Bangladesh J Microbiol, Volume 37, Number 2, December 2020, pp 38-41

Original Article

Comparison of Immunogenicity and Protective Efficacy of the Intranasal and Intraperitoneal Immunization Routes of *Escherichia albertii* Strain DM104 in Mouse Model

Fatema Moni Chowdhury[1,2,3], Nils-Kare Birkeland[2] and Chowdhury Rafiqul Ahsan[1*]

[1]Department of Microbiology, University of Dhaka, Dhaka 1000, Bangladesh, [2]Department of Biology, University of Bergen, Bergen, Norway, [3]Present Address: Department of Microbiology, Jagannath University, 9-10 Chittaranjan Road, Dhaka 1100, Bangladesh

In recent years, our group isolated the *Escherichia albertii* strain DM104 and characterized it as a vaccine strain against *Shigella dysenteriae* type 4 in the guinea pig eye model. Protective efficacy of different routes of immunization such as intranasal, oral, and intrarectal routes were also determined and compared by challenging immunized guinea pigs against live *S. dysenteriae*. In the current study, we compared the intranasal and intraperitoneal routes of immunizations with the DM104 vaccine strain in mice to understand the better route of administration of the DM104 vaccine and its immunogenicity as well as protective efficacy in mouse model. The results indicate that the immune response elicited by the DM104 strain is strongly dependent on the immunization route, with the intranasal route being more effective than the intraperitoneal route following intraperitoneal live *S. dysenteriae* challenge. Intranasal immunization yielded 80% protective efficacy in immunized mice whereas, intraperitoneal immunization could not provide any protection. Protection generated by intranasal immunization was accompanied by high titre of anti-whole cell lysate IgG and IgA in DM104 immunized sera compared to sera collected from mice of control group. All these data demonstrate the intranasal route of the vaccine DM104 strain in mouse model to be a better immunization route to protect the animals against live *S. dysenteriae* challenge.

Keywords: *Escherichia albertii*, *Shigella*, shigellosis

Introduction

Shigellosis is endemic throughout the world where it is held responsible for severe dysentery with blood and mucus in the stools, the overwhelming majority of which occur in developing countries and involve children less than five years of age[1]. About 2 million people were estimated to die from *Shigella* infection each year, with a third of these associated with young children[2]. Shigellosis is transmitted from human-to-human by the fecal-oral route via contaminated food and water or through person-to-person contact, as often observed in institutionalized populations and readily disseminate in settings where hygiene is poor[3]. The disease is characterized by a short period of watery diarrhoea with intestinal cramps and general malaise, soon followed by emission of bloody, mucoid, often mucopurulent stools. Acute complications may occur that include peritonitis and septicaemia, especially in malnourished children, and the severe haemolytic uremic syndrome (HUS) with renal failure[4]. In the absence of an existing effective vaccine, the ever-increasing frequency of antimicrobial-resistant *Shigella* strains worldwide has become a major source of concern[5].

In recent years, we have isolated a number of *Shigella*-like bacteria from freshwater environments in Bangladesh that serologically cross-reacted with different *Shigella* spp.[6,7]. One of these strains, DM104 was phylogenetically identified as *Escherichia albertii* and showed a similar lipopolysaccharide (LPS) gel banding profile to that of *S. dysenteriae* type 4[8]. The DM104 isolate was found to be non-invasive, did not produce any entero- or cytotoxins, and showed negative results in the mouse lethal activity assay[9]. As an ocularly administered vaccine in the guinea pig eye model against *S. dysenteriae* type 4 challenge, the non-pathogenic DM104 strain gave, a high protective efficacy and also induced a high titer of serum IgG against *S. dysenteriae* type 4 whole cell lysate (WCL) and LPS. Protective efficacy of different routes of immunization such as intranasal, oral, and intrarectal routes was also determined and compared by challenging immunized guinea pigs against live *S. dysenteriae* type 4 and by measuring both the serum IgG and mucosal IgA antibody titers[10].

In the present study, immunogenicity and protective efficacy of DM104 strain using intranasal and intraperitoneal routes of immunizations were determined and compared in mouse model against live *S. dysenteriae* type 4 challenge.

