Dear editor

We are much pleased to submit our paper to your esteemed Genes and Immunity.

This report suggests possibility of therapeutic application with *salmonella* harboring IL-12 expressing plasmid. In mouse model, *Salmonella typhimurium* with IL-12 expressing plasmid were showed induced attenuation of lethal infection and protection against wild type *salmonella* challenge. Our data imply that the *S. typhimurium* IL-12 might be a safer and even more effective therapeutic agent for rapid vaccine development. Moreover, Vaccine development using IL-12 expressing plasmid can be made at a lower cost and in a shorter period of time. This concept may be applied the vaccine development processes.

We would like you to accept our manuscript for publication in Genes and Immunity. Looking forward to receiving your response, we heartily thank you in advance for your considerations.

Sincerely yours,

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Salmonella typhimurium harboring plasmid expressing interleukin-12 induced attenuation of infection and protective immune responses

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Abstract

IL-12 is known to be an essential cytokine which appears to provide protective immunity against intracellular bacteria, such as *Salmonella*. In this study, we investigated the possibility of developing a vaccine using IL-12 against virulent *Salmonella*. We used the host defense system activated by cytokine IL-12. The highly virulent *Salmonella* strain (*Salmonella typhimurium* UK-1) was transformed with cytokine-expressing plasmids. These live, wild-type pathogens were used as vaccine strains without undergoing any other biological or genetic attenuating processes. The newly developed strains induced partial protection from infections (30-40%). Of note, the interleukin-12 transformed pathogen was safe upon immunization with low doses (10^3 CFU), induced IgG responses, and stimulated protective immune responses against *Salmonella* Typhimurium in mice (80-100%). These results suggest that IL-12 induced attenuation of wild-type *Salmonella* in the host infection stage and vaccine development using the wild-type strain harboring IL-12 secreting plasmids may be considered as an alternative process for intracellular bacterial vaccine development without the inconvenience of time-consuming attenuation processes.

Keyword : *salmonella typhimurium*; vaccine; interleukin-12; attenuation

Introduction

IL-12, a heterodimeric cytokine, is composed of two subunits, p40 and p35. This cytokine is produced mainly by monocytes/macrophages and promotes the development and activity of cytotoxic T-lymphocytes, including natural killer cells, lymphokine-activated killer cells, and macrophages. Consequently, IL-12 is now recognized as a critical cytokine for intracellular pathogens in immune responses [1]. IL-12 has also been shown to have an adjuvant activity [2]. *Salmonella* strains are intracellular pathogens inducing Th1 and Th2 immune responses [3, 4]. The immune response to *Salmonella* infection has been studied extensively in mice. A child with Il-12 deficiency may be predisposed to severe infections due to poorly virulent *Salmonella*. It is suggested that IL-12 is essential to and appears specific for protective immunity to intracellular bacteria, such as *Salmonella*.

Salmonella species are a common cause of enteric infection in humans and are associated with significant mortality all over the world. *Salmonella* strains were first used as a bioterror agent in 1984 [5]. This agent was a new, modified strain of existing bacteria. Similarly to these *Salmonella* strains, bioterror agents are modified to evade detection by existing diagnostic, as well as treatment measures [6]. In the age of the bioterror menace, bioterror agents could be modified to cause outbreaks of infectious

diseases which cannot be cured with existing treatments. The development of rapid and new treatment measures against unknown and modified bioterror agents is necessary to cope with this bioterror situation [1, 2, 3]. Existing vaccines, however, are developed by the attenuation of pathogens through the genetic modification process. In addition, vaccines were prepared from the DNA of pathogens or from killed pathogens [4, 5, 6]. These vaccine development processes were extremely time-consuming. Furthermore, existing vaccines are useless for protecting the public and the military health systems against bioterror that requires rapid, new vaccine development against pathogens used as bioweapons during wartime [7, 8]. We used a wild-type organism without the chemical and biological attenuation process. The use of the wild-type strain can shorten the vaccine development process. Instead of the attenuation of pathogens or other processes required for the preparation of vaccine, we used a means of stimulating the host defense system at infected sites to prepare the vaccine. We speculated that the host defense system could play a more crucial role in the prevention of infectious agents than the virulence of unknown infectious agents. Thus, we postulated that the infection of this lethal strain with host-stimulating cytokine might induce protective immunity.

