Veterinary Immunology and Immunopathology 131 (2009) 278–284

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

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

Short communication

Gene expression study of two widely used pig intestinal epithelial cell lines: IPEC-J2 and IPI-2I

Valentina Mariani *, Simona Palermo, Silvia Fiorentini, Alessandra Lanubile, Elisabetta Giuffra

Parco Tecnologico Padano – CERSA - Centro Ricerche e Studi Agroalimentari, Livestock Genomics I Group, Via A Einstein - Loc. Cascina Codazza, 26900 Lodi, Italy

ARTICLE INFO

Article history:
Received 1 September 2008
Received in revised form 7 April 2009
Accepted 14 April 2009

Keywords:
Pig
Intestinal epithelial cells
IPEC-J2
IPI-2I
Gene expression

ABSTRACT

The intestinal epithelial cells (IEC) play an important role in the immune system of swine, protecting against infectious and non-infectious environmental insults. The IEC participate in the innate immune response of the intestine through different mechanisms such as barrier function, mucus secretion, antibacterial peptide synthesis and participation in the cytokine/chemokine networks.

Most of the current knowledge of intestinal cell functions has come from studies conducted on cell cultures generated from human cancers or from classical animal models. However, because the molecular and cellular elements of the immune system have been selected over evolutionary time in response to the species-specific environment, models of immune function based on mouse and human need to be applied cautiously in pig. Few models of swine small intestine epithelium exist and these are poorly characterised. In the present study we characterised the basal expression of epithelial and immune-related genes of two pig small intestine cell lines, IPEC-J2 and IPI-2I, under different culture conditions. These data represent essential background information for future studies on pig-intestinal pathogen interactions.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

The intestinal tract represents one of the primary routes of entry of infective agents into an organism from food. The epithelial cells of the gut form a monolayer that covers the intestine and has a primary role in protecting against pathogenic insults. Due to their exposed location and their constant contact with commensal microbiota and pathogens, the intestinal epithelial cells (IEC) have developed different mechanisms and functions to reduce the risk of infection such as barrier function, mucus secretion, antibacterial peptide synthesis and participation in the cytokine/chemokine networks (Oswald, 2006).

Most of the current knowledge of intestinal cell functions has come from studies of cell cultures generated from human cancers or experimental animals, however, the results obtained on these models cannot be transferred directly to swine. The porcine immune system differs in many aspects from that of humans and mice. Examples of differences between these models and pigs include the morphological and functional peculiarities of the porcine lymphatic system and the immune cell population (Scharek and Tedin, 2007), the presence in the peripheral blood of adult pigs of a sizeable population of T cells positive for both CD4 and CD8, that play an unknown role in gut immunity (Zuckermann and Gaskins, 1996), and the expression of genes of unknown function in pig Peyer's patch (Dvorak et al., 2006). Moreover, Sonntag et al. (2005) have identified different adhesion patterns and lysis between human and porcine epithelial cells after challenge with pathogenic *E. coli*. Taken as a whole, these findings

* Corresponding author. Tel.: +39 0371 4662 420; fax: +39 0371 4662 349.
E-mail address: valentina.mariani@tecnoparco.org (V. Mariani).

0165-2427/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetimm.2009.04.006

clearly show that the use of human and mouse cell lines to study the mechanism of pathogenesis of swine is not advisable.

The IPEC-J2 and IPI-2I cell lines, derived from pig small intestine, are being increasingly used in challenge studies to investigate the epithelial interactions between cells of the host and enteric pathogens (Kaeffer et al., 1993; Rhoads et al., 1994; Sonntag et al., 2005; Boyen et al., 2006; Schierack et al., 2006; Skjolaas et al., 2006, 2007; Veldhuizen et al., 2006; Brown and Price, 2007; Ho et al., 2007; Volf et al., 2007; Schmidt et al., 2008). The non-transformed intestinal cell line IPEC-J2 was derived from the jejunum of an animal less than 12-h old (Rhoads et al., 1994), while the IPI-2I cell line (ECACC 93100622) was established from the ileum of an adult boar and immortalized by transfection with an SV40 plasmid (pSV3) (Kaeffer et al., 1993). For both cell lines few data are available on the constitutive "baseline" expression of genes known to be involved in the main epithelial and immune functions, and thus may possibly influence the outcome of differential gene expression measurements in infection experiments. It is well known that culture conditions could stress the cells and influence their basal gene expression (Calder et al., 2005; Tian et al., 2005; Ye and Lotan, 2008), however little attention has been paid to the effect of these variations on the data currently available.