Materials and Methods

Bacterial Strains

The DM104 strain was originally isolated from river water and the 16S rRNA gene sequence (GenBank accession number,

*Corresponding author:
Chowdhury Rafiqul Ahsan, Department of Microbiology, University of Dhaka, Dhaka 1000, Bangladesh, E-mail: crahsan@du.ac.bd.

JQ996386) identified it as *E. albertii*[8]. The DM104 isolate along with *S. dysenteriae* type 4 strain used in this study were obtained from the stock culture of the Department of Microbiology, University of Dhaka.

Animals and ethical issues

Swiss albino mice were obtained from the Animal Resources Branch of International Center for Diarrhoeal Diseases Research, Bangladesh (icddr,b) and maintained in the Department of Microbiology, University of Dhaka, under proper hygienic conditions. All animal experiments were performed following the guidelines set by the Ethical Review Committee for Animal Experimentation of the Faculty of Biological Sciences, University of Dhaka.

Preparation of bacterial whole cell lysate (WCL)

One ml of a 6 h culture of *S. dysenteriae* type 4 in Brain Heart Infusion broth (BHIB) was inoculated into 200 ml of BHIB and incubated further for overnight. The cultures were centrifuged at 8,000 rpm for 10 min at 4ºC and the supernatant was discarded. The cell pellet obtained after centrifugation, were re-suspended in 3 ml PBS, sonicated at 30 Hz for 5 min on ice and centrifuged at 10,000 rpm for 10 min[11]. The supernatant containing the WCL was filtered through a 0.45 µm pore size membrane filter and stored at -20ºC.

Immunization of the animals

A total of 18 mice were immunized in each group of intranasal or intraperitoneal immunization with live DM104[12]. Groups of mice that received identical doses of PBS in each immunization schedule, were used as controls.

Intranasal immunization:

The intranasally immunized group of mice received anesthesia using chloroform followed by a single dose of the DM104 strain (1.2×10^9 CFU/ml) diluted in PBS (10 µl each nare, 20 µl total) administered with a pipette into the nares of the mice. All the mice received identical vaccine dose, two times at 2-week intervals and one booster dose 30 days after the second dose[12].

Intraperitoneal immunization:

The intraperitoneally immunized groups were administered with the live DM104 suspension (1.2×10^9 CFU/ml) at a volume of 100 µl delivered with a 25 gauge needle. All the mice received identical vaccine dose, two times at 6-week intervals[12].

Collection of sera before bacterial challenge experiment

Four immunized mice from each group of intranasal and intraperitoneal immunization, were taken and lateral tail vein bleeds were performed 72 h prior to bacterial challenge experiment for collection of sera. Sera samples were kept at -20ºC and later assayed for both IgG and IgA antibody responses against sonicated WCL of the *S. dysenteriae* type 4 using ELISA[13]. Sera from control mice were used as control.

Bacterial challenge experiment

Remaining mice from DM104 immunized (both through intranasal and intraperitoneal route) and control groups were intraperitoneally challenged with a dose of 100¼l containing 1.5 $\times 10^8$ CFU/ml of live *S. dysenteriae* type 4, 4 weeks after the last dose of immunization. Challenged mice were observed daily for any death or taken for other experiments as described below.

Recovery of bacteria from tissues and organs of immunized mice

After 24 h of challenge experiment, four mice from intranasally immunized group were taken for the recovery of experimental bacteria. Blood was collected from each mouse and then sacrificed by cervical dislocation for collection of organs. The abdominal cavity was aseptically opened and spleen, liver and kidney were removed and homogenized separately for counting of viable *S. dysenteriae* type 4 bacteria as described previously[14]. Similarly, two mice from control group were taken for control experiment.

Statistical analysis

Mean ± SEM or mean ± SD were determined and ELISA OD titers were compared by Student's t test. A statistical comparison of protection data was determined using a Fisher's exact t test. P values of <0.05 were considered significant. An unpaired Student's t test was used to compare the mean value of CFU of bacteria recovered from infected tissues and the significance of the differences between the two immunization routes.

