INSTITUT PASTEUR

Microbes and Infection 10 (2008) 159–165

www.elsevier.com/locate/micinf

Original article

Modulation of chemokine gene expression by Shiga-toxin producing *Escherichia coli* belonging to various origins and serotypes

Alain P. Gobert [a,*], Alix Coste [a,1], Carlos A. Guzman [b], Marjolaine Vareille [a], Thomas Hindré [a], Thibaut de Sablet [a], Jean-Pierre Girardeau [a], Christine Martin [a]

[a] *Institut National de la Recherche Agronomique (INRA), UR454 Unité de Microbiologie, Centre de Theix, 63122 Saint-Genès-Champanelle, France*
[b] *Department of Vaccinology, Helmholtz Centre for Infection Research, 38124 Braunschweig, Germany*

Received 17 July 2007; accepted 31 October 2007
Available online 9 November 2007

Abstract

Infection with Shiga-toxin producing *Escherichia coli* (STEC) may result in the development of the haemolytic–uremic syndrome (HUS), the main cause of acute renal failure in children. While O157:H7 STEC are associated with large outbreaks of HUS, it is difficult to predict whether a non-O157:H7 isolate can be pathogenic for humans. The mucosal innate immune response plays a central role in the pathogenesis of HUS; therefore, we compared the induction of IL-8 and CCL20 in human colon epithelial cells infected with strains belonging to different serotypes, isolated from cattle or from HUS patients. No correlation was observed between strain virulence and chemokine gene expression. Rather, the genetic background of the strains seems to determine the chemokine gene expression profile. Investigating the contribution of different bacterial factors in this process, we show that the type III secretion system of O157:H7 bacteria, but not the intimate adhesion, is required to stimulate the cells. In addition, H7, H10, and H21 flagellins are potent inducers of chemokine gene expression when synthesized in large amount.

Keywords: Epithelial cells; Shiga-toxin producing *Escherichia coli*; Chemokine; Locus of enterocyte effacement; Flagellin

1. Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are emerging pathogens responsible for foodborne infections. Healthy rearing animals are the main STEC reservoir and human infection occurs through the ingestion of contaminated food. The presence of these bacteria in the gut can be asymptomatic or lead to diseases ranging from watery diarrhoea to hemorrhagic colitis and life-threatening complications, such as haemolytic–uremic syndrome (HUS) [1,2]. Human isolates from patients are also referred to as enterohemorrhagic *E. coli* (EHEC). STEC strains belonging to the O157:H7 serotype are frequently responsible for the majority of the cases of disease worldwide and for large outbreaks, whereas non-O157:H7 serotypes are often responsible for sporadic cases. The bacterial factors associated with pathogenicity are Shiga-toxin (Stx) 2 and intimin [3]. Moreover, it has been recently demonstrated that the chromosomal insertion site of Stx-encoding bacteriophage in O157:H7 bacteria might determine its virulence or transmissibility to humans [4]. Nonetheless, in non-O157:H7 strains it is difficult to predict whether an isolate could represent a risk to human health.

STEC possess numerous virulence factors that allow colonization and pathogenesis. Genes in the locus of enterocyte effacement (LEE) code for proteins implicated in the intimate adhesion of the bacteria to eukaryotic cells, i.e. intimin encoded by *eae* and its receptor Tir [5,6], and for a type III secretion system (TTSS). EspA is the main component of the pilus-like appendage, which forms the needle of the

* Corresponding author. Tel.: +33 4 73 62 45 34; fax: +33 4 73 62 45 81.
E-mail address: agobert@clermont.inra.fr (A.P. Gobert).
[1] Present address: IMUL-CHUV, 1011 Lausanne, Switzerland.

1286-4579/$ - see front matter © 2007 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.micinf.2007.10.018

TTSS [7]. EspD protein is essential for the formation of surface appendages and is integrated in the cytoplasmic membranes of eukaryotic cells [8]. Two additional proteins, SepL and EscD, also called Pas, are involved in the translocation of Tir and secretion of Esp proteins [9,10]. TTSS-secreted proteins interact with the host signal transduction, leading to actin polymerization and microvilli effacement [1]. However, it is not known whether attachment/effacement is a prerequisite for the development of severe diseases, notably because LEE-negative EHEC strains are often isolated from cases of HUS [1]. Additionally, the EHEC virulence factors associated with severe human diseases are Stx1 and Stx2; these toxins are produced in the lower intestine, translocate across intestinal epithelium, and induce necrosis or apoptosis of vascular endothelial cells by inhibiting protein synthesis [11]. Stx may also modulate the innate immune response of human enterocytes [12,13] and favour the attachment of the bacteria to the colonic epithelium [14].

