Veterinary Immunology and Immunopathology 146 (2012) 212–224

Contents lists available at SciVerse ScienceDirect

Veterinary Immunology and Immunopathology

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

Research paper

Immune dynamics following infection of avian macrophages and epithelial cells with typhoidal and non-typhoidal *Salmonella enterica* serovars; bacterial invasion and persistence, nitric oxide and oxygen production, differential host gene expression, NF-κB signalling and cell cytotoxicity

Ahmed Setta[a,c], Paul A. Barrow[a], Pete Kaiser[b], Michael A. Jones[a,*]

[a] *Animal Infection and Immunity Research Group, School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, Leicestershire, LE12 5RD, UK*
[b] *Roslin Institute, University of Edinburgh, Easter Bush Veterinary Centre, Roslin, Midlothian, EH25 9RG, UK*
[c] *Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, P.O. Box 12211, Giza, Egypt*

ARTICLE INFO

Article history:
Received 15 June 2011
Received in revised form 18 February 2012
Accepted 8 March 2012

Keywords:
Cytokines
Immunity
Chicken
Salmonella
Macrophages
Epithelial cells

ABSTRACT

Poultry-derived food is a common source of infection of human with the non-host-adapted salmonellae while fowl typhoid and pullorum disease are serious diseases in poultry. Development of novel immune-based control strategies against *Salmonella* infection necessitates a better understanding of the host–pathogen interactions at the cellular level. Intestinal epithelial cells are the first line of defence against enteric infections and the role of macrophages is crucial in *Salmonella* infection and pathogenesis. While gene expression following *Salmonella* infection has been investigated, a comparison between different serovars has not been, as yet, extensively studied in poultry. In this study, chicken macrophage-like cells (HD11) and chick kidney epithelial cells (CKC) were used to study and compare the immune responses and mechanisms that develop after infection with different *Salmonella* serotypes. *Salmonella* serovars Typhimurium, Enteritidis, Hadar and Infantis showed a greater level of invasion and/or uptake characters when compared with *S.* Pullorum or *S.* Gallinarum. Nitrate and reactive oxygen species were greater in *Salmonella*-infected HD11 cells with the expression of iNOS and nuclear factor-κB by chicken macrophages infected with both systemic and broad host range serovars. HD11 cells revealed higher mRNA gene expression for CXCLi2, IL-6 and iNOS genes in response to *S.* Enteritidis infection when compared to *S.* Pullorum-infected cells. *S.* Typhimurium- and *S.* Hadar-infected HD11 showed higher gene expression for CXCLi2 *versus S.* Pullorum-infected cells. Higher mRNA gene expression levels of pro-inflammatory cytokine IL-6, chemokines CXCLi1 and CXCLi2 and iNOS genes were detected in *S.* Typhimurium- and *S.* Enteritidis-infected CKC followed by *S.* Hadar and *S.* Infantis while no significant changes were observed in *S.* Pullorum or *S.* Gallinarum-infected CKC.

1. Introduction

Salmonella enterica subspecies *enterica* (*S. enterica*), a member of family *Enterobacteriaceae*, is a major pathogen affecting a wide range of hosts and is one of the leading

* Corresponding author. Fax: +44 0115 951 6440.
E-mail address: michael.a.jones@nottingham.ac.uk (M.A. Jones).

0165-2427/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetimm.2012.03.008

causes of food-borne infections in humans. Poultry products, meat and eggs, are well-known as a common source of human infections as the majority of human food poisoning cases is usually associated with consumption of poultry-derived food (Burr et al., 2005; Little et al., 2007; Mcpherson et al., 2006).

After oral infection, *Salmonella* colonizes the intestinal tract followed by adhesion to and invasion of the intestinal epithelium which may be followed by the development of enteritis in susceptible mammalian hosts, including man (reviewed by Wallis and Galyov, 2000). The nature and severity of the disease in chickens are dependent on the infecting serovar, breed and the genetic background, age and immune status of the bird (Gast, 2003). Serotypes of *S. enterica* can be classified into two categories based on the nature of the disease produced and the range of hosts being infected (Barrow, 2007). In poultry, *S.* Gallinarum and *S.* Pullorum are poultry-specific serotypes which cause systemic diseases, fowl typhoid and pullorum disease, respectively. The remaining group of broad host-range serotypes, including *S.* Typhimurium and *S.* Enteritidis, produce little systemic disease in normal, healthy adult birds but colonise the alimentary tract and thus can contaminate the carcass and thereby enter the human food chain causes food poisoning. Other serovars in this group, such as *S.* Hadar and *S.* Infantis, are less virulent but colonise the gut very well and have become the centre of attention for the EU (Mochizuki et al., 1992; Wilkins et al., 2002; European Food Safety Authority, 2004).

