Veterinary Immunology and Immunopathology 166 (2015) 58–69

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Review Paper

In vivo porcine lipopolysaccharide inflammation models to study immunomodulation of drugs

H. Wyns*, E. Plessers, P. De Backer, E. Meyer[1], S. Croubels[1]

Department of Pharmacology, Toxicology and Biochemistry, Ghent University, Faculty of Veterinary Medicine, Salisburylaan 133, 9820 Merelbeke, Belgium

ARTICLE INFO

Article history:
Received 19 June 2014
Received in revised form 7 May 2015
Accepted 2 June 2015

Keywords:
LPS
Acute phase response
Immunomodulation
Pigs
(N)SAIDs
Antimicrobial drugs

ABSTRACT

Lipopolysaccharide (LPS), a structural part of the outer membrane of Gram-negative bacteria, is one of the most effective stimulators of the immune system and has been widely applied in pigs as an experimental model for bacterial infection. For this purpose, a variety of *Escherichia coli* serotypes, LPS doses, routes and duration of administration have been used.

LPS administration induces the acute phase response (APR) and is associated with dramatic hemodynamic, clinical and behavioral changes in pigs. Pro-inflammatory cytokines, including tumor necrosis factor α (TNF-α), interleukin (IL)-1 and IL-6 are involved in the induction of the eicosanoid pathway and the hepatic production of acute phase proteins, including C-reactive protein (CRP), haptoglobin (Hp) and pig major acute phase protein (pig-MAP). Prostaglandin E_2 (PGE_2) and thromboxane A_2 (TXA_2) play a major role in the development of fever and pulmonary hypertension in LPS-challenged pigs, respectively.

The LPS-induced APR can be modulated by drugs. Steroidal and nonsteroidal anti-inflammatory drugs ((N)SAIDs) possess anti-inflammatory, antipyretic and analgesic properties through (non)-selective central and peripheral cyclooxygenase (COX) inhibition. Antimicrobial drugs, especially macrolide antibiotics, which are commonly used in veterinary medicine for the treatment of bacterial respiratory diseases, have been recurrently reported to exert clinically important immunomodulatory effects in human and murine research.

To investigate the influence of these drugs on the clinical response, production of pro-inflammatory cytokines, acute phase proteins (APP) and the course of the febrile response in pigs, *in vivo* LPS inflammation models can be applied. Yet, to date, *in vivo* research on the immunomodulatory properties of antimicrobial drugs in these models in pigs is largely lacking.

This review provides acritical overview of the use of *in vivo* porcine *E. coli* LPS inflammation models for the study of the APR, as well as the potential immunomodulatory properties of anti-inflammatory and antimicrobial drugs in pigs.

Contents

* Corresponding author. Tel.: +32 9 264 73 50; fax: +32 9 264 74 97.
E-mail address: Heidi.Wyns@UGent.be (H. Wyns).
[1] These authors equally contributed.

http://dx.doi.org/10.1016/j.vetimm.2015.06.001
0165-2427/

1. General introduction

1.1. Lipopolysaccharide and the acute phase response

Lipopolysaccharide (LPS) is a structural part of the outer membrane of Gram-negative bacteria. When bacteria multiply or die and lyse, LPS is released from their surface. To distinguish LPS from the actively secreted exotoxins, it is termed endotoxin (Rietschel et al., 1993, 1994). Conversely, if LPS remains membranar, activation of the innate immune system is weak (Miyake, 2004; Jerala, 2007).

Innate immunity is the first, non-specific line of defence against invading micro-organisms based on pattern-recognition systems (Heumann and Roger, 2002; Brown et al., 2011). The extracellular recognition of LPS and following intracellular signal transduction leads to activation of the transcription factor nuclear factor-κB (NF-κB) and induction of the acute phase response (APR). In brief, LPS binding protein (LBP) circulates and recognizes LPS in the blood after which it is initially transferred to cluster of differentiation (CD) 14 and subsequently to the secreted glycoprotein myeloid differentiation (MD)-2. The latter then forms a complex with the extracellular domain of toll-like receptor (TLR) 4. Dimerization of this TLR4–MD-2–LPS-complex leads to the recruitment of adaptor proteins to the intracellular domain of TLR4, initiating the intracellular signaling cascade. Following a coordinated cooperation of membrane-bound and cytosolic adaptor proteins and kinases, the intracellular LPS signaling cascade ultimately leads to the release and nuclear translocation of NF-κB to bind the promoters of responsive genes. The latter includes pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin (IL)-1 and IL-6 as well as cyclooxygenase (COX)-2 and the subsequent induction of the eicosanoid pathway, including prostaglandins (PGs), thromboxanes (TXs) and leukotrienes (LTs), which are collectively referred to as eicosanoids (Adams, 2001; Triantafilou and Triantafilou, 2002; Pålsson-McDermott and O'Neill, 2004; Jerala, 2007; Verstrepen et al., 2008; Peri et al., 2010).

Arachidonic acid (AA), which is incorporated into membrane phospholipids, is the main source of eicosanoids. In response to phospholipase (PL) A2, AA is released from the cell membrane after which it is subjected to a rapid oxidative catabolism by two separate enzymatic pathways, a COX and a lipoxygenase (LOX) pathway (Adams, 2001).

While both neutrophils and monocytes are important cells in the innate immune response, the blood monocyte or tissue macrophage generally triggers the APR cascade (Heinrich et al., 1990; Baumann and Gauldie, 1994; Netea et al., 2000). It should be mentioned that the existence of monocyte subsets has been recognized and also characterized by Fairnbairn et al. (2011, 2013).

Following initiation of the APR, an important upregulation and synthesis of a variety of proteins, the acute phase proteins (APP), occurs in the liver. In pigs, C-reactive protein (CRP), serum amyloid A (SAA), haptoglobin (Hp) and pig major acute phase protein (pig-MAP) have been identified as major positive APP (Lampreave et al., 1994; González-Ramón et al., 1995; Eckersall et al., 1996; Heegaard et al., 1998; Petersen et al., 2004; Sorensen et al., 2006).

In pigs, bacterial infections are responsible for considerable economic losses and reduced animal welfare, due to decreased growth rate, morbidity, mortality and medication costs. Bacteria, such as *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Haemophilus parasuis* and *Mycoplasma hyopneumoniae* are major pathogens involved in swine respiratory disease (SRD). *Escherichia coli* on the other hand, is one of the most important causes of postweaning diarrhea in pigs (Fairbrother et al., 2005; Rose et al., 2013).

Pigs are anatomically, genetically and physiologically very similar to humans. Since the porcine immune system as well as the response to the intravenous (i.v.) administration of endotoxin is analogous to that of man, pigs also represent an excellent animal model to study various human bacterial infectious diseases (Schrauwen et al., 1984; Meurens et al., 2012; Mair et al., 2014). It should be remarked that the use of these models to study human inflammation and infections is out of the scope of this review. Excellent literature is available as reviewed by Meurens et al. (2012) and Mair et al. (2014).

Compared to a bacterial infection model, an LPS model is more standardized and reproducible, less expensive to develop and validate, and properly accessible (Schrauwen et al., 1986; Olson et al., 1995; Myers et al., 2003). Moreover, LPS is a stable and purified molecule, which can be easily stored in its lyophilized form until use (Fink and Heard, 1990).

1.2. Lipopolysaccharide and septic shock

Sepsis is defined as a complex dysregulation of inflammation, ultimately affecting multiple organ systems and leading to