Results

Immune responses and protection following intranasal immunization

Mice were immunized intranasally with 20 µl of bacterial preparations containing 1.2×10^9 CFU/ml of DM104. Both immunized and control mice were challenged 4-weeks after the last immunization and 80% (8 out of 10 mice) mice were found to survive in the DM104 immunized group whereas all control mice died between days 4 and 8 post-challenge (Fig. 1). Also, increased level of IgG and IgA against WCL were found in blood sera of all the live DM104 immunized mice (Fig. 2).

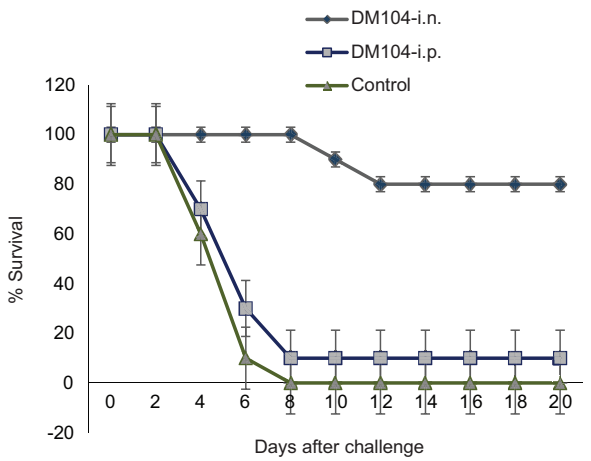

Fig. 1. *Protective efficacy of DM104 strain in mice model. Mice (n=10) were immunized with live DM104 bacteria, either intranasal (i.n.) or intraperitoneal (i.p.) route and were challenged with 1.5 × 10^8 CFU/ml of live S. dysenteriae type 4. Intranasally immunized mice showed 80% protection while almost all control or intraperitoneally immunized mice died within 8 days of challenge.*

Immune responses and protection following intraperitoneal immunization

Mice were immunized intraperitoneally with two doses each of 100µl bacterial suspensions containing 1.2×10^9 CFU/ml of live DM104, given at 6 weeks intervals. Both immunized and control mice were challenged intraperitoneally with approximately 1.5×10^8 CFU/ml of live *S. dysenteriae* type 4. However, none of the intraperitoneally immunized mice survived the challenge experiment (Fig.1). Intraperitoneal immunization of the DM104 induced strong IgG responses to their respective WCL antigen, although the IgA response was very low (Fig. 2).

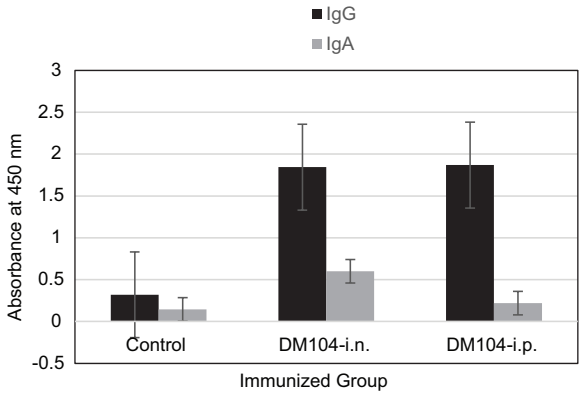

Fig. 2. *Immunogenicity of the DM104 in mice model. Increased level (mean ± S.D.) of IgG and IgA were found in blood sera of all live DM104 immunized mice through intranasal (i.n.) and intraperitoneal (i.p.) route. On the other hand, the level of antibodies in control mice without any immunization showed lower level.*

Recovery and estimation of live bacteria from tissues and organs of infected mice

At 24 h post challenge 67±3, 33±5 and 33±3 CFU/organ of *S. dysenteriae* type 4 were isolated from the spleen, liver and kidney of control mice, respectively (Fig 3). However, in case of intranasally immunized mice, 55±2 CFU/organ of bacteria were isolated only from spleen whereas no bacteria were isolated from liver or kidney. To test for the presence of live *Shigella* in the blood of immunized and control mice, blood samples taken at 24 h after challenge, were separately plated on MacConkey agar but resulted without any bacterial growth. It might have been interesting to check whether antibody profile shows any significant change after the challenge experiment.

