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Cellular immune response induced by surface immunogenic protein with AbISCO-100 adjuvant vaccination decreases group B *Streptococcus* vaginal colonization

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ABSTRACT

Group B Streptococcus (GBS) represents one of the most common causes of bacterial infection in neonates; it is also associated with premature childbirth and stillbirth. A vaccine against GBS is needed, but no approved vaccines are yet available. The Surface Immunogenic Protein (SIP) of GBS is conserved in all serotypes and had been reported to be a good vaccine prototype in a mouse model of GBS infection. Also, we have previously shown that both subcutaneous and oral immunization with rSIP can induce an efficient immune response that decreases GBS vaginal colonization in mice. In this study, we show that a vaccine based on a mixture of rSIP and AbISCO-100 adjuvant reduces GBS vaginal colonization in mice and induces antibodies with opsonophagocytic activities. Moreover, the passive transfer of sera and total T-cells from mice immunized with rSIP mixed with AbISCO-100 to unvaccinated mice decreases vaginal GBS colonization in an infected mouse. This is the first report of cellular immunity associated with rSIP-based vaccine testing in a mouse model of GBS infection.

1. Introduction

Streptococcus agalactiae (Group B *Streptococcus*, GBS) is an opportunistic pathogen capable of colonizing up to 50% of the gastrointestinal and genitourinary tracts in healthy adults (Ippolito et al., 2010; Teatero et al., 2017). GBS is the main cause for neonatal infections causing diseases such as pneumonia, septicemia, and meningitis in newborns (Vornhagen et al., 2017; Russell et al., 2017). High-income countries have experienced significant reductions in the incidence of early-onset disease in women with GBS detection between the 35th and 37th weeks of pregnancy. However, this intrapartum prophylaxis has not been shown to be effective in the prevention of LOD, stillbirth, early delivery, and antibiotics resistance are being reported (Teatero et al., 2017). Therefore, there is a long-standing interest in the development of a GBS vaccine for women (Vekemans et al., 2018).

The development of a GBS vaccine has mainly focused on the study of the ability to produce functional antibodies with opsonizing capacity, while the effect on T-cells response by a prototype vaccine against GBS has not been studied (Sadarangani, 2018). To date, T-cells are seen as essential to the induction of high-affinity antibodies and immune memory, directly contributing to the protection conferred by current vaccines´ targets with predominant intracellular localization (Muruganandah et al., 2018). In this context, hyper-virulent GBS exhibits intracellular persistence, and antibiotic evasion (Gendrin et al., 2017).

Structurally conserved protein antigens from GBS can induce a strong immune response, emerging as an attractive and cost-effective vaccine candidate. Particularly, the surface immunogenic protein (SIP) has a molecular weight of 53 kDa and is considered a great target because of its high immunogenic capacity, and conserved sequence among GBS serotypes (Brodeur et al., 2000; Martin et al., 2002; Xue et al., 2010). Previously, we showed that subcutaneous immunization using rSIP without adjuvant induces high antibody levels and a diminished GBS vaginal colonization (Diaz-Dinamarca et al.,

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2018a,b,c,d). We also showed that oral rSIP vaccination decreases GBS vaginal colonization, and antibodies with opsonizing activities were observed (Diaz-Dinamarca et al., 2018a,b,c,d).

The protein-based vaccine requires adjuvants, and the ISCOMATRIX has been linked to innate and adaptive immunity, creating a favorable milieu, mainly Th1 type immune responses (Bengtsson et al., 2011; Duewell et al., 2011). Several vaccine prototypes using AbISCO (similar to ISCOMATRIX) were tested in an animal model (Martinez et al., 2015; Vasquez et al., 2015; Diaz-Dinamarca et al., 2018a,b,c,d) and recently a veterinary vaccine using that type of adjuvant was licensed (Dilai et al., 2018).

In this study, using a mouse model, we show that vaccination with SIP mixed with AbISCO (a saponin-based adjuvant) by a subcutaneous route, induces antibodies with opsonizing activities, and promotes a reduction in GBS vaginal colonization. Moreover, we observed the relationship between cellular immunity induced by this vaccination and the effects on GBS vaginal colonization.

2. Materials and methods

2.1. Recombinant SIP purification and vaccine formulation

The rSIP was expressed and purified following the experimental procedures previously developed in our laboratory (Diaz-Dinamarca et al., 2018a,b,c,d). Briefly, the rSIP was expressed as a soluble protein and purified using low-pressure chromatography, using the His-Tag resin (six histidine residues). Additional purification was carried out by HPLC through molecular exclusion resin. The rSIP was quantified using Bradford reagent, and the identity was analyzed by the western blot technique using specific polyclonal antibodies available in our laboratory. The vaccine was formulated by mixing 20 µg of rSIP with either 12 ng of AbISCO-100 (ISCONOVA) or 2 mg of Aluminum hydroxide (Alum, SIGMA).