Clinical studies highlight that the host inflammatory response is induced during the course of EHEC infection and correlates with the development of HUS [15,16]. This inflammation depends on the EHEC-induced mucosal innate immune response. Activated enterocytes synthesize chemokines and cytokines in response to pathogen-associated molecular patterns to attract and activate leukocytes to the site of infection. In fact, histological analysis of gut tissues harvested from O157:H7-infected patients has shown neutrophils infiltrating the lamina propria and crypts [17]. It has been proposed that the colonic mucosa damaged by the inflammation represents a way for Stx to cross the epithelial barrier. In vitro studies have shown that STEC strains induce the expression in intestinal epithelial cells of IL-8 and CCL20 [13,18], which can recruit neutrophils and dendritic cells, respectively. In this context, the ability of STEC strains to induce the host innate immune response could be a reliable marker of bacterial virulence.

Thus, studies were performed to evaluate the induction of IL-8 and CCL20 in human epithelial cells in response to STEC strains isolated from either the animal reservoir or HUS patients and belonging to O157:H7, O91:H21, O91:H10, O113:H21, or O6:H10 serotypes. In addition, we analysed the potential contribution of different bacterial factors to the stimulation of the innate immune response using mutant strains lacking the main STEC virulence factors.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Bacterial strains used in this study are described in Table 1 [19–22]. Bacteria were isolated on Luria–Bertani (LB) agar plates. One clone of each strain was grown overnight in LB broth with agitation at 37 °C, then diluted ($OD_{600\ nm} \approx 0.03$) and grown for 2 h until the exponential growth phase ($OD_{600\ nm} \approx 0.3$) in DMEM (Invitrogen). Media were supplemented with ampicillin (50 μg/ml), kanamycin (50 μg/ml), and/or IPTG (see Table 2) when required. For the motility experiments, LB agar was overlaid by LB soft agar. Bacterial concentration was estimated to be 5×10^8 bacteria per millilitre per OD unit at 600 nm, as calculated by plating.

Table 1
Bacterial strains

Strain	Serotype	Origin	Genetic characteristic			Reference
			LEE	*stx1*	*stx2*	
EDL933	O157:H7	HC/outbreak	+	+	+	[21]
Sakaï	O157:H7	HUS/outbreak	+	+	+	[22]
86-24	O157:H7	HC/outbreak	+	−	+	T.S. Whittam[a]
ChVi-1	O157:H7	HUS	+	−	+	[20]
Ch1898	O157:H7	HUS	+	−	+	[20]
NV95	O157:H7	Bovine	+	+	+	[20]
CHO14	O91:H21	HUS	−	−	+	[20]
B2F1	O91:H21	HUS	−	−	+	[19]
VTH13	O91:H21	HC	−	+	+	J. Blanco[b]
NV127	O91:H21	Bovine	−	−	+	[20]
NV197	O91:H21	Bovine	−	−	+	[20]
NV20	O91:H21	Bovine	−	−	+	[20]
CHO13	O91:H10	HUS	−	−	+	[20]
CB67-74	O91:H10	HUS	−	+	−	L. Beutin[c]
CB67-75	O91:H10	HUS	−	−	+	L. Beutin[c]
NV130	O91:H10	Bovine	−	−	+	[20]
NV148	O91:H10	Bovine	−	−	+	[20]
NV280	O91:H10	Bovine	−	−	+	[20]
CL3	O113:H21	HUS	−	−	+	T.S. Whittam[a]
CL15	O113:H21	HUS	−	−	+	T.S. Whittam[a]
87-307	O113:H21	HUS	−	−	+	T.S. Whittam[a]
NV254	O113:H21	Bovine	−	−	+	[20]
NV298	O113:H21	Bovine	−	−	+	[20]
NV299	O113:H21	Bovine	−	−	+	[20]
NV106	O6:H10	Bovine	−	−	+	[20]
NV183	O6:H10	Bovine	−	−	+	[20]
NV268	O6:H10	Bovine	−	−	+	[20]
DH5α	NA	NA	NA	NA	NA	Invitrogen

[a] STEC center (http://shigatox.net).
[b] Laboratorio de Referencia de *E. coli*, Universidade de Santiago de Compostela, Lugo, Spain.
[c] National Reference Laboratory for *Escherichia coli*, Federal Institute for Risk Assessment, Berlin, Germany.

2.2. Mutagenesis and complementation

The mutant and complemented STEC strains used in this study are presented in Table 2. Deletion of the *eae* and *fliC* genes of EDL933 and of the *fliC* genes of CHO14, CHO13, and NV268, were obtained by allelic exchange with a kanamycin cassette using the one-step PCR-based method of Datsenko and Wanner [23]. For complementation of the mutants, the genes of interest were cloned under the control of the IPTG-inducible *trc* promoter in the expression vector pTrc99A.

Mutations and complementations of *fliC* mutants were checked by measuring the motility of the strains in soft agar (Table 2). For the *eae* gene, wild-type (WT), mutant, and complemented strains were grown in DMEM to the early exponential phase; subsequently, intimin protein in crude bacterial lysates was analysed by Western blotting using polyclonal anti-intimin antibody provided by Josée Harel (Groupe de Recherche sur les Maladies Infectieuses du Porc, Université de Montréal, Sainte-Hyacinthe, Canada). Intimin was detected