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Coinfection of pigs with porcine respiratory coronavirus and *Bordetella bronchiseptica*

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Abstract

Coinfection with two or more pathogens is a common occurrence in respiratory diseases of most species. The manner in which multiple pathogens interact is not always straightforward, however. *Bordetella bronchiseptica* and porcine respiratory coronavirus (PRCV) are respiratory pathogens of pigs whose relatives, *B. pertussis* and the SARS virus, cause respiratory disease in humans. In an initial experiment, the effect of coinfection of PRCV and *B. bronchiseptica* was examined in thirty, 4-week-old pigs (10 pigs/group) that were infected with either PRCV or *B. bronchiseptica*, or both PRCV and *B. bronchiseptica*. An additional 10 pigs served as sham infected controls. Five pigs from each group were euthanized at 4 and 10 days post-infection. Gross and histopathological lung lesions were more severe in the coinfected group as compared to the groups infected with *B. bronchiseptica* or PRCV alone. In order to investigate the potential role of proinflammatory cytokines in disease severity after coinfection, a second experiment was performed to examine cytokine transcription in alveolar macrophages from single and dually infected pigs. A total of 48 pigs were divided equally into groups as above, but 4 pigs from each group were euthanized at 1, 4 and 10 days post-infection. Coinfected pigs showed a greater and more sustained transcription of proinflammatory cytokines, especially IL-6 and MCP-1, than pigs infected with either PRCV or *B. bronchiseptica* alone. Thus, there appears to be a synergistic effect between PRCV and *B. bronchiseptica* with regards to proinflammatory cytokine transcription that may partially explain the increased severity of pneumonia in coinfected pigs.
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Keywords: Porcine respiratory coronavirus; *Bordetella bronchiseptica*; Porcine respiratory disease complex

1. Introduction

Respiratory disease in swine, as with other species, is generally considered a multifactorial problem caused by a combination of viral and bacterial infectious agents, as well as adverse environmental conditions.

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The list of infectious agents that cause respiratory disease in swine is extensive and includes both viral agents, such as porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV) and porcine respiratory coronavirus (PRCV); and bacterial agents, such as *Mycoplasma hyopneumoniae*, *Bordetella bronchiseptica*, *Pasteurella multocida* and *Actinobacillus pleuropneumoniae*. Although any one of these pathogens can potentially cause disease on its own, more serious and chronic respiratory disease results, and more economic losses are incurred, when infection with multiple pathogens occurs. Although the multifactorial nature of respiratory disease is well accepted, the specific mechanisms by which respiratory pathogens interact with each other or the host to cause more severe disease are poorly understood.

Coronaviruses are enveloped, single-stranded, RNA viruses which cause respiratory and enteric disease in animals, including the common cold and severe acute respiratory syndrome (SARS) in humans. Porcine respiratory coronavirus is a naturally occurring deletion mutant of transmissible gastroenteritis virus (TGE), an enteric coronavirus of swine (Rasschaert et al., 1990; Britton et al., 1991; Wesley et al., 1991). A deletion in the spike gene of TGE has altered the tropism of PRCV from primarily enteric to primarily respiratory in nature. Although experimentally PRCV has been shown to cause bronchointersitial pneumonia, the clinical relevance of this virus has been debated, since many farms seroconvert without overt clinical signs of disease. What role, if any, PRCV plays in multifactorial respiratory disease is still being investigated. Prior work has shown that exposure to PRCV decreases replication of SIV, *in vivo*, and mixed results have been reported with coinfections of PRCV and PRRSV (Van Reeth and Pensaert, 1994; Van Reeth and Nauwynck, 2000). However, experimental exposure of pigs to bacterial lipopolysaccharide (LPS) following PRCV infection led to more severe respiratory disease and increased tumor necrosis factor-alpha (TNF-alpha) and interleukin (IL)-1 levels than exposure to either LPS or PRCV alone (Van Reeth et al., 2000). Thus, infection with PRCV and gram-negative bacteria may result in a more severe disease.

The species belonging to the genus *Bordetella* are closely related gram-negative bacteria which cause respiratory disease in animals. *Bordetella pertussis* is the obligatory human pathogen that causes whooping cough, and *B. bronchiseptica* is the more promiscuous pathogen that is the etiologic agent of respiratory disease in a number of animals including swine. Although *B. bronchiseptica* can cause pneumonia in neonatal pigs and rhinitis and bronchitis in older swine on its own, its role in multifactorial disease is also under investigation. Prior infection with *B. bronchiseptica* in swine has led to increased colonization and more severe disease with other bacterial agents such as *P. multocida*, *Streptococcus suis* and *Haemophilus parasuis* (Chanter et al., 1989; Vecht et al., 1992; Brockmeier et al., 2001; Brockmeier, 2004), and coinfection of *B. bronchiseptica* with PRRSV in swine exacerbated clinical disease and induced *B bronchiseptica* pneumonia (Brockmeier et al., 2000).

The innate immune response of the lung is not only important for initial protection from infectious agents and stimulation of the adaptive immune response, but the nature of the innate response can dictate the character of the pathology observed and the outcome of infection. Proinflammatory cytokines, such as TNF-alpha, IL-1 and IL-6, as well as the chemokines IL-8 and monocyte chemotactic protein-1 (MCP-1) are often produced after recognition of pathogen associated molecular patterns for early host protection and recruitment of effector cells. Exposure to multiple infectious agents may result in distinct cytokine patterns and thus alter the severity of disease compared to that observed during infection with individual agents. In this report we determine whether coinfection with PRCV and *B. bronchiseptica* leads to increased severity of respiratory disease, and if so, whether or not the local proinflammatory cytokine response helps to elucidate the mechanism of this interaction. The data reported here demonstrates that coinfection results in a proinflammatory response that is different from each individual infection, and this proinflammatory response likely contributes to the differences in disease pathology observed during infection with PRCV and *B. bronchiseptica*.

2. Materials and methods

2.1. PRCV and B. bronchiseptica inocula

The PRCV isolate used was isolated from a pig on a farm in Iowa that was experiencing respiratory