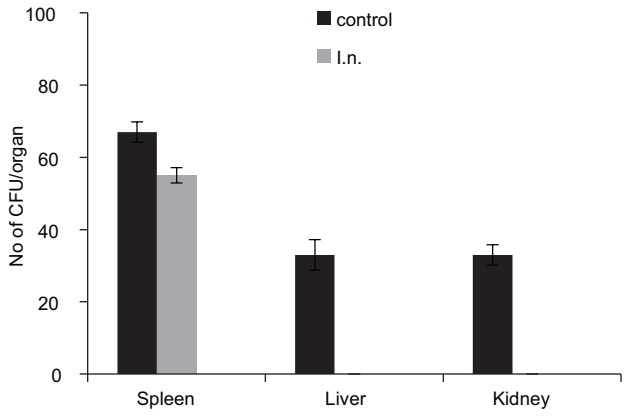

Fig. 3. *Survival and dissemination of bacteria in spleen, liver and kidney of control and immunized mice. Data shown represent the mean number of CFU calculated per organ which was 67, 33 and 33 in spleen, liver and lung respectively, of control mice and 55 only in the spleen of intranasally (i.n.) immunized mice with DM104 after 24 h of challenge. SDs are shown.*

Discussion

The protective immunity provided by DM104 to challenge with *S. dysenteriae* type 4, as was seen by our previous experiments in guinea pig models, was confirmed by the mice model. In this study, we compared the intranasal and intraperitoneal routes of immunizations with the DM104 vaccine strain in mice to understand the better route of administration of the DM104 vaccine and its immunogenicity and protective efficacy in mouse model. Our results indicate that the immune response elicited by vaccine candidate strain DM104 is strongly dependent on the immunization route, with the intranasal route being more effective than the intraperitoneal route following intraperitoneal challenge. Intranasal immunization yielded 80% protective efficacy in immunized mice whereas, intraperitoneal immunization could not provide any protection. Protection generated by intranasal immunization was accompanied by high titre of anti-WCL IgG and IgA in DM104 immunized sera compared to sera collected from mice of control group. In a potency assessment with immunized mice via the intraperitoneal route, the live cells of DM104 induced high antibody titers to the pathogen. The IgG titers were quite high following two intraperitoneal injections, although none of these animals were protected against mortality in the intraperitoneal challenge model.

In previous studies[9, 10], an extensive characterization was carried out with *S. dysenteriae* type 4 vaccine strain, DM104 and it clearly showed the protective efficacy of a vaccine in the guinea pig model. High protection in guinea pigs and also in mice model done in the present study induced by the DM104 strain along with non-pathogenic properties strongly suggest that the *Shigella*-cross reacting DM104 strain could be used as a promising vaccine candidate against shigellosis.

Further, organ burden was estimated after lethal challenge with *S. dysenteriae* type 4 in the intranasally immunized and control mice. Significant decrease in the bacterial load was observed in the liver and kidney of immunized mice as compared to the control. The immune response developed by the vaccinated mice with DM104 is featured by strong mucosal and humoral immunity.

The emergence of multidrug resistant *Shigella* strains underscores the need for a safe and effective vaccine that is encouraged and supported by the Global Program for Vaccines and Immunization (GPV). The World Health Organization (WHO) defined the

objective and new strategies for accelerating *Shigella* vaccine development[15]. Our approach was shown to be a promising one for the development of a safe and effective vaccine against *S. dysenteriae* type 4, the epidemic strain. Sanitation and improved hygiene and quality of water would help to control the disease, but progress is slow in the poor communities where the disease is most prevalent. Therefore, the only hope to control *S. dysenteriae* infection is to develop an effective vaccine[5, 16]. Therefore, this vaccine approach using *Escherichia albertii* strain DM104 against shigellosis caused by *S. dysenteriae* type 4 might have considerable practical value.

