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IgA and IgG antibody responses following systemic immunization of cattle with native H7 flagellin differ in epitope recognition and capacity to neutralise TLR5 signalling

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ABSTRACT

Systemic immunization of cattle with H7 flagellin results in induction of both H7-specific IgA and IgG antibodies but only partially protects against subsequent colonization with *Escherichia coli* O157:H7. Recent studies indicate that anti-flagellin antibodies directed against TLR5 binding domains located in the conserved N- and C-terminal domains of flagellin can neutralise TLR5 activation and impair vaccine efficacy. In the current study we determined whether systemic immunization of cattle with H7 flagellin induces antibodies capable of interfering with flagellin-mediated TLR5 activation. Both anti-H7 IgG1 and IgG2 but not IgA antibodies recognised epitopes within the conserved N- and C-terminal domains of H7 flagellin, and purified H7-specific IgG but not IgA was capable of inhibiting H7-mediated TLR5 activation *in vitro*. These results suggest that (i) IgA and IgG isotypes originated from different populations of B cells and (ii) systemically induced H7-specific IgG but not IgA may impair innate immune responses to *E. coli* O157:H7 via neutralisation of TLR5 activation and subsequently reduce vaccine efficacy.

1. Introduction

Enterohaemorrhagic *Escherichia coli* (EHEC) is a zoonotic pathogen of worldwide importance, causing haemorrhagic colitis and, in a small percentage of cases, haemolytic–uraemic syndrome in humans. The predominant EHEC serotype in North America and Europe is O157:H7. Ruminants, in particular cattle, are an important reservoir of EHEC, and human infections are frequently associated with direct or indirect contact with cattle faeces [1–4]. Reducing carriage of EHEC in cattle has been predicted to be a key control point in reducing EHEC infections in humans [4] and as a result a number of cattle EHEC vaccines have been evaluated.

EHEC infections in cattle are generally asymptomatic and the bacteria are restricted to the intestinal epithelium and gut lumen [4]. We have recently shown that the flagellum of *E. coli* O157:H7 (H7 flagellum) acts as an important adhesin to bovine intestinal epithelium during the early stages of colonization [5]. H7 flagella form physical contact points with the rectal epithelial cells and once bacterial anchoring has occurred, flagellar expression is down-regulated and other adhesion mechanisms such as the TTSS take over [5,6]. In addition to epithelial cell binding, H7 flagella also bind to bovine mucus [7]. Therefore it appears that H7 flagella play a crucial role in initiating binding to the mucosal surface, either by anchoring to mucus, epithelium or both, and represent an additional target for *E. coli* O157:H7 vaccination in cattle.

Flagella consist of three main structures: a basal body within the bacterial cell wall which functions as a motor, a torsion hook and a helical hollow filament [8,9]. This filament consists almost entirely of flagellin subunits which are secreted through the central channel of the growing filament to be assembled in a helical structure at the growing distal end. These flagellin monomers themselves consist of N- and C-terminal domains which are highly conserved between *E. coli* strains and several other bacterial species, and a central hypervariable domain [10,11]. The conserved N- and C-terminal domains of flagellin form α-helix structures which are positioned in the filament core, whereas the central variable domain is exposed as a β-sheet folded structure on the filament outer surface. These domains of flagellin interact differently with