Here, we characterised the expression of a selected panel of genes known to be involved in the main epithelial and immune-related gut functions in the IPEC-J2 and IPI-2I cell lines by real-time quantitative PCR (qPCR). The aim was to identify differences between the two systems and to obtain new information that could help to choose the best model for the gene expression studies of porcine gut immunity *in vitro*. Moreover, we investigated the effect of the presence of serum in the growth medium of IPI-2I cells on the expression of immune-related transcripts.

2. Materials and methods

2.1. Cell lines and culture conditions

The culture media for both cell lines were chosen on the basis of protocols described in literature. The IPEC-J2 cells were maintained in Dulbecco's MEM nutrient mix F12 (DMEM-F12) (1:1) (Euroclone, Pero, Italy) supplemented with 5% FBS (Euroclone), while the IPI-2I cells were grown in Dulbecco's modified Eagle medium (DMEM) (Euroclone) supplemented with 10% FBS, 4 mM L-glutamine (Sigma–Aldrich, St. Louis, MO, USA), Insulin 0.024 U/ml (Sigma–Aldrich) (medium A).

In order to test the effect of serum on gene expression, the IPI-2I cells were also grown in UltraCulture serum-free medium (Lonza Group Ltd., Visp, Switzerland) supplemented with 1.7 mM L-glutamine (medium B) and in medium B supplemented with 10% FBS (medium C).

Both IPEC-J2 and IPI-2I cells were seeded into 34.8 mm, six well plates (Corning Inc., Corning, NY, USA) at 2.5×10^5–5×10^5 cells per well in a 2 ml volume and allowed to adhere for 48 h before being washed and re-fed every other day until confluence. The cells were grown at 37 °C in a humidified atmosphere of 5% CO_2.

2.2. Real-time quantitative PCR

IPEC-J2 and IPI-2I cells were grown to confluence, then washed two times with PBS (Euroclone) and immediately lysed and collected using TRIzol reagent (Invitrogen Life Technologies Ltd., Carlsbad, CA, USA). Each culture

Table 1
List of genes, gene sequences and primers (F: forward, R: reverse) used for real-time qPCR of epithelial and immune-related genes using the Human Roche Universal ProbeLibrary. The last column reports the library probe number.

Gene symbol	Gene name	Sequence reference (gi)	Primer sequence	Probe no.
CCL2	Chemokine (CC motif) ligand 2	47523511	F 5′-TGT GCC TGC TGC TCA CTG-3′ R 5′-GCA GCA GGT GAC TGG AGA AT-3′	61
CCL20	Chemokine (CC motif) ligand 20	66793464	F 5′-TGA AAA TGA TGT GCA GTA GCA AG-3′ R 5′-TTG CTG CAG AGG TAG AGC AG-3′	71
CD47	Integrin-associated protein (IAP)	47522789	F 5′-TGG CCT TAA CCT GAG TAA TGT G-3′ R 5′-TGT ACT CAA TTT GTT ATT CCC TTG A-3′	68
CD58	Lymphocyte function-associated antigen, type 3 (LFA)	47523253	F 5′-CGT AGC GGA TAA ACC CAC AG-5′ R 5′-GCT TGG TTT CCC CTT CTG TT-3′	89
CLDN3	Claudin 3	48374875	F 5′-GAT GCA GTG CAA AGT GTA CGA-3′ R 5′-GTC CTG CAC GCA GTT GGT-3′	18
CLDN4	Claudin 4	75992462	F 5′-TAT CAT CCT GGC CGT GCT A-3′ R 5′-CAT CAT CCA CGC AGT TGG T-3′	77
FCGR3B	Fc fragment of IgG, low affinity IIIb, receptor (CD16b)	47523835	F 5′-TCT ACC TTG GCA CCA AAT CA3′ R 5′-GTG TCC ACT GCA AAC AGG AAT-3′	9
GM-CSF	Colony-stimulating factor 2 (granulocyte–macrophage)	47523043	F 5′-TGG CCA AGG CTG TAA TGG-3′ R 5′-GGC CTG TAT CAG GGT CAA CA-3′	37
ICAM1	Intracellular adhesion molecule 1	55742637	F 5′-GAG GAG CTG TTC AGG CAG TC-3′ R 5′-CAT CCG GAA CGT GAC ATT G-3′	58

Download English Version:

https://daneshyari.com/en/article/2462575

Download Persian Version:

https://daneshyari.com/article/2462575

Daneshyari.com