2.2. Mouse immunization and GBS colonization

Six- to eight-week-old female mice C57BL6/J (n = 6) from Instituto de Salud Pública de Chile were subcutaneously immunized in the right dorsal flank with different vaccine formulations. The immunization model consisted of 42 individuals, which were randomly arranged in 6 experimental groups of 6 mice each. The groups were immunized with 20 µg of rSIP mixed with 12 ng of AbISCO-100; 20 µg of rSIP mixed with 2 mg of Alum; 20 µg of rSIP without adjuvant; 12 ng of AbISCO and 100 µL PBS. Also, an unimmunized and unchallenged control group was used (Table 1). The subcutaneous immunization was performed on days 1, 14 and 28. The GBS-infection was performed using the GBS strain (NCBI Code: KU736792) and following the infection procedure described in our laboratory (Soto et al., 2018; Diaz-Dinamarca et al., 2018a,b,c,d). Briefly, a dose of 1×10^7 colony forming units (CFU) of GBS bacterial strain was introduced through the vaginal tract at day 33 post the first subcutaneous immunization. On day 38, the animals were euthanized (using Ketamine 10% and Xylazine 2%), and vaginal swabs were taken and plated on Todd-Hewitt agar base supplemented with 5% horse blood. The β-hemolytic colonies were selected to perform the counting of bacterial CFUs. Moreover, blood and spleen samples were taken. Weight loss was measured on a daily basis.

Table 1
Experimental groups used in immune response evaluation.

	Infection doses	*Injected volume*
Group 1	rSIP (20 µg) + AbISCO 100® (12 ng) + PBS 1X	100µL
Group 2	rSIP (20 µg) + PBS 1X	100 µL
Group 3	rSIP (20 µg) + Aluminum hydroxide + PBS 1X	100 µL
Group 4	PBS 1X	100 µL
Group 5	AbISCO 100® (12 ng) + PBS 1X	100 µL
Group 6	Unchallenged mice	/

n = 6 for each experimental group, with an average weight of 18 g per group. In Group 3, the proportion of Aluminum hydroxide added was 1:1 regarding rSIP.

All mice used in the experimental design were approved in accordance with the guidelines of the Ethics Committee on animal care at the Public Health Institute of Chile (ISPCH). Daily food and water ingestion were performed to *ad libitum* in accordance with the animal handling policies proposed by the NCh 2856/2 regulation.

2.3. Immunoglobulin detection by ELISA

IgA antibodies specific against rSIP were measured in the genitourinary tract wash samples using an indirect ELISA. Briefly, the plates were coated with 1 µg of rSIP for 14–16 h at 4 °C, blocked with 3% PBS-BSA for 1 h, and finally, washed 3 times with 0.1% PBS-Tween 20. The genitourinary tract washed samples were coated with the plate where serial dilution was performed using sterile PBS 1X from 1/50 to 1/1600. Afterward, anti-mouse IgA antibodies conjugated with alkaline phosphatase (1/1000 dilution) were employed to analyze the absorbance (ABS) in ELISA plate at 405 nm.

In order to evaluate the immunoglobulin in serum, blood samples were obtained by retro-orbital bleeding from each animal and incubated at 37 °C for 30 min. Then, the samples were centrifuged at 1466 x *g* for 15 min, and sera were collected and stored at -20 °C. The IgG against rSIP analysis was performed using similar conditions as the IgA ELISA described above (plates were coated with 1 µg of rSIP). Sera serial dilutions were made from 1/100 to 1/3200. A secondary antibody 1/1000 diluted (rat anti-mouse IgG) conjugated with alkaline phosphatase was utilized to see a positive reaction by ELISA. The IgG1 and IgG2a isotypes also were evaluated by ELISA following the protocol described in the Kit Mouse Immunoglobulin Isotyping ELISA kit (BD PharmingenTM). Briefly, serum samples were incubated for 1 h at room temperature (RT). Subsequently, anti-IgG1 and anti-IgG2a antibodies were diluted 1/5 and incubated for 1 h at RT. Finally, the ELISA plate was incubated with anti-IgG rat antibody conjugated with peroxidase with a 1/100 dilution and analyzed at 450 nm.