In this study, we investigated the possibility of vaccine development using IL-12 against virulent *Salmonella*. We used the host defense system activated by cytokine IL-12. The

highly virulent *Salmonella* strain (*Salmonella typhimurium* UK-1) was transformed with cytokine-expressing plasmids. These live wild-type pathogens were used as vaccine strains without other biological and genetic attenuating processes.

To test this hypothesis, we applied this concept to the preparation of *Salmonella typhimurium* vaccine in mice. *S. typhimurium* UK-1 is highly virulent for chickens and mice.

Material and Methods

Bacterial strains and transformation of S. typhimurium. Salmonella typhimurium wild-type strain UK-1 was grown in Luria-Bertani (LB) medium. Cytokine-expressing plasmids (Bank for Cytokine Research, Korea) were transformed into *E.coli* DH5a. To form the transformed DH5a, plasmids were extracted and again transformed into *S. typhimurium* SF586. The plasmids which had formed the transformed SF586 were again transformed to *S. typhimurium* UK-1.

Bacterial challenges. Bacterial inocula of *S. typhimurium* were grown in Luria-Bertani (LB) medium to mid-log phase from single colonies. Bacteria were pelleted, re-suspended in PBS, and quantified. Oral inoculation from a disposable syringe was used for oral challenges (10^3-10^8 c.f.u. of bacteria in 100µl PBS). The mice were returned to cages with food and bedding and carefully monitored. They were killed if they became moribund. For bacterial translocation studies, the mice were treated as above, and 7 days after a single inoculation of 10^3 c.f.u, they were killed, their spleens were isolated and homogenized, and bacteria were plated in dilution on LB agar.

T cell proliferation assay. For *salmonella* antigen presentation in mice inoculated with *salmonella* harboring IL-12 secreting plasmid, CD4 T cells were obtained from spleens from *salmonella* inoculated mice by MACS systems(Miltenyi Biotec, Germany). Syngeneic unprimed T cells as APC treated with mitomycin C(0.5mg/ml) at 1×10^6 cells/ml, pulsed with *salmonella* whole cell antigens (2ug/ml). Isolated CD4 T cells APCs were mixed 1:1 and incubated for 4days. Cell proliferation data obtained with Cell titer 96 AQuous One Solution Cell Proliferation assay(Promega, Co. USA).

Cytokine and antibody ELISA. Sera were collected by eye-bleeding 2 weeks after the last inoculation. Sera were assayed for detection of IL-12 with a commercially available kit (Endogen, Boston, MA), according to the manufacturer's directions. HRP-conjugated streptoavidin and tetramethylbenzydime were used in the cytokine ELISA. The absorbance was read on a Universal Microplate reader (EL800) at 630nm. Elisa was also used to assay antibody responses to *S. typhimurium*. Polystyrene 96-well flat-bottom microtiter plates were coated with lysates of *S. typhimurium* (2□g per well). Sera obtained from the same experimental group were pooled. A 100ul volume of diluted samples was added to individual wells in duplicate and incubated for 2h at 37□. Plates were then washed and HRP-conjugated goat anti-mouse IgG and IgA which had

been diluted 1:500 in blocking buffer were used as secondary antibodies.

Results

Efficient induction of immune response with DNA plasmids orally delivered by Salmonella.

When eukaryotic expression plasmid-bearing *S. typhimurium* were fed to BALB/c mice, the transformants would cross the intestinal epithelium via M cells. In the Peyer's Patches, they would then be phagocytosed by macrophages and dendritic cells, where the bacteria would die and release their plasmids. This acted to transfect the host cells and ultimately led to antigen expression. At the same time, the phagocytic cells would be activated due to the endotoxins of the bacterial carrier and, therefore, efficiently induce cytotoxic T-cells. The cytotoxic T-cells would, in turn, lyse the phagocytes. The release of antigen would subsequently induce helper T-cells and antibody production[REF+]. Thus, all of the specific arms of the immune system should be induced. Fig. 1 shows that this was, indeed, the case. In mice that had been immunized with *Salmonella* transformants that carried expression plasmids encoding IL-12, More IL-12 induction in blood production than with wild-type *salmonella* control strain was detected.

Survival of wild-type S. typhimurium with cytokine-secreting plasmids.

