Journal of Microbiology, Immunology and Infection (2014) **47**, 57—62

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.e-jmii.com

ORIGINAL ARTICLE

The molecular adjuvant mC3d enhances the immunogenicity of FimA from type I fimbriae of *Salmonella enterica* serovar Enteritidis

Hassan-Hussein Musa [a,b,d], Wei-Juan Zhang [a,d], Jing Lv [a], Xiao-Li Duan [a], Yang Yang [a], Chun-Hong Zhu [a,c], Hui-Fang Li [c], Kuan-Wei Chen [c], Xia Meng [a], Guo-Qiang Zhu [a,*]

[a] *College of Veterinary Medicine, Yangzhou University, Yangzhou, China*
[b] *Faculty of Veterinary Science, University of Nyala, Sudan*
[c] *Jiangsu Institute of Poultry Science, Yangzhou 225003, China*

Received 17 August 2012; received in revised form 30 October 2012; accepted 20 November 2012

KEYWORDS
FimA;
mC3d;
recombinant protein;
Salmonella enterica serovar Enteritidis

Background: The fimbriae of *Salmonella enterica* serovar Enteritidis are used for colonization and invasion into host cells, and have drawn considerable interest because fimbriae can serve as potential immunogens against many pathogenic bacteria that colonize on epithelial surfaces. The purpose of the study is to use a molecular adjuvant, C3d, to enhance the immunogenicity of FimA proteins against *Salmonella enterica* serovar Enteritidis.
Methods: FimA of type I fimbriae from *Salmonella enteritidis* and FimA with one copy of m*C3d*, two copies of m$C3d_2$ and three copies of m$C3d_3$ were cloned into the expression vector pCold-TF. Soluble fusion proteins of FimA with different copy of *mC3d* were induced by IPTG and expressed into *Escherichia coli* BL21 (DE3). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) showed that the recombinant proteins from pCold-TF-fimA, TF-fimA-*mC3d*, TF-fimA-$mC3d_2$, TF-fimA-$mC3d_3$ were 70 kDa, 100 kDa, 130 kDa and 160 kDa, respectively. The fusion protein was recognized by rabbit anti-fimbriae polyclonal antibodies, and then visualized by goat anti-rabbit polyclonal antibodies with a chrome appearance by enzyme-subtract interaction. The recombinant proteins were purified by Ni-TED (tris-carboxymethyl ethylene diamine), immobilized metal ion affinity chromatography (IMAC). Balb/c mice were subcutaneously immunized with the purified proteins and the immune response was monitored by an enzyme-linked immunosorbent assay (ELISA) for FimA-specific antibody. The immunized mice were challenged with a 10-fold LD_{50} dose (i.e., 100 CFU) of *Salmonella enterica* serovar Enteritidis standard strain (SD-2) 1 week after the second immunization.

* Corresponding author. College of Veterinary Medicine, Yangzhou University, Yangzhou, China.
E-mail addresses: hassan_hm30@yahoo.com (H.-H. Musa), yzgqzhu@hotmail.com (G.-Q. Zhu).
[d] The co-first authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jmii.2012.11.004

Results: The immunized mice with the fusion proteins FimA-mC3d$_2$ and FimA-mC3d$_3$ had increased levels of ELISA titer of antibody that were 2 and 4 logs, respectively, more immunogenic than the TF-FimA protein alone. The challenge results showed that immune protection rate in the mice immunized with 10 μg of FimA, FimA-mC3d$_2$, and FimA-mC3d$_3$ were 50%, 75% and 100%, respectively.
Conclusion: We conclude that mC3d can be expressed in a prokaryotic vector and enhance the immune response of the recombinant protein. FimA-mC3d$_3$ is potentially a subunit vaccine against *S. enterica* serovar Enteritidis infection.