2.4. Cytokine analysis by flow cytometry

Spleen cells were recovered from the euthanized mice and were perfused with a sterile PBS 1x solution. Cells were centrifuged at 300 x *g* for 5 min at 4 °C. The pellets were re-suspended in an ACK solution (NH4Ac, KHCO3, EDTA), incubated for 3–5 min at RT and the volume was fitted to 15 mL with a sterile 1X PBS. The cellular suspension was centrifuged at 366 x *g*, and the cell pellet was re-suspended in a 3 mL of RPMI medium (Gibco) supplemented with 10% of Fetal Bovine Serum (FBS, SIGMA) and 1% of penicillin/streptomycin. A total of 2×10^6 cells/mL were cultured per well and stimulated with 1 µg of rSIP and incubated for 72 h in the presence of CO_2 (5%), and the culture supernatant was collected to measure the different cytokine secretion by flow cytometry using the CBA commercial kit (BD Biosciences). A standard curve was made using serial dilutions from 1/2 to 1/256. The captured beads mixture (IL-2, IL-4, and INF-γ) was combined with 25 µL in each sample (incubated for 2 h at RT) and rinsed with 1 mL of wash buffer to be centrifuged for 3 min at 200 x *g*. The supernatant was discarded, and the pellet was re-suspended with 150 µL of wash buffer solution. Samples were then analyzed using FCAP software on FACS Verse cytometer (BD). Data obtained were graphed as fold increase with respect to the uninfected group. The average of the value obtained from the uninfected group was used to make the comparison to the other experimental groups.

2.5. Opsonophagocytosis assay

Hemolytic GBS strain (Genbank Code: KU736792) was grown in sealed tubes containing Todd-Hewitt Broth. GBS was washed with PBS-

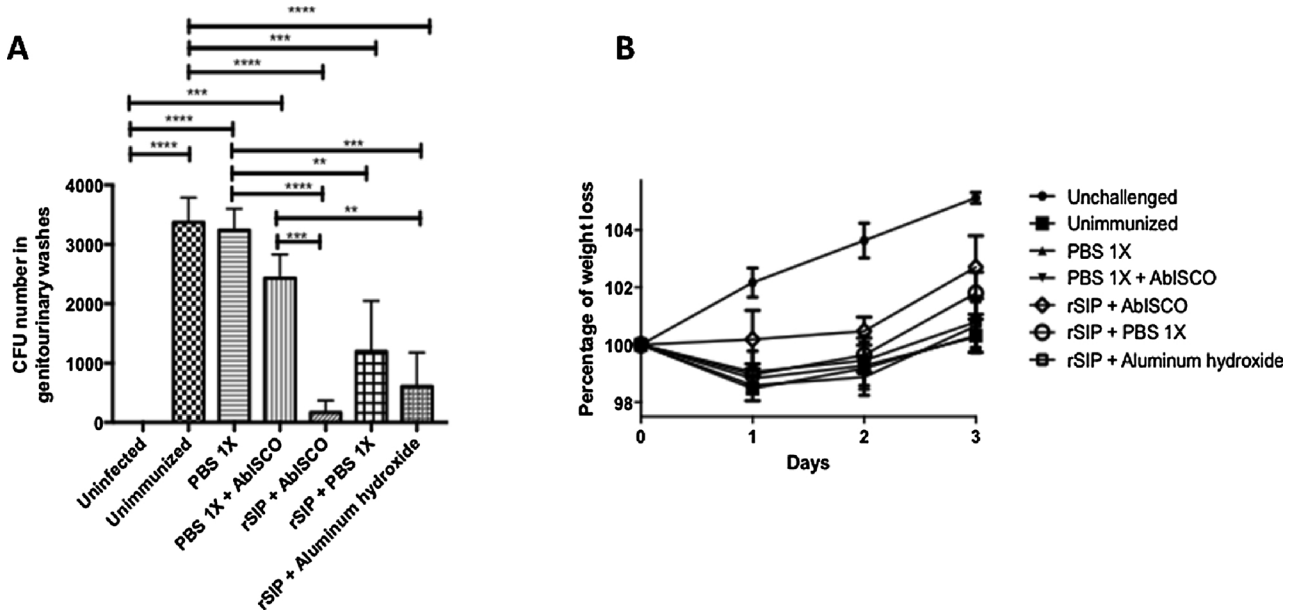

Fig. 1. Analysis of vaginal GBS load and weight loss in an animal experimental model. The number of β-hemolytic colonies was measured from genitourinary rinses at the end of experiment (A), and the weight levels percentage for immunized mice and challenged groups with GBS was evaluated over 3 days (B). Each group (n = 6) was immunized with one immunization and two booster doses. Weights were measured from the day of the challenge until the day of euthanization as stated in the Material and Methods section. Differences were evaluated by a one-way ANOVA (* = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; **** = $p \leq 0.0001$).

1X, re-suspended in modified Eagles medium and used in the assay. Promyelocytic HL-60 cells (ATCC, CCL-240) were grown and differentiated, as described above. The opsonophagocytosis assay (OPA) was conducted on serum from immunized mice as described by Guttormsen et al., 2008. Briefly, the reaction was performed in 96 well plates (Nunc), in HBSS (Hank's Balanced Salt Solution, Gibco). For each reaction mixture, heat inactivated test serum (56 °C for 30 min; HI), GBS bacteria, differentiated HL-60 cells, and 10% baby rabbit complement (Cedarlane) were added. Control reactions lacked complement and/or antibody, effector cells or all components except for GBS. The effector cell to GBS cell ratio was 90:1. Reaction mixtures were incubated at 37 °C for 1 h with agitation. Aliquots were removed before and after incubation and plated on blood agar plates. The plates were incubated overnight at 37 °C and 5% CO_2. Percentage of 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 that included all four components of the assay.

