Irradiated Group B Streptococcal Vaccine provides Effective Cell-mediated Immune Response in Mice

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1. Introduction

Many vaccines used today rely on technologies developed over 100 years ago, and involve some form of attenuation or inactivation, where chemical or physical methods are used to kill virulent pathogenic strains) [1-4]. Nevertheless, the aim remains to maximize the effectiveness and quality of currently available or new vaccines, because current methods of vaccine manufacture are not cost-effective, are susceptible to chemical contamination, are difficult to match to current circulating strains, and are susceptible to other manufacturing issues. Radiation technology is of interest to vaccine manufacturers, because it can remove chemical contaminants and penetrate pathogens to damage the DNA [5-7]. However, the molecular and immunological mechanism of irradiated vaccine has not been studied well. In this study, we compared the humoral and cellular immune response by chemically and radiation inactivated vaccine to elucidate the efficacy of these vaccine.

2. Methods and Results

2.1 Irradiated Group B Streptococcal Vaccine

Cultures of GBS NCTC 10/84 were harvested at A600 = 0.5-0.8 by centrifugation. To produce irradiated killed vaccine, the pellets were lyophilized using chemical free freeze dryer -120 ℃ (Operon, Korea) and exposed to 9 kGy gamma irradiation at RT using ^{60}Co source irradiator (Point source, AECL, IR-79; MDS Ordion International Co., Ltd., Ottawa, Canada). For chemically killed vaccine, lyophilized bacteria was treated with 0.2% formaldehyde (37% in H_2O, contains 10-15% methanol as stabilzer; Sigma, MO, USA) for 2 hrs in 37 ℃ shaking incubator. The irradiated and chemical treated bacteria were then washed with sterile phosphate-buffered saline (PBS) twice and suspended in PBS (final concentration, 10^{10} CFU/ml). One hundred microliter of vaccine stocks were plated on blood agar plate to confirm the inability of the GBS replication. The GBS vaccines were stored at -80 ℃ until use. At a dose of 5kGy, we found all bacteria was completely inactivated.

2.2 GBS-specific Immuoglobulin levels

Groups of 5 or 10 male ICR mice (4-6 weeks old) were were vaccinated by three intraperitoneal inoculations, on days 0, 7 and 14, with either 1x10^6 CFU-equivalent, 1x10^7 CFU-equivalent or 1x10^8 CFU-equivalent of gamma-irradiated or chemical treated GBS NCTC 10/84 with aluminium hydroxide gel 2% (Invivogen; San Diego, USA). A group of mice inoculated with PBS and aluminium hydroxide gel was used as a negative control.

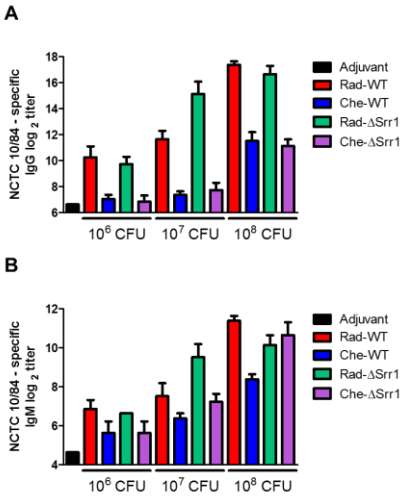

Fig. 1. Serum levels of latch specific IgG (*A*) and IgM (*B*) were analyzed at 7 days after the 2nd immunization. Bars indicate the mean of a representative experiment. Error bars indicate SD. $^{**}P < 0.001$ and $^{***}P < 0.005$ compared with adjuvant.

Levels of live GBS-specific total immunoglobulin (total Ig), IgM, IgG and selected IgG isotypes were determined by ELISA as described previously [8]. In brief, live GBS (final concentration, 1X10^6 CFU/100 μl) in PBS was immobilized in 96-well immunoplate (SPL, Korea) by overnight incubation at 4 ℃. The wells were washed three times with PBS-T (PBS with 0.05% Tween 20) and blocked with 300 μl of 1% BSA in PBS for 1 h at room temperature. The plates were then washed three times with PBS-T, and mouse serum was added over a range of serial dilution (100 μl/well). The plates were then incubated for 2 hrs at RT. Unbound antibody was removed by washing with PBS-T, and the plates were incubated with HRP-conjugated rabbit anti-mouse Ig, IgG, IgM, IgG1, IgG2a, IgG2b, IgG3 diluted 1:5000 in PBS-T for 1h at RT. Wells were washed 7 times and 100 μl of substrate solution (TMB) was added to each well. Fifty microliter of stop solution (2N H_2SO_4) was further added to the wells in order to stop the enzymatic reaction, and the absorbance (450 nm) was recorded using a microtiter plate reader

(Perkinelmer). As shown in Figure 1, all mouse groups immunized with either chemically or irradiated GBS vaccine showed significantly higher levels of GBS specific IgG and IgM in serum compared to the mice immunized with adjuvant. But, irradiated GBS vaccine elicited higher levels of both Ig than chemical vaccine.

