host epithelium. In this issue, Mimuro et al. shed light on mechanisms by which *Helicobacter pylori* attenuates apoptosis in the gastric epithelium to facilitate its persistence within the human stomach.

Helicobacter pylori (*H. pylori*) has infected human stomachs for more than 55,000 years, with colonization typically lasting for the lifetime of its host (Linz et al., 2007). This bacterium colonizes more than 50% of the world's population, thereby establishing itself as one of the most successful human pathogens. *H. pylori* has developed numerous strategies to colonize and thrive within the hostile environment of the stomach. These include evasion of the host immune response, gaining access to nutrients, protection against the inherent acidic environment, adherence to the gastric epithelium, and resisting peristalsis and rapid cellular turnover. Several lines of evidence have also indicated that, within gastric mucosa, *H. pylori* is able to modify signaling events that couple epithelial and mesenchymal cells in order to tailor an environment optimal for its own survival. However, although all colonized individuals develop chronic gastritis, only a mere subset will develop gastric adenocarcinoma (Peek and Crabtree, 2006). *H. pylori* virulence constituents clearly modify disease risk, and strains that possess the *cag* island, a type IV secretion system that functions to translocate its substrate CagA into host cells, further