2.6. Purification of T-cells by MACS column

$CD4^+$ and $CD8^+$ T-cells were purified from perfused spleen cell cultures of immunized mice with rSIP mixed with AbISCO and untreated mice. First, cells were centrifuged at 300 x *g* for 10 min, and the pellet was re-suspended in a 1% PBS-BSA 0.2 mM EDTA buffer. Afterward, 10 µL of anti-biotin Microbead Cocktail (MACS Miltenyi Biotec) was added to 1×10^7 cells and incubated for 10 min at 4 °C in darkness. Subsequently, 30 µL of buffer and 20 µL of Anti-biotin Microbeads (MACS Miltenyi Biotec) were added to 1×10^7 cells and incubated for 15 min at 4 °C. Then, the cells were washed and centrifuged at 300 x *g* for 10 min, and the recovered pellet was re-suspended in 500 µL of the buffer. The total cells mixture was put into the MS column (MACS Miltenyi Biotec) to purify the cells by negative selection. The purified $CD4^+$ and $CD8^+$ were tested by flow cytometry to analyze the purified grade.

2.7. Adoptive transfer of T-cells and Serum into naïve mice

Six- to eight-week-old female (C57BL/6) mice were used to evaluate the passive transfer of immunity. Serum or T-cells ($CD4^+$/$CD8^+$) from immunized mice were used in the transfer to the naïve mice by the intravenous injection. The number of cells transferred was equivalent to 1×10^6 per mouse.

The passive transfer of immunity model consists of 36 individuals, which were randomly arranged into 6 experimental groups of 6 mice each. We used basal group (no treatment) and three control groups; without serum/T-cells transfer, PBS transfers and serum/T-cells transfers purified from unvaccinated mice. The other two experimental groups were a serum and a T-cells transfer from vaccinated mice. One day post passive transfer; all experimental groups, except for the first one, were infected with the GBS bacterial strain as described above. After infection, body weight was recorded daily for all groups. In addition, vaginal swabs were taken, and GBS β-hemolytic colonies were analyzed.

2.8. Statistical analysis

The results are presented as the mean – standard deviation. The statistical analysis of the data was performed using the "Student's" *t*-test or ANOVA; p-Values < 0.05 were considered statistically significant. The analyses were performed using GraphPad Prism (GraphPad Software, Inc.). All experiments were repeated at least three times.

3. Results

3.1. Vaccination with rSIP mixed with AbISCO-100 adjuvant reduces vaginal colonization in mice

In order to analyze the subcutaneous vaccination effect after GBS vaginal colonization, we followed the procedure described in the methodology. After infection, genitourinary tract swab samples were obtained from all groups of mice and used to evaluate the GBS colony number on blood agar plates. For the mice immunized with rSIP alone, or mixed with either AbISCO or Alum, we observed a significant decrease in GBS colonization as compared with the control groups (Fig. 1A). In addition, we recorded the body weight loss of mice post GBS colonization. We observed a weight loss decrease in all

experimental mice groups, but the mice vaccinated with rSIP-AbISCO or SIP alone showed a more significant recovery (Fig. 1B).

These findings suggest that the weight loss observed in the control mice post-GBS infection might be related to GBS colonization. These observations support the idea that vaccination with rSIP formulations can decrease GBS colonization.

3.2. The rSIP + AbISCO immunization induces an anti-rSIP humoral response

Humoral immune response contributes to the clearance of GBS vaginal colonization (Baker et al., 2017) and the vaccination with rSIP without adjuvant induces a decreased GBS vaginal colonization (Diaz-Dinamarca et al., 2018a,b,c,d). To evaluate whether the animals vaccinated with rSIP alone, or mixed with AbISCO or Alum, might induce a humoral immune response, we collected sera and genitourinary swab samples from all experimental groups. First, we evaluated the IgA secretion on vaginal swab samples and could identify that rSIP AbISCO–, rSIP Alum–, and rSIP–immunized mice had higher IgA levels versus the control groups, which were statistically significant. Also, the rSIP AbISCO– immunized mice presented higher levels in all serum dilution when compared to the rest of the experimental groups (Fig. 2A). A similar response was identified for serum IgG against rSIP (Fig. 2B). As expected, the three-rSIP formulations were able to promote the more important IgG levels compared to the rest of the control groups. Additionally, the rSIP AbISCO group presented the higher titers of IgG in all serum dilution following the rSIP Alum– and rSIP– immunized groups respectively. Finally, we evaluated the IgG1 and IgG2a isotypes levels (Fig. 2C-D), which are associated with a Th2 or Th1 immune response, respectively. We found a similar level of both isotypes' antibody in all of the treatments, observing significant differences between the rSIP formulation treatments as compared with the lack of rSIP protein in their formulation. These data suggest the importance of the Th1– and Th2– humoral response against GBS colonization promoted by the immunization with rSIP.

