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Oral vaccine based on a surface immunogenic protein mixed with alum promotes a decrease in *Streptococcus agalactiae* vaginal colonization in a mouse model

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ABSTRACT

The Surface Immunogenic Protein (SIP) of Group B Streptococcus (GBS) had been described as a good target for vaccine development. To date, SIP has been reported as a highly conserved protein, and in a mouse model it induces protection against lethal GBS challenge. Also, similar effects have been described by intranasal immunization with a SIP-based vaccine. In this study, we show the immune response induced by an oral SIP-based vaccine formulated on alum in a mouse model. Our vaccine can reduce vaginal GBS colonization and induce specific SIP-antibodies with opsonophagocytosis activities against GBS. Moreover, we observed the activation of T-cells producing IFN-γ, TNF-α, IL-10, IL-2, and increased expression of the transcription factor T-bet, suggesting a Th1-type humoral response. The oral SIP-based vaccine is a novel alternative in the development of a vaccine against GBS.

1. Introduction

Streptococcus agalactiae, also known as the Group B Streptococcus (GBS) can be transmitted to the newborn at the time of vaginal delivery, or to the fetus through the ascension of the pathogen from the vaginal mucosa to the placenta or amniotic fluid (Desa and Trevenen, 1984; Patras and Nizet, 2018). Colonization of the maternal genital tract is the leading risk factor for infection and occurs in 10–40% of pregnant women (Natarajan et al., 2006; Vekemans et al., 2018). Ten GBS serotypes have been isolated based on the antigenic variation of the capsular polysaccharides (Slotved et al., 2007). The development of vaccines against GBS, for maternal immunization purposes, has been identified as a priority for the World Health Organization (WHO) Initiative for vaccine research, based on a large unmet medical need (World Health Organization, 2017). To date, there is no licensed vaccine against GBS, and two types of vaccines are in clinical phase, based on five polysaccharides conjugated to a carrier protein, and based on a chimeric protein vaccine (Heath, 2016; Lin et al., 2018).

Vaccination through a mucosal route is shown to offer advantages for enhanced mucosal immune responses that result in better local protection. Also, to obtain antibodies on mucosal surfaces, the vaccine could be delivered to the common mucosal immune system (CMIS), for example orally, nasally, or rectally (Zhu and Berzofsky, 2013). The oral route is especially desirable for vaccine administration, due to its convenience (McConnell et al., 2008). Moreover, oral immunization can generate a systemic immunity, and mucosal specifically in vaginal fluids (Lebre et al., 2016). For this purpose, an oral vaccine that generates protective immunity in the vaginal mucosa may be a good alternative in developing a vaccine against GBS infection.

The Surface Immunogenic Protein (SIP) is an immunogenic and conservative antigen. The subcutaneous and intranasal immunization with recombinant SIP elicited specific antibodies that confer protection against the challenge of GBS strains of different serotypes (Brodeur et al., 2000; Martin et al., 2002; Xue et al., 2010). Moreover, subcutaneous immunization using SIP without adjuvant generates decreased vaginal GBS colonization in a murine model (Diaz-Dinamarca et al., 2018). In this study, we analyzed the immune response on the oral SIP-based vaccine mixed with alum in a mouse model. Our vaccine

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can decrease vaginal GBS colonization and induce a humoral Th1-immunity.

2. Materials and methods

2.1. Obtaining recombinant SIP

The SIP was obtained as described above (Diaz-Dinamarca et al., 2018). Briefly, the sip gene was cloned from a Chilean GBS strain serotype III available in our laboratory (GenBank Code: KU736792.1). The sip gene was amplified by PCR and cloned into *pET21*a plasmid DNA to obtain the *pET21a::sip*. The cloned sip gene was analyzed by DNA sequencing and submitted to GenBank (Code: KX363665.1). The *Escherichia coli* strain BL21-CodonPlus (DE3) was used to express the rSIP. Purification of the recombinant SIP was performed by the Ni-NTA affinity column (Ni-NTA; Invitrogen Purification System), following the manufacturer's instructions. Characterization and the level of recombinant SIP purification were previously described (Diaz-Dinamarca et al., 2018). The screening for expression of rSIP was performed by SDS-PAGE and Western blot using a polyclonal antibody against GBS. The purified protein from Ni–NTA resin was analyzed on HPLC, Smarline UV detector 2520 (Knauer, WissenschaftlicheGeräte GmbH, Germany). The system consisted of GPC/SEC column (5 µm, 300 mm × 8 mm, 100 Å), a mobile phase composed of phosphate buffer (34 mM) and sodium chloride (0.5 M) at pH = 6.6. The flow rate was 0.8 ml/min, and detection was performed at 210 nm using a UV detector at ambient conditions".

Protein standards (PSS Polymer Standards Service GmbH) were used for the construction of the calibration curve.