Introduction

The prevalence of *Salmonella enterica* serovar Enteritidis has dramatically increased worldwide; it is reportedly the most common serotype in the United States. Approximately 1000 people die of the infection each year.[1] Fimbriae are used for colonization and invasion into host cells. They have drawn considerable interest because the fimbriae are potential immunogens against many pathogenic bacteria that colonize epithelial surfaces.[2] The main fimbriae of *S. enterica* serovar Enteritidis are SEF14, SEF17, and SEF21, which are composed of SefA, AgfA, and FimA fimbrial proteins, respectively.[3] Because of fimbriae structure and localization, they are excellent targets for the host immunological system. Fimbria-based vaccines are hypothesized to protect the host against the adherence of pathogens by blocking the organisms from attaching to the intestinal mucosa.[4] Scientific interest has recently focused on effector molecules generated by the innate immune response and on their role in shaping acquired immunity.[5] A recombinant protein consisting of an antigen fused to C3d may elicit a more robust immune response than the antigen alone. Dempsey et al.[6] demonstrated that a recombinant protein containing three copies of C3d attached to the carboxy terminus of hen egg lysozyme (HEL) could elicit a primary immune response is 10,000-fold greater with the modified HEL protein, compared to the unmodified HEL protein. Similar results were achieved by conjugating C3d to viral, bacterial, parasitic, and cellular (i.e., self) antigens.[7,8] Three repeats of C3d conjugated to a soluble trimeric form of the HIV-1 Env was more effective at inducing neutralizing antibodies to primary isolates than plasmids encoding for non-C3d conjugated Env glycoproteins.[9,10] Purified E2 proteins fused to murine and bovine homolog C3d3 were 10,000 more immunogenic than E2 alone or anti-E2 antibodies neutralized virus infection.[8,10] This immunization mode demonstrated the ability of C3d to enhance secondary humoral (i.e., IgG) immune responses and the maturation of antibody avidity, and the ability of the antibody to neutralize infection and stimulate B-cell proliferation.[11,12] In previous studies, mC3d conjugated to prokaryote antigen was expressed on a cell culture, whereas in the present study mC3d conjugated to prokaryote antigen was clones into a pCold-TF expression vector and successfully expressed in *Escherichia coli* BL21(DE3). We therefore used the active C3d as a molecular adjuvant to enhance the immunogenicity of FimA proteins against *Salmonella enterica* serovar Enteritidis. This will help in the design of more efficient vaccines against *Salmonella enterica* serovar Enteritidis.

Materials and methods

Bacterial growth and DNA extraction

The *Salmonella enterica* serovar Enteritidis were cultured overnight at 37°C with vigorous agitation. The DNA then was extracted. The FimA gene was amplified by an upper primer with the NdeI restriction enzyme site: 5′-CGC CATATG AAA CAT AAA TTA ATG ACC TCT A-3′ and a lower primer with the NotI and BamHI restriction enzymes site: 5′-TCG GCG GCC GCG GAT CCT TCG TAT TTC ATG ATA AAG GTG-3′. Polymerase chain reaction (PCR) was performed in a volume of 25 μL that contained 2.5 μL of the DNA template, 1.5 μL of 2.5 mM dNTP mixture, 2.5 μL of 10 × PCR buffer, 1 μL upper primer, 0.5 μL lower primer, 0.5 μL Ex Taq polymerase, and 16.5 μL sterilized distilled water. The PCR conditions were an initial denaturing at 94°C for 4 minutes, followed by 30 cycles at 94°C for 1 minute. The product was annealed at 56°C for 1 minute and extended at 72°C for 1 minute. The PCR product was finally extended at 72°C for 10 minutes. The 550 bp of the FimA gene was excised from the gel and purified using DNA purification kit. The purified product was then cloned into the PMD18-T simple vector and transformed into *Escherischia coli* DH5a-competent cells. Recombinant plasmids were extracted and analyzed by restriction enzyme digestion and sequencing.

Construction of recombinant protein

The recombinant plasmids PMD18-T-FimA and the expression vector pCold TF were digested with NdeI and BamHI restriction enzymes. The FimA gene and pCold TF expression vector both recovered from the gel in the agarose gel DNA purification kit. Purified FimA DNA was cloned into pCold-TF expression vector. The recombinant FimA-pCold-TF was transformed into the *E. coli* DH5a-competent cells; the positive clones were selected and determined by restriction enzyme analysis with NdeI and BamHI.

The clones of C3d into the pUC plasmid were generated according to the strategy of Dempsey et al[6] and were a gift