3.3. The rSIP + AbISCO vaccination promotes IFN-γ, IL-4 and IL-2 secretion against the GBS infection, with a balance skewed toward Th1

To understand the effect induced by our vaccine prototype, and having correlated our antibody secretion data with the polarization profile, we evaluated the cytokine pattern of IFN-γ, IL-4 and IL-2 on the supernatants from splenocytes stimulated with rSIP, as described in the methodology. We found a significant increase in the IFN-γ secretion, in the splenocytes culture from the rSIP + AbISCO-100 group compared to the other experimental groups. Additionally, the IFN-γ, secreted by the other experimental groups was similar, and almost 4- or 5-fold lower than the rSIP + AbISCO-100 group (Fig. 3A). A similar trend was observed when IL-4 secretion was evaluated in the splenocytes culture where the rSIP + AbISCO-100 group showed the highest response as compared to the other groups (Fig. 3B). However, this response was slightly lower than that observed when evaluating the IFN-γ secretion (Fig. 3A and B). Also, an increased IL-4 secretion in the PBS 1X-AbISCO-100, rSIP + PBS 1X and rSIP + Aluminum hydroxide groups were observed with respect to the IFN-γ secretion evaluated in the same experimental groups. Finally, IL-2 secretion was measured in order to corroborate the T cell proliferation. The results showed a similar level of IL-2 in all the groups in relation to the uninfected group, suggesting that the T-cell proliferation was equivalent between the experimental groups (Fig. 3C). The cytokine measured prompts a balance between the Th1-and Th2-like profiles. However, the Th-1-like profile could be preferably activated in response to infection with GBS.