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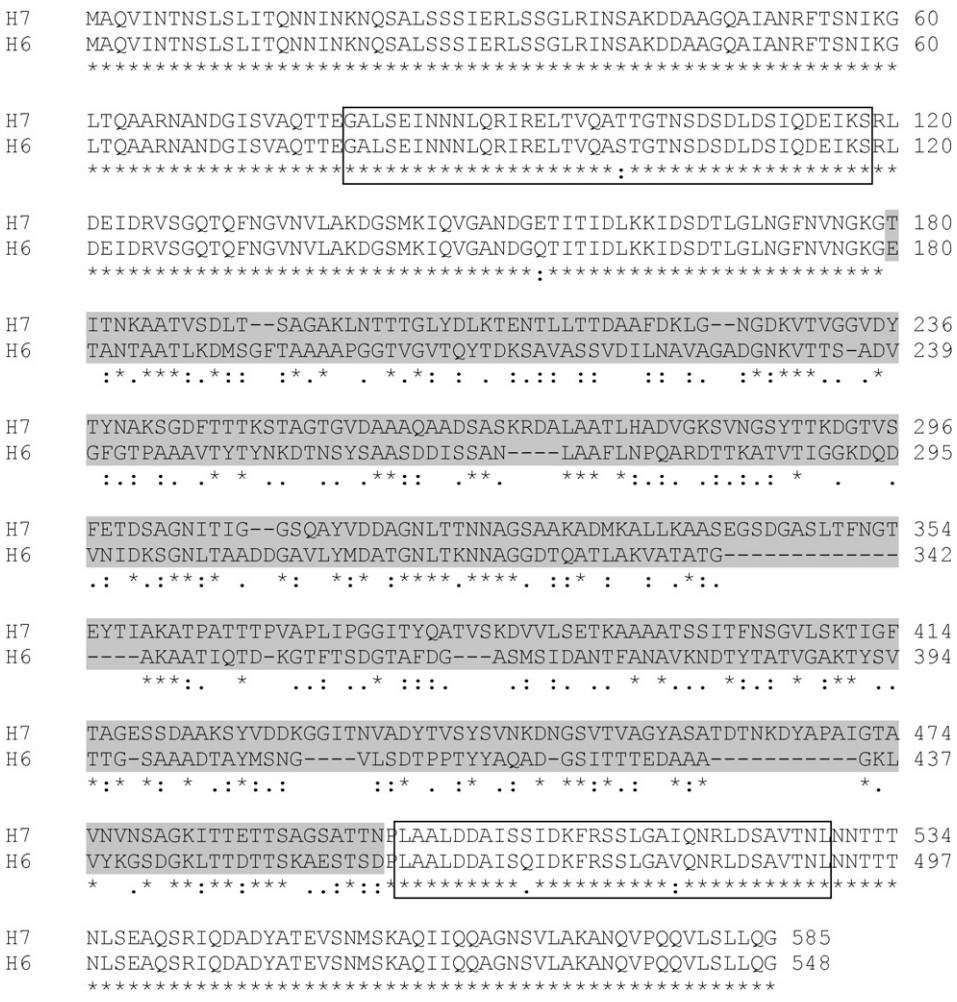

Fig. 1. Alignment of H7 flagellin (accession no. AM228903) and H6 flagellin (available at www.sanger.ac.uk/cgi-bin/blast/submitblast/escherichia_shigella) amino acid sequences using the multiple sequence alignment program ClustalW2. The variable domain of H7 flagellin is highlighted in grey. Boxed areas represent putative TLR5 binding domains.

the immune system. For example, the variable domain possesses sero-dominant epitopes which are the basis for H-serotyping of *E. coli* [12], whereas toll-like receptor 5 (TLR5) binding domains have been mapped to regions within the conserved N- and C- terminal domains [13,14]. TLR5 is an important innate immune receptor and interaction of flagellins with TLR5 expressed on the surface of epithelial cells, macrophages or dendritic cells (DC) initiates a pro-inflammatory cell signalling cascade mediated through the myeloid differentiation factor 88 (MyD88) adaptor molecule and NF-κβ transcriptional activator [15,16]. This results in the stimulation of innate immune responses characterised by production of pro-inflammatory cytokines and has been shown to potentiate induction of antigen-specific adaptive immune responses [17–19].

As H7 flagella appear to be important in initiating colonization of cattle with *E. coli* O157:H7, we have recently evaluated the efficacy of a vaccine based on H7 flagellin [20]. Intra-muscular immunization of calves with purified H7 flagellin induced both systemic and mucosal IgA and IgG antibodies and resulted in a significant reduction in the number of animals that were successfully colonized following subsequent bacterial challenge. However, in animals that did become colonized, peak levels of bacterial shedding were identical in magnitude to those in unvaccinated animals and were maintained for a longer period (approximately 6 days compared to 3–4 days for unvaccinated controls) [20]. These results support a role for H7 flagella in initial colonization of the bovine host by *E. coli* O157:H7 but also suggest that once colonization has occurred, anti-H7 flagellin immune responses have little suppressive effect on subsequent bacterial growth at the epithelial surface and may actually impair the ability of the host to mount a protective immune response against the bacterium.

It has recently been shown that immunization of mice with full-length *Pseudomonas aeruginosa* flagellin results in the generation of antibodies directed towards the conserved N- and C-terminal domains which interfere with TLR5 activation [21]. This antibody-mediated-neutralisation of TLR5 correlated with reduced vaccine efficacy against subsequent homologous bacterial challenge. As we had similarly immunized cattle with full-length flagellin, the aim of this study was to determine if the antibodies induced following immunization of cattle with H7 flagellin in our previous vaccine trial [20] were directed against the conserved domains of H7 by determining antibody reactivities to a closely related flagellin and to recombinant fragments of H7 flagellin, and whether these antibodies had any effect on subsequent TLR5 activation.

2. Materials and methods

2.1. Purification of native flagellins

H6 and H7 Flagellins were isolated from *E.* coli O127:H6 strain E2348/69 and *E. coli* O157:H7 (stx-) strain ZAP984, a LEE4 deletion mutant derived from strain Walla 3 [22], respectively by acid dissociation, neutral pH re-association and ammonium sulfate