To test whether such a strain would result in an immune response that would confer safety against a lethal infection with a pathogenic microorganism, we immunized mice with the recombinant *Salmonella,* as described above, and challenged them *with various doses(*10^3 - $10^{8)}$

Mice infected with *S. typhimurium* containing the interleukin-12 (IL-12) gene survived longer than mice infected with *S. typhimurium* containing other cytokine genes (data not shown). It is well known that IL-12 induces IFN-g production, which is thought to be crucial for protective immunity against intracellular microorganisms. This is supported by studies which used mice absent IL-12 or absent the IL-12 receptor[16]. These reports are in agreement with our results of the experimental infection of mice with *Salmonella* harboring IL-12 plasmids. However, the limited survival of mice infected with 10^8 CFU *S. typhimurium* harboring IL-12 plasmids had limited its therapeutic effectiveness. To determine the safe dose of our *Salmonella* strains in mice, we administered *Salmonella* orally at doses equivalent to 10^3, 10^4, 10^5, 10^6, 10^7, and 10^8 colony-forming units. All of the mice that received *Salmonella* survived longer than untreated control mice upon subsequent *Salmonella* infection. A single oral administration of a higher dose of *Salmonella* could not protect mice against the virulence of the *Salmonella*. Thus, we determined 10^3 CFU per mice to be the safety dose. Treated with this dose, nearly 100%

survival rate was obtained in mice infected orally with *S. typhimurium* strains (Fig. 2).

Analysis of antibody responses to Salmonella following immunization with S. typhimurium UK-1 with IL-12 plasmids.

To see whether bacteria containing cytokine genes could be used as vaccines, we orally immunized mice with the 10^3 CFU of *S. typhimurium* UK-1 harboring cytokine plasmids. The serum sample was collected at 2, 4 weeks after immunization and tested for antibodies to *S. typhimurium* in the ELISA. Increased levels of serum IgG were detected in mice infected with *S. typhimurium* UK-1 harboring cytokine plasmids (Fig. 3A). The specific response to the *Salmonella* antigen was also evaluated by measuring the specific antibody in the serum. The level of IgG in the serum was significantly higher in mice treated with *Salmonella* than in control mice. Additionally, an elevation of IgA anti-*Salmonella* levels was observed after the immunization in all experimental groups (Fig 3B.)

Proliferative responses to Salmonella antigen for Salmonella with IL-12 plasmids induced protection.

To evaluate the ability of *S. typhimurium UK-1* with IL-12 plasmids to activate T

lymphocytes, we cultured spleen cells of immunized animals and measured the proliferative response based on MTT-based assay. *Salmonella* with IL-12 plasmid-primed splenocytes proliferated in response to *Salmonella* lysate antigen or ConA stimulation (Fig. 4). This finding demonstrates that *Salmonella* with IL-12 plasmids is able to activate primed murine T-cells more than non-transforming *Salmonella* that is a critical arm of the immune system involved in host protection against this parasitic infection.

Adjuvant effect of IL-12 on Salmonella with IL-12 plasmid-induced protection.

To measure the protection level induced by *S. typhimurium UK-1* with IL-12 plasmid immunization associated to different adjuvant formulations, BALB/c mice were infected with a dose of 10^3 *S. typhimurium* UK-1. The protective effect was observed during the 30-day post-challenge observation period. 80-100% of mice immunized with a single dose of *S. typhimurium* UK-1 harboring IL-12 plasmids were protected against the oral challenge of wild-type *S. typhimurium* UK-1 (Fig. 5). Vaccination with the attenuated strain (BRD509) alone at the same low dose induced 25% protection compared to their respective controls. These results indicate that immunization with IL-12 plasmid-transforming *S. typhimurium* could be used as an effective vaccination.