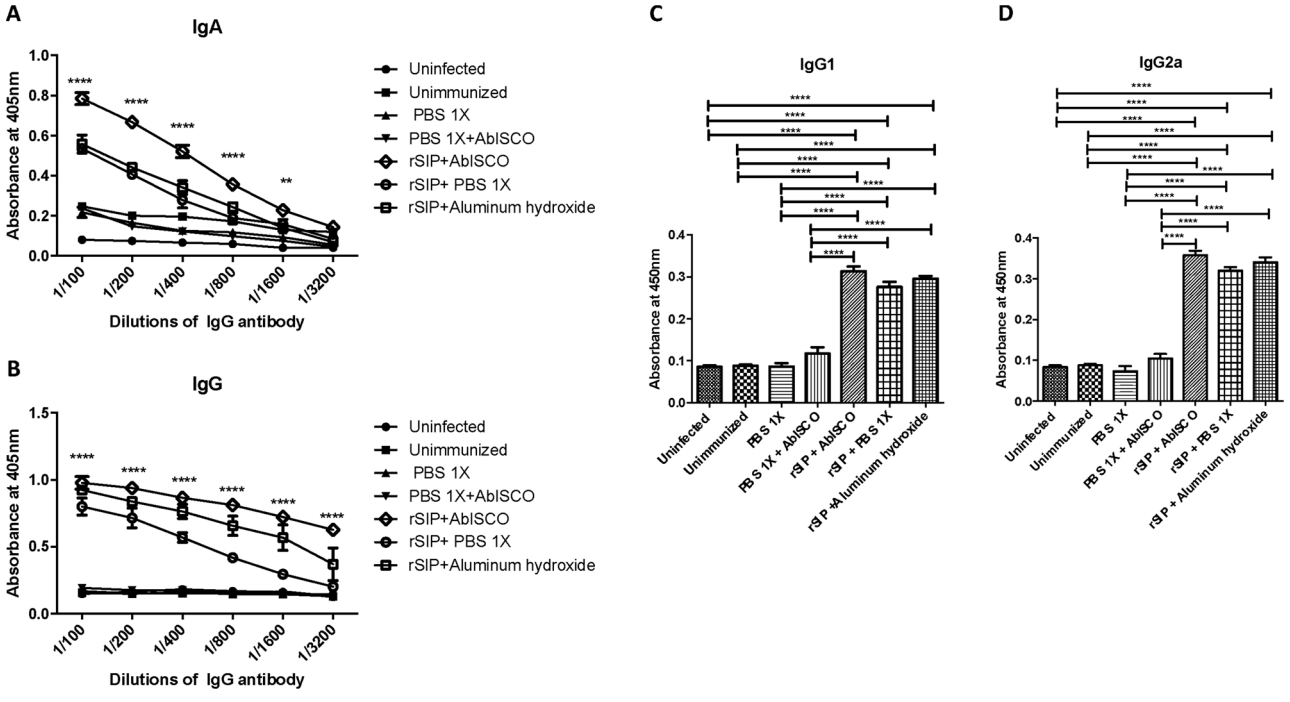

Fig. 2. Antibody levels secreted against the rSIP protein of groups immunized and challenged with GBS. Different specific antibodies types against rSIP were measured from samples of sera or genitourinary tract. The samples evaluated corresponding to samples collected to the end of the experiment. The IgA titration was evaluated from genitourinary rinses using different serial dilutions (A) while the IgG was measured from sera samples using similar serial dilution (B). IgG1 (C) and IgG2a (D) were measured from sera samples. ELISA plates were measured at 450 nm. Positive controls correspond to an antigen from IgG-Subclass measuring kit (BD Pharmingen™). Differences in Figs. 2A and 2B were evaluated by a two-way ANOVA (* = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; **** = $p \leq 0.0001$). Statistics reported corresponding to the comparison between unimmunized and rSIP + AbISCO-100 groups. Differences in Figs. 2C and 2D were evaluated by a one-way ANOVA (* = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; **** = $p \leq 0.0001$).

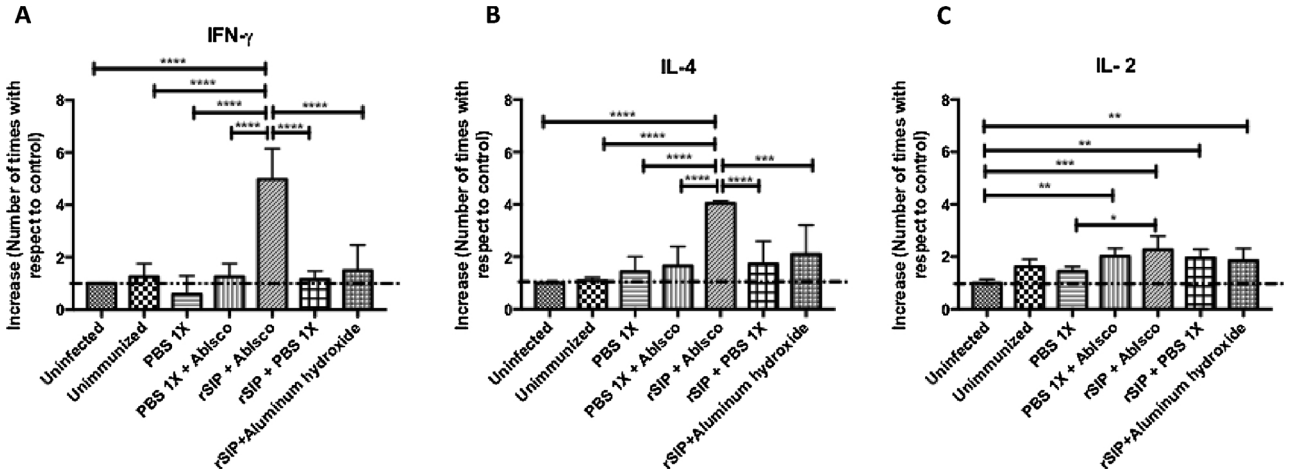

Fig. 3. Cytokine profile evaluation from immunized and GBS-infected mice. The cytokine levels of IFN-γ (A), IL-4 (B) and IL-2 (C) were measured using supernatant collected from splenocytes cultures stimulated with 1 μg of rSIP. The cultures were incubated for 72 h and the supernatant collected and stored at -80 °C. The negative control corresponds to a culture with the absence of a stimulus (rSIP) and unchallenged with GBS. Differences were evaluated by a one-way ANOVA (* = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; **** = $p \leq 0.0001$).

3.4. Subcutaneous immunization with rSIP + AbISCO-100 induces opsonizing antibodies

Based on the decreased GBS vaginal colonization and the higher antibody levels from mice immunized with the rSIP + AbISCO-100 formulation, we evaluated the functional capacity of the serum antibody from the rSIP + AbISCO-100 group of mice using the opsonophagocytosis assay (OPA), as described in the Material and Methods section. Fig. 4 shows that the sera from rSIP + AbISCO-100 immunized mice presented an opsonophagocytic capacity promoting the killing of GBS compared to the pre-immune control group using three different relations between GBS and the sera (1/10, 1/100, 1/1000). The maximum opsonic killing activities of the antibodies at 1:10 dilution were 71% for the anti-SIP serum. A reduction of killing was observed in a dose-dependent fashion using increasingly higher dilutions of sera from mice immunized with rSIP + AbICO (Fig. 4).