References

1. Rossi O, Molesti E, Saul A, Giannelli C, Micoli F and Necchi F. 2020. Intra-laboratory evaluation of luminescence based high-throughput serum bactericidal assay (L-SBA) to determine bactericidal activity of human sera against *Shigella*. *High-Throughput*. **9**(2), 14.
2. GBD 2017 Causes of Death Collaborators. 2018. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet*. **392**: 1736–1788.
3. Chakraborty S, Harro C, DeNearing B, Bream J, Bauers N, Dally L, Flores J, Van de Verg L, Sack DA and Walker R. 2016. Evaluation of the safety, tolerability, and mmunogenicity of an oral, inactivated whole-cell *Shigella flexneri* 2a vaccine in healthy adult subjects. *Clin Vaccine Immunol*. **23**(4): 315-325.
4. Taneja N and Mewara A. 2016. Shigellosis: epidemiology in India. *Indian J Med Res*. **143**: 565-576.
5. Kotloff KL, Riddle MS, Platts-Mills JA, Pavlinac P and Zaidi AKM. 2018. Shigellosis. *Lancet*. **391**: 801-812.
6. Rahman MZ, Sultana M, Khan SI and Birkeland NK. 2007. Serological cross-reactivity of environmental isolates of *Enterobacter*, *Escherichia*, *Stenotrophomonas*, and *Aerococcus* with *Shigella* spp.-specific antisera. *Curr Microbiol*. **54**: 63–67.
7. Azmuda N, Rahman MZ, Sultana M, Jenssen EL, Khan SI and Birkeland NK. 2012. Evidence of interspecies O antigen gene cluster transfer between *Shigella boydii* 15 and *Escherichia fergusonii*. *APMIS*. **120**: 959–966.
8. Rahman MZ, Akter S, Azmuda N, Sultana M, Weill FX, Khan SI, Grimont PA and Birkeland NK. 2013. Serological cross-reaction between O-antigens of *Shigella dysenteriae* type 4 and an environmental *Escherichia albertii* isolate. *Curr Microbiol*. **67**(5): 590–595.
9. Chowdhury FM, Rahman MZ, Khan SI, Ahsan CR and Birkeland NK. 2014. An environmental *Escherichia albertii* strain, DM104, induces protective immunity to *Shigella dysenteriae* in guinea pig eye model. *Curr Microbiol*. **68**: 642–647.
10. Chowdhury FM, Rahman MZ, Sarkar MMH, Rabbi F, Khan SI, Ahsan CR and Birkeland NK 2017. Protection against shigellosis caused by *Shigella dysenteriae* serotype 4 in guinea pigs using *Escherichia albertii* DM104 as a live vaccine candidate strain. *Acta Microbiol Immunol Hung*. **9**: 1-14.
11. Swain P, Nayak SK, Sahu A, Meher PK and Mishra BK. 2003. High antigenic cross-reaction among the bacterial species responsible for diseases of cultured freshwater fishes and strategies to overcome it for specific serodiagnosis. *Comp Immunol Microbiol Infect Dis*. **26**, 199-211.
12. Osorio M, Bray MD and Walker RI. 2007. Vaccine potential for inactivated shigellae. *Vaccine*. **25**: 1581-1592.
13. Ranallo RT, Thakkar S, Chen Q and Venkatesan MM. 2007. Immunogenicity and characterization of WRSF2G11: a second generation live attenuated *Shigella flexneri* 2a vaccine strain. *Vaccine*. **25**: 2269-2278.
14. Oliveira AF, Ruas LP, Cardoso SA, Soares SG and Roque-Barreira MC. 2010. Vaccination of mice with salmonella expressing VapA: mucosal and systemic Th1 responses provide protection against *Rhodococcus equi* infection. *PLoS One*. **5**: e8644.
15. WHO. 2006. Future needs and directions for *Shigella* vaccines. *Wkly Epidemiol Rec*. **81**: 51-58.
16. Baker S and The HC. 2018. Recent insights into Shigella. *Curr Opin Infect Dis*. **31**: 449–454.