2.2. Mice immunization and GBS challenge

Six-to-eight-week-old female C57BL/6 mice (obtained from the Instituto de Salud Pública de Chile) were acclimated and randomly distributed into experimental groups. The animals were kept under standard pathogen-free conditions and provided with free access to food and water during the experiment. Mice were handled and disposed of according to the guidelines of the Institutional Ethics Committee. The immunization model consists of 24 female mice of C57BL/6 strain from 6 to 8 weeks of age, which were randomly arranged in 4 experimental groups of 6 individuals each. The groups were orally immunized with SIP alone (20 µg), alum (2 mg), SIP (20 µg) + alum (2 mg). Also, an unimmunized control group was added (Basal-control Group). Oral immunization was performed using an orogastric tube on days 1, 3, 5, 19 and 26. Before immunization, the animals were kept fasting for 5 h. On day 29, we started the GBS challenge based as described above (Diaz-Dinamarca et al., 2018) (Fig. 1). Briefly, from days 29–33 the animals received a daily dose of gentamicin (100 mg/kg) and on day 32 a dose of 17β-estradiol (0.1 mg). On day 33, the genitourinary tract of all animals was inoculated with 1×10^7 CFU of GBS and gelatin (10%). The model ended on day 38 when animals were anesthetized and euthanized; obtaining samples of the vaginal swab, genitourinary tract, blood, and spleen for the immunological evaluation.

2.3. Immunoglobulin detection by ELISA

The specific anti–SIP IgG and IgA were evaluated by ELISA from the serum mice ten days after GBS challenge. A 96-well plate was activated overnight with 200 ng/well of rSIP in a carbonate buffer (0.1 mM of sodium carbonate [pH 9.5]) and blocked for 1 h at room temperature (RT) with PBS-3% BSA. The mice serum was added to each well in serial dilutions from 1:100 to 1:1600 and then incubated to RT for one hour. After several washes, the specific anti-SIP Igs were detected with Goat anti-mouse horseradish-linked IgG and IgA. The specific IgG and IgA were measured in serum of immunized and unimmunized mice.

The Igs isotypes in serum were measured with the mouse Ig isotyping ELISA kit (BD Biosciences Pharmingen) on day 33, following the manufacturer's instructions. Briefly, a 96-well plate was activated overnight at 4 °C with 1:5 anti-mouse capture Ig isotype in PBS buffer. The wells were blocked for 1 h at RT with PBS-3%. Serial dilutions of serum were added to the wells and incubated for 1 h to RT. For detection of Ig, HRP-linked goat anti-mouse IgGs were added to each well and incubated for 1 h to RT. The color was formed by adding a substrate in the kit, and the OD was determined at 405 nm.

2.4. Opsonophagocytosis assay

Hemolytic GBS strain (Genbank Code: KU736792) was grown in sealed tubes containing Todd-Hewitt broth. GBS was washed with PBS-1X, resuspended in modified Eagles medium and used in the assay. Promyelocytic HL-60 cells (ATCC, CCL-240) were grown and differentiated, as described above (Guttormsen et al., 2008; Millius and Weiner, 2010). The opsonophagocytosis assay (OPA) was conducted on serum from immunized mice as described by (Guttormsen et al., 2008). Briefly, the reaction was performed in a 96-well plate (Nunc), in HBSS (Hank's Balanced Salt Solution, Gibco). For each reaction mixture, heat inactivated (HI; 56 °C for 30 min) test serum, GBS bacteria, differentiated HL-60 cells, and 10% baby rabbit complement (Cedarlane) were added. Control reactions lacked complement and/or antibody, effector cells and all components except GBS. The effector cell to GBS cell ratio was 90:1. Reaction mixtures were incubated at 37 °C for 1 h with shaking. Aliquots were removed before and after incubation and placed on blood agar plates. Wells were incubated overnight at 37 °C and 5% CO_2. Percentage killing was assessed as described by Romero-Saavedra et al., 2015, by comparing the colony counts at 60 min (t60) which did not contain PMNs (PMNneg) to colony counts of a tube including all four components of the assay.

2.5. Mouse vaginal tract immune globulin measure

Vaginal tissue was processed according to the PERFEXT method (Johansson et al., 1998; Villavedra et al., 1997). Before extracting serum from each mouse, we proceeded to extract the genitouterine tract from the mice that were orally immunized with the SIP protein plus

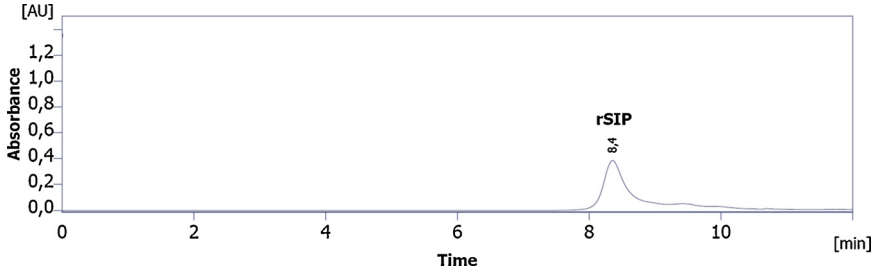

Fig. 1. Characterization of the purity of recombinant SIP. Size exclusion chromatography separation was used to determine the level of purity of the recombinant protein purified by Ni-NTA column. Data were collected digitally with Clarity chromatography software.