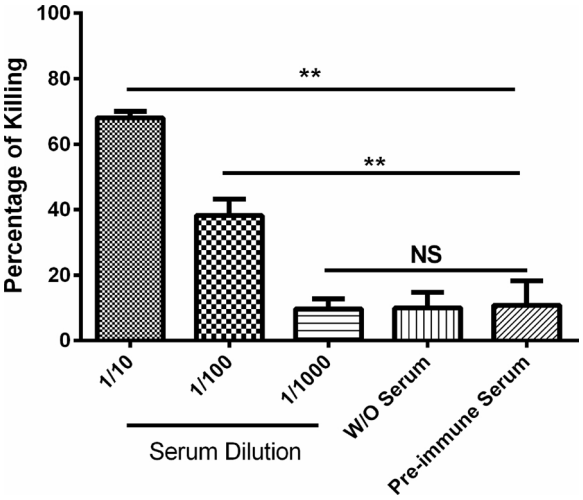

Fig. 4. Opsonophagocytosis Assay (OPA) used to test the opsonic killing of GBS from antibodies secreted by the rSIP + AbISCO-100 treatment. The bars show the median OPA of sera in the presence of rabbit complement C′, and HL-60 cells were used as an effector to GBS cell ratio of 90:1. The sera and the corresponding dilution used in the OPA are indicated as a percentage of killing. The control reactions contained dilution that lacked antibody and/or complement, or that contained normal rabbit serum resulted in GBS growth (not significant [ns] by "Student´s" *t*-test between serum dilution 1/1000 compared with pre-immune serum,** = $p \leq 0.01$ by "Student´s" *t*-test for serum dilution 1/10 and 1/100 compared with pre-immune serum).

3.5. Passive sera and T-cells transfer from rSIP + AbISCO immunized mice promote a decreased GBS vaginal colonization and prevent weight loss

Subsequently, we tested whether the transfer of immune sera and total T-cells from rSIP-AbISCO immunized mice could decrease the GBS vaginal colonization in naïve mice. For this purpose, the sera and the total T-cells mix from the rSIP-AbISCO immunized group were adoptively transferred as were described in the methodology. One-day post-transfer, the mice were infected, and weight loss was measured daily. The weight loss kinetics showed that only the T-cell transferred mice registered a significant decrease in their weight post-infection, but we observed a trend to recovery one day post-infection (Fig. 5A). Interestingly, the sera-transferred group presented a one-day post-infection weight loss, but showed a slower weight recovery versus the T-cell transferred group; however, on day 3 post-infection, weight recovery was similar to the T-cell transferred group.

When analyzing the genitourinary swabs, it was possible to find a significantly decreased number of β-hemolytic colonies from the T-cell and sera transferred mice compared to the other GBS-infected controls (Fig. 5B). We do not observe significant differences between sera and T-cell experimental groups

4. Discussion and Conclusion

GBS is the main etiological agent causing neonatal sepsis in newborns (Russell et al., 2017). Currently, two vaccines are in clinical phase testing against GBS-infections (Lin et al., 2018). In order to generate a vaccine for pregnant women that could prevent infection of the newborn through the birth canal, functional antibodies against GBS have been evaluated in clinical and preclinical studies (Sadarangani, 2018). GBS could have a virulence potential that could invade the epithelial cells from the vaginal mucosa of an asymptomatic woman (Vornhagen et al., 2017). Then, a humoral and cellular immune response stimulated by a GBS vaccine could be desired.

We used a clinical strain from a newborn with septicemia that was utilized to develop GBS vaginal colonization in a murine model. This study evaluated, for the first time, the cellular and humoral immune response induced in female mice having passively adopted transferred T-cell and serum from mice immunized with the recombinant SIP.

We have previously shown noted that SIP without adjuvant reduces GBS vaginal colonization in mice (Diaz-Dinamarca et al., 2018a,b,c,d).