Macrophages are an important cellular component of the immune system and play a primary role in the development of both innate and adaptive immune responses. Macrophages are not only phagocytic cells which are responsible for clearance and elimination of harmful pathogens but also function as antigen-presenting cells (APCs) for B and T lymphocytes and participate in the development and stimulation of the adaptive immune system. Activated macrophages produce several antimicrobial molecules as well as different immune mediators to control infections (Qureshi et al., 2000; Taylor et al., 2005). These include nitric oxide (NO), reactive oxygen species (ROS) and a group of cytokines and chemokines. As a facultative intracellular bacterial pathogen, *Salmonella* is capable of surviving within infected host cells, including phagocytes, through adaptation to the intracellular environment (Okamura et al., 2005). The ability of *Salmonella* to survive and multiply within chicken macrophages is crucial for *Salmonella* pathogenesis and the establishment of systemic infection (Barrow et al., 1994; Chappell et al., 2009). The intestinal epithelium is an integral part of the intestinal mucosal surface and together with the gut associated lymphoid tissues (GALT) they represent the first line of defence against infection (Lillehoj and Trout, 1996). GALT participates in gut immunity in many ways including the presence of APCs and modulation of immune responses *via* production of key mediators, cytokines and chemokines. HD11 cells, a transformed cell line of chicken macrophages, together with chick kidney epithelial cell (CKC) models were chosen to study their response to *Salmonella* infection. CKC cells are robust standard models for *in vitro* interactions of this sort (Kaiser et al., 2000). Invasion of CKC by many *Salmonella* serotypes induces a strong pro-inflammatory response indicated by high levels of IL-6 cytokine induction (Kaiser et al., 2000). The production of pro-inflammatory cytokines has been associated with infection of HD11 with many bacterial pathogens including *Salmonella* (Zhang et al., 2008), *Campylobacter* (Smith et al., 2005) and *Chlamydophila* (Beeckman et al., 2010). Also, up-regulation of interferon-γ (IFN-γ) has been correlated with baculovirus infection of HD11 (Han et al., 2009).

In the present study, we have compared four serotypes of *Salmonella* known to cause food poisoning in humans and two poultry specific serotypes known to produce systemic disease in chickens in terms of the immune responses and mechanisms that develop after infections of avian epithelial and macrophage cell lines. Given that the biology of the various pathotypes of *S. enterica* is so different, we decided to investigate the host response to individual representative strains from these pathotypes.

2. Materials and methods

2.1. Bacterial strains

The infection studies were carried out using spontaneous nalidixic acid-resistant (Nal^r) serovars of *S.* Typhimurium 4/74 (Foster et al., 2006), *S.* Enteritidis P125109 (Thomson et al., 2008), *S.* Pullorum 449/87 (Berchieri et al., 2001; Suar et al., 2006), *S.* Gallinarum 287/91 (Thomson et al., 2008), *S.* Hadar 18 (Berndt et al., 2007) and *S.* Infantis 1326.28 (Barrow et al., 1988; Berndt et al., 2007). Prior to infection, bacteria were grown in nutrient broth at 37 °C in an orbital shaking incubator at 150 rpm/min.

2.2. Tissue cultures

HD11 cells were cultured as previously described (Kaiser et al., 2000). Briefly, cells were seeded at 4×10^5 cells/ml in 24-well plates (1 ml/well) and grown at 41 °C in 5% CO_2 for 48 h in RPMI 1640 medium supplemented with 20 mM L-glutamine, 2.5% foetal bovine serum (FBS), 2.5% chicken serum, 10% tryptose phosphate broth (TPB) and 100 U/ml penicillin/streptomycin (P/S). Primary CKC were prepared from the kidneys of 1–3 weeks old Ross 308 broiler chicks supplied by PD Hook Hatcheries (Oxfordshire, UK) as essentially described by (Barrow and Lovell, 1989). CKC cells were seeded in 24-well plates at 1×10^6 cells/ml (1 ml/well) in Dulbecco's modified Eagle's medium (DMEM) containing 12.5% FBS, 10% TPB, 25 U/ml nystatin and P/S and incubated for 72 h at 37 °C in 5% CO_2.

At 2 h before the invasion assays of cells, media were replaced with either RPMI (HD11 cells) or DMEM (CKC) without antibiotics.

2.3. Invasion assays

Infections were carried out using a multiplicity of infection (MOI) 10:1. Bacteria were suspended in phosphate buffered saline (PBS) according to the required challenge dose which was calculated by measuring the optical density of bacterial cultures at 600 nm and comparing the