Discussion

Cytokines are the key communication molecules in host cells to defend against the enteric *Salmonella* pathogen. Infected with *Salmonella*, intestinal epithelial cells and macrophages produce multiple chemokines and proinflammatory cytokines in culture[9]. Among these, IL-12 is involved in differentiation of naïve CD4+ lymphocyte to the Th1 subset, which produces, IFN-gamma, as well as other cytokines. Many previous studies had tested this cytokine as an adjuvant in experimental infection models in which induction of Th1 responses is known to be critical for protective immunity. In particular, IL-12 is essential for the induction of protective immunity against intracellular bacteria, such as mycobacteria and *Salmonella*[10, 11]. More recently, the clinical observation that severe *Salmonella* disease is more likely in patients with IL-12/IL-23 component deficiency than in patients with IFN-gamma component deficiency suggests that IL-12/IL-23 is a key cytokine for immunity against *Salmonella* in humans and merits both further investigation into possible IFN-gamma independent IL-12/IL-23 driven mechanisms of immunity, and dissection of the contributory role of IL-12 and IL-23. It also suggests a possible role for recombinant IL-12/IL-23 as immunotherapy for severe *Salmonella* disease.

In the present study, we transformed plasmids with the cytokine gene, but did not use

general attenuation or modification processes. The process could be applied to develop vaccines against unknown pathogens, as it requi−res neither characterization, nor genomic analysis. We used many cytokine genes for this *Salmonella* construction, selecting efficient strains harboring cytokine gene plasmids that induced protection in mice (data not shown). This wild-type pathogenic *S. typhimurium UK1* harboring Il-12 expressing plasmids induced protection upon administration with a lethal dose (Fig. 5). In the *Salmonella* harboring IL-12 plasmid vaccine model, IL-12 is involved in the protective response induced by administration and the level of protection is increased with co-administration of rIL-12.

In the present study, we showed the ability of wild-type *S. typhimurium* UK1 (20-100%) in presence of IL-12 to induce partial protection against challenge in mice.

The level of IL-12 which had increased more in blood following IL-12 administration is not the only mechanism involved in the enhanced protection observed in the *S. typhimurium* with IL-12 immunized group. T-cell proliferative responses from these individuals to *Salmonella* with plasmids totally enhanced the responses and are dependent on IFN-gamma, which suggests a Th1 pattern of immune response in the control of this parasitic disease.

Although the vaccine presented here was not protective against challenge with high

doses of pathogens, the safety problem and the effectiveness with higher doses could be overcome by lowering the dose of wild bacteria for immunization or by using plasmids expressing high concentrations of protein.

This investigation established that co-administration of IL-12expressing plasmids in the highly susceptible BALB/c mouse strain enhances survival against challenge with *S. typhimurium*. The enhanced protective effect was accompanied by an increased production of IL-12 in blood-associated susceptibility and immunity against salmonellosis.

We also observed that the *Salmonella* harboring IL-12 plasmids induced antibody responses at low dose immunization, induced mucosal and humoral immunity, and showed protection (80-100%) against salmonellosis in mice, like other live attenuated vaccines.

In conclusion, we have shown in this study that IL-12 is an important cytokine for increasing protection induced by a *Salmonella* harboring IL-12 expressing plasmid administration strategy in mice. These features would indicate that *Salmonella* harboring IL-12 plasmids are good candidates for the construction of live wild-type vaccine for *Salmonella*.

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References

1 Jouanguy E, Doffinger R, Dupuis S, Pallierc A, Altare F and Casanova JL, IL-12 and IFN-γ in host defense against mycobacteria and *Salmonella* in mice and men. *Curr. Opin. Immunol.* 11 (1999), pp. 346–351.

2 Arulanandam BP, Van Cleave VH and Metzger DW , IL-12 is a potent neonatal vaccine adjuvant. *Eur. J. Immunol.* 29 (1999), pp. 256–264.

3 Mizuno Y, Takada H, Nomura A, Jin CH, Hattori H, Ihara K, Aoki T, Eguchi K, Hara T, Th1 and Th1-inducing cytokines in *Salmonella* infection. Clin Exp Immunol. 131, (2003) 111-117

4 Mittrucker HW, Kaufmann SH, Immune response to infection with *Salmonella* typhimurium in mice. J Leukoc Biol. 67, (2000) 457-463

5 Torok TJ, Tauxe RV, Wise RP, Livengood JR, Sokolow R, Mauvais S, Birkness KA, Skeels MR, Horan JM, Foster LR, A large community outbreak of salmonellosis caused by intentional contamination of restaurant salad bars. JAMA. 278, (1997) 389-395

6 Smith SM, Palumbo PE, Edelson PI, *Salmonella* strains resistant to multiple antibiotics: therapeutic implications. Pediatr Infect Dis. 3, (1984) 455-460

