Expression of mRNA Encoding Interleukin (IL)-10, IL-12p35 and IL-12p40 in Lungs from Pigs Experimentally Infected with *Actinobacillus pleuropneumoniae*

W.-S. Cho, K. Jung, J. Kim, Y. Ha and C. Chae*

Department of Veterinary Pathology, College of Veterinary Medicine and School of Agricultural Biotechnology, Seoul National University, Kwanak-Gu 151-742, Seoul, Republic of Korea

*Correspondence: E-mail: swine@plaza.snu.ac.kr


ABSTRACT

The expression of mRNA encoding interleukin (IL)-10, IL-12p35 and IL-12p40 was studied, by reverse transcription–polymerase chain reaction and by *in situ* hybridization with a non-radioactive digoxigenin-labelled cDNA probe, in formalin-fixed, paraffin-wax-embedded lung tissue from pigs experimentally infected with *Actinobacillus pleuropneumoniae*. Forty-eight 7-week-old colostrum-deprived pigs were randomly allocated to infected (n = 24) or control (n = 24) groups. Three pigs from each group were euthanized at 3, 6, 9, 12, 24, 36, 48 and 60 h post inoculation (hpi). IL-10 mRNA was detected in the lung at 3 hpi, numbers of cells positive for IL-10 increasing at 36 hpi. IL-12p35 mRNA was detected in the lung at 3 hpi, numbers of cells positive for IL-12p35 increasing at 36 and 48 hpi and rapidly decreasing thereafter whereas IL-12p40 mRNA was constitutively expressed at low levels during the experiment. Hybridization signals for IL-10, IL-12p35 and IL-12p40 were always associated with inflammation, in particular with macrophages and neutrophils within alveolar spaces. Expression of these cytokines was minimal in non-lesional lung of *A. pleuropneumoniae*-infected pigs and in normal lung from control pigs. *In situ* hybridization of *A. pleuropneumoniae* and these cytokines in serial sections of lung tissues indicated close co-localization of *A. pleuropneumoniae* and these cytokines in pleuropneumonia. The results suggest that the expression of IL-10 and IL-12 play a role in pathogenesis of *A. pleuropneumoniae* infection.

Keywords: *Actinobacillus pleuropneumoniae*, *in situ* hybridization, interleukin-10, interleukin-12, pathogenesis

Abbreviations: BCIP, 5-bromocresyl-3-indolyl phosphate; cfu, colony-forming unit; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; hpi, hours post inoculation; IL, interleukin; LPS, lipopolysaccharide; IFN-γ, interferon-γ; RT-PCR, reverse transcription–polymerase chain reaction; SSC, saline sodium citrate; T_{H1}, T helper; TNF-α, tumour necrosis factor-α

INTRODUCTION

*Actinobacillus pleuropneumoniae* is the most common causative agent of pleuropneumonia in pigs characterized by acute fibrinohaemorrhagic or chronic localized necrotizing pneumonia with pleuritis. Pleuropneumonia results from the uncontrolled
release of pro-inflammatory mediators and cytokines in response to *A. pleuropneumoniae* or its product lipopolysaccharides (LPS) (Udeze et al., 1987; Baarsch et al., 1995; Choi et al., 1999). The LPS excreted by *A. pleuropneumoniae* appears to be associated with the early inflammatory response (Bertram, 1985, 1986; Udeze et al., 1987; Idris et al., 1993; Baarsch et al., 1995). Overproduction of macrophage-derived mediators such as oxygen radicals, nitric oxide, prostaglandins and pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α) and interleukin (IL)-1 has been shown to be responsible for the inflammatory reactions (Bertram, 1988; Xing et al., 1994; Baarsch et al., 1995; Morrison et al., 2000).

IL-10 is an anti-inflammatory cytokine that is produced by T cells, B cells, monocytes and macrophages and functions generally to suppress immune and inflammatory reactions (Spits and de Waal Malefyt, 1992). IL-10 is a critical component of the host's natural defence against the development of pathological responses to LPS (Berg et al., 1995). IL-12 is a heterodimeric cytokine that consists of disulphide-linked p35 and p40 subunits. IL-12 is a pro-inflammatory cytokine produced by macrophages, neutrophils and other antigen-presenting cells in response to bacterial products such as LPS (D'Andrea et al., 1992; Trinchieri, 1995). IL-12 plays a key role in LPS-induced shock by inducing interferon-γ (IFN-γ) production, which in turn enhances the release of TNF-α (Wysocka et al., 1995). It has been demonstrated using *in situ* hybridization and reverse transcription–polymerase chain reaction (RT-PCR) that TNF-α is expressed in the pleuropneumonic lung in response to *A. pleuropneumoniae* infection (Choi et al., 1999; Cho and Chae, 2002).

IL-10 and IL-12 are most noted for their ability to regulate the balance between T helper 1 (Th1) cells and Th2 cells (Moore et al., 1993; Trinchieri, 1995; Stern et al., 1996; Gately et al., 1998). Th1 cells secrete IL-12 and IFN-γ, thus promoting cell-mediated immunity; whereas Th2 cells produce IL-4, -5, -6, -10 and -13, thereby facilitating humoral immunity. Balance between IL-10 and IL-12 modulates expression of pro-inflammatory cytokines, soluble mediators and cell surface molecules by cells of myeloid origin, with important consequences for their ability to activate and sustain immune and inflammatory responses (Moore et al., 2001). However, very little is known regarding IL-10 and IL-12 expression in *A. pleuropneumoniae* infection. The objective of this study was to determine, by RT-PCR and *in situ* hybridization, the expression of IL-10 and IL-12 mRNA in the lungs of pigs experimentally infected with *A. pleuropneumoniae*.

**MATERIALS AND METHODS**

*Experimental design*

Forty-eight 7-week-old colostrum-deprived pigs were randomly allocated to infected (n = 24) or control (n = 24) groups. In the infected group, pigs were inoculated intratracheally with *A. pleuropneumoniae* serotype 2 as previously described (Baarsch et al., 1995). For this, *A. pleuropneumoniae* organisms were washed once with phosphate-buffered saline (PBS, pH 7.4) and diluted in PBS containing 5% bovine