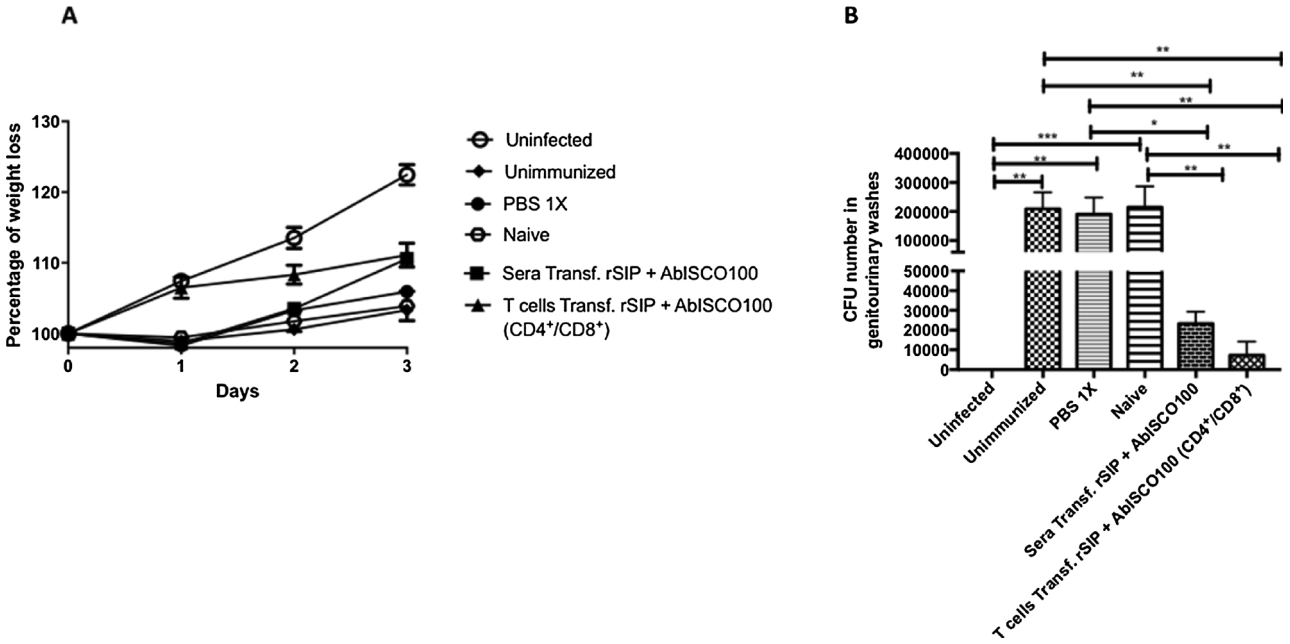

Fig. 5. Determination of disease and infection parameters in naïve mice passively transferred and GBS-infected. Initial weight level percentage was measured in passively transferred mice with sera or T-cells from rSIP-AbISCO immunized mice. One day post-transfer the mice were infected with GBS, and the weight loss was measured over 3 days (A). In addition, the number of β-hemolytic colonies was measured from genitourinary rinses at the end of experiment (B). Differences were evaluated by a one-way ANOVA (* = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; **** = $p \leq 0.0001$).

Moreover, antibodies against SIP have been characterized as highly immunogenic against GBS (Brodeur et al., 2000).

Our results demonstrated that the humoral immune response induced by the rSIP-AbISCO immunization showed a significant increase in the IgA level compared to rSIP-Alum and rSIP alone, but these showed a significant increase in relation to control groups (Fig. 2A). Also, we observed an increase in IgG, IgG1, and IgG2a immunoglobulin levels with respect to the control groups, but no significant difference was observed between these experimental groups (Fig. 2B–D).

Moreover, vaginal GBS infection could generate a decrease in body weight, associated with a parameter of a greater degree of colonization (Soto et al., 2018). Consistent with this idea, the mice immunized with SIP-AbISCO generate a larger recovery in body weight (1B). Also, we observed a reduction of the GBS bacterial load in the genitourinary tract from GBS-infected female mice, which exhibited significant differences when compared to the control groups (Fig. 1A). Finally, serum from rSIP-AbISCO mice was protective according to OPA.

To date, the OPA activities are considered a conditional factor to GBS vaccine (ref), but the reduction of vaginal GBS load may also be considered because the newborn is infected at childbirth.

On the other hand, the rSIP-AbISCO formulation generates the secretion of IL-4 and IFN-γ, and shows a high level in INF-Y (Fig. 3A–B), suggesting a tendency towards a Th1 type profile (Lefeber et al., 2003). This property of AbISCO-100 was correlated with previous studies (Pearse and Drane, 2005; Diaz-Dinamarca et al., 2018a,b,c,d)

The adoptive transfer of vaginal infection in the murine model has already been studied in another vaginal tract infection study (Soerens et al., 2016). The adoptive transfer of T-cell and sera from animals immunized with rSIP-AbISCO generated a recovery in body weight and a decrease in vaginal colonization by GBS, implying that our prototype vaccine has a cellular and humoral immune response against GBS. Further work is needed to determine how T-cells are involved in reducing vaginal colonization by GBS.

To date, the functional activity of the vaccine-induced antibody is a necessary component of any vaccine against GBS. In general, there are often situations where not all bacteria could be neutralized by antibodies (Sallusto et al., 2010). Prototype vaccines against GBS have not studied the effect on T-cell immune response. Recently, it has been shown that GBS could behave as an intracellular pathogen (Gendrin et al., 2017), where a vaccine that stimulates cellular immune response could be positive by killing the infected cells and inhibiting the survival of the pathogen inside the cell (Sallusto et al., 2010). In order to potentiate cellular and humoral immune responses, we use an AbISCO-100 adjuvant, which stimulates Th1/Th2 response (Morein et al., 2004; Wilson et al., 2012). In the case of a cellular immune response, this is favorable for the decrease of GBS vaginal colonization by the transfer of CD4 + and CD8 + T-cells from the mice vaccinated with SIP-AbISCO. Also, this vaccination generates a humoral immune response characterized by the OPA, IgA, IgG, cytokine profiles and weight loss recovery. Furthermore, the transfer of both T-cells and sera had a positive response on body weight recovery, suggesting that the cellular immune response could be desirable against GBS vaginal colonization. Therefore, given the potential of rSIP as a vaccine described in this study, this could be a promising prophylactic tool to prevent and control the GBS disease burden.

Competing interests

The authors declare that they have no financial or commercial conflicts of interest.

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