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Research Article

Construction of a Nanobodies Phage Display Library From an *Escherichia coli* Immunized Dromedary

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Background: Diarrhea caused by *Escherichia coli* is a major cause of morbidity and mortality in young animals. Few treatment options are available, mainly antibiotic therapy increasingly limited by resistance to commonly used drugs.
Objectives: The aim of this work was to develop immunotherapy based on the use of camel VHH antibody fragments, or nanobodies, to target pathogenic *E. coli* surface antigens.
Material and methods: We immunized a camel with a killed strain we had previously isolated from a diarrheic camel calf and identified as expressing the F17 fimbriae antigen.
Results: The immunized animal developed an anti-*E.coli* immune response including heavy-chain antibodies. Lymphocytes from this animal were purified and RNA isolated to create a VHH library by phage display with a size of about 10^9 individual transformants. Panning on live *E. coli* cells resulted in the isolation of VHH fragments specific to the cell surface antigens.
Conclusion: The identification of these antigens can lead to the development of new diagnostic and therapeutic tools against diarrhea.
Key words: *Escherichia coli*; diarrhea; nanobodies; phage display

1. Background

Neonatal diarrhea is a major cause of death in herds and is responsible for considerable economic losses. *Escherichia coli* (*E. coli*) is a major etiological agent of diarrhea in young animals. It is part of the intestinal commensal microflora of most warm-blooded animals (1). However, *E. coli* can also be an opportunistic or obligate pathogen, able to multiply and persist in the host's digestive tract by bypassing immune defenses and inducing cell damage (2).

The study of the different modes of interactions between the host and the bacterium during infection makes it possible to classify *E. coli* strains into several pathogenic variants called pathovars or pathotypes. This classification is based on the combination of particular properties associated with virulence factors expressed by a strain, the infection route and the associated clinical signs (3).

There are currently nine pathovars of pathogenic *E. coli* that can be classified into two groups: Intestinal *E.coli* comprising seven pathovars responsible for intestinal disorders: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic (EHEC) (or Shiga toxin-producing *E. coli*, STEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC or EAggEC) and diffuse adhesion *E. coli* (DAEC). Extra-intestinal *E. coli* including two pathovars, *E. coli* associated with neonatal meningitis (NMEC) and uropathogenic *E. coli* (UPEC) (4).

Recently, we demonstrated that out of 120 fecal samples from camel calves aged 1 day to 3 months (62 healthy and 58 diarrheic), 70 strains of *E. coli* were isolated with a prevalence of 67% and 50% in diarrheic and healthy calves, respectively. Molecular identification of *E. coli*-associated virulence genes, using the PCR technique, showed that the prevalence of the gene encoding F17 fimbriae was significantly higher in diarrheic than in clinically healthy calves (46.5% vs. 14.5%)(5).

In addition to conventional antibodies, camelids (camels and llama) produce antibodies devoid of the light chain and the CH1 domain called heavy chain antibodies (HCAbs) (6). VHH fragments or nanobodies are derived from these HCAbs. They are highly soluble, physicochemically stable and can be produced with high yields in bacteria, fungi or plants (7, 8). Thanks to their monomeric structure, VHH are easily engineered to create multimers or to be more stable, especially to proteases(9) These favorable properties have led to the development of several nanobodies for use in a wide variety of research, diagnosis or therapeutic applications (10).