2.3 Protective effect of irradiated GBS vaccine

Mice immunized with either chemically or radiation inactivated GBS vaccine were i.v. challenged with GBS strain NCTC 1/84 (serotype V), and their survival was monitored. 40-50% mice immunized with adjuvant or chemically inactivated GBS vaccine died by 36 hours post-GBS infection (hpi) (Figure 2). In contrast, 80% of mice immunized with radiation inactivated GBS vaccine survived through the end of the experiment.

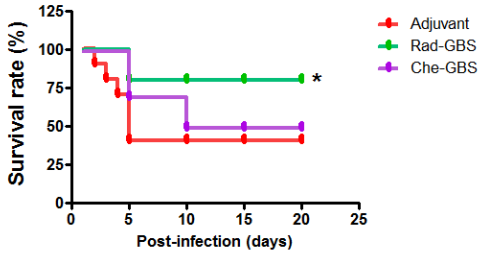

Fig. 2. Protective effects of immunization with Rad-GBS against GBS infection. At seven days after the 2nd immunization, mice were intravenously challenged with GBS NCTC10/84, and mouse survival was recorded * $p<0.05$ compared with mice immunized with adjuant alone.

2.4 Shaping Amplifier Model

Sera and spleens were collected from mice immunized with chemically or radiation inactivated vaccine as described above. Spleen cell suspensions were made by passing through a nylon cell strainer (BD; La Jolla, CA), and red blood cells were lysed with FACS™ Lysing Solution (BD). Splenocytes were washed and then resuspended in RPMI1640 (Gibco; Grand Island, NY) containing 10% fetal bovine serum. $CD4^+$ T cells were purified using $CD4^+$ T cell isolation affinity columns (Miltenyi Biotech; Bergisch Gladbach, Germany), according to manufacturer instruction. $CD4^+$ T cells (4×10^5 cells/mice) or sera (100 µl) were administered i.p. to naïve CD-1 mice. At 12 hours after serum or cell administration, mice were i.v. infected with GBS and survival was monitored for 72 hours. As shown Fig 3, 100% of mice that received unvaccinated naïve serum had died at 24 hpi, whereas adoptive transfer of radiation or chemically inactivated vaccine serum provided complete protection against GBS infection. Adoptive transfer of $CD4^+$ T cells isolated from the spleens from radiation inactivated vaccine immunized mice significantly higher survival rates following GBS infection than those receiving $CD4^+$ T cells from unimmunized mice (naïve $CD4^+$ T cells) and chemically inactivated vaccine immunized mice (Fig. 3B). Collectively, these data provide experimental evidence that radiation inactivated vaccine is highly immunogenic in mice and that immunization therewith induces high antigen-specific antibody responses and T-cell mediated effective cellular immune responses against GBS infection.

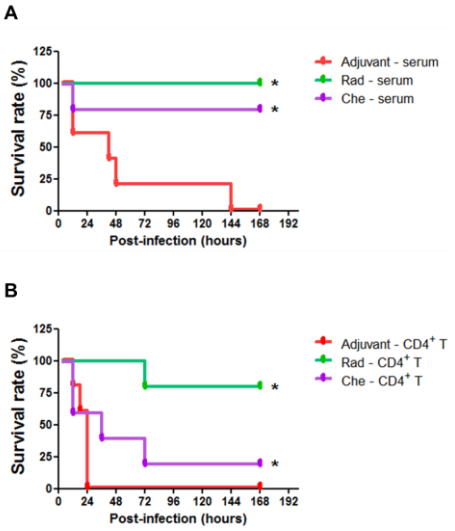

Fig. 3. Protective effect of radiation inactivated vaccine specific $CD4^+$ T cells against GBS.

3. Conclusions

Irradiated vaccine showed dramatic increase of mice cellular and humoral immune responses than conventional vaccine developing method (chemical).

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