7 Fidler DP, Facing the global challenges posed by biological weapons. Microbes and Infection 1, (1999) 1059-1066

8 Cohen J, Marshall E, Bioterrorism. Vaccines for Biodefense: A System in Distress SCIENCE 294, (2001) 498-501

9 Deresinski S, Coccidioides immitis as a Select Agent of bioterrorism. Appl Microbiol. 91, (2001) 602-605

10 Cerquetti MC, Gherardi MM, Orally administered attenuated *Salmonella* enteritidis reduces chicken cecal carriage of virulent *Salmonella* challenge organisms. 76. Vet Microbiol. (2000) 185-192

11 Garmory HS, Brown KA, Titball RW, *Salmonella* vaccines for use in humans: present and future perspectives. FEMS Microbiol Rev. 26, (2002) 339-53

12 Yoon WS, Park SH, Park YK, Park SC, Sin JI, Kim MJ, Comparison of responses elicited by immunization with a Legionella species common lipoprotein delivered as naked DNA or recombinant protein. DNA Cell Biol. 21, (2002) 99-107

13 Titball RW, Williamson ED, Vaccine development for potential bioterrorism agents. Curr Drug Targets Infect Disord. 3, (2003) 255-262

14 Polgreen PM, Helms C, Vaccines, biological warfare, and bioterrorism. Prim Care. 28, (2001) 807-821

15 Raupach B, Kaufmann SH, Bacterial virulence, proinflammatory cytokines and host immunity: how to choose the appropriate *Salmonella* vaccine strain? Microbes Infect. 3, (2001) 1261-1269

16 Fieschi C, Casanova JL, The role of interleukin-12 in human infectious diseases: only a faint signature. Eur J Immunol. 33, (2003) 1461-1464

17 Trinchieri G, Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nat Rev Immunol. 3, (2003) 133-146

18 Fewtrell C Ca2C oscillations in non-excitable cells. Annu Rev Physiol 1993;55:427e54.

19 Lewis RS, Cahalan MD. Potassium and calcium channels in lymphocytes. Annu Rev Immunol 1995;13:623e53.

20 Premack BA, Gardner P. Signal transduction by T-cell receptors: mobilization of Ca and regulation of Ca-dependent effector molecules. Am J Physiol; 1992;263:C1119e40.

21 Rasmussen H, Rasmussen JE. Calcium as intracellular messenger: from simplicity to complexity. Curr Top Cell Regul 1990;31: 1e109.

22 Weiss A, Imboden JB. Cell surface molecules and early events involved in human T

lymphocyte activation. Adv Immunol 1987;41:1e38.

23 Dolmetsch RE, Lewis RS, Goodnow CC, Healy JI. Differential activation of transcription factors induced by Ca2C response amplitude and duration. Nature 1997;386:855e8.

24 Sloan-Lancaster J, Allen PM. Altered peptide ligand-induced partial T cell activation: molecular mechanisms and role in T cell biology. Annu Rev Immunol 1996;14:1e27.

25 Guse AH, Roth E, Emmerich F. Intracellular Ca2C pools in Jurkat T-lymphocytes. Biochem J 1993;291:447e51.

26 Ritter M, Menon S, Zhao L, Xu S, Shelby J, Barry WH. Functional importance and caffeine sensitivity of ryanodine receptors in primary lymphocytes. Int Immunopharmacol 2001;339e47.

27 Finkel MS, Oddis CV, Jacob TD, Watkins SC, Hattler BG, Simmons RL. Negative inotropic effects of cytokines on the heart mediated by nitric oxide. Science 1992;257:387e9.

28 Ing DJ, Zang J, Dzau VJ, Webster KA, Bishopric NH. Modulation of cytokine-induced cardiac myocyte apoptosis by nitric oxide, Bak and Bcl-x. Circ Res 1999;84:21e33.

29 Krown KA, Page MT, Nguyen C, Zechner D, Gutierrez V, Comstock KL, et al. Tumor necrosis factor alpha-induced apoptosis in cardiac myocytes: involvement of the sphingolipid signalling cascade in cardiac cell death. J Clin Invest 1996;98:2854e65.

30 Pulkki KJ. Cytokines and cardiomyocyte death. Ann Med 1997;29:339e43.

31 Zhang J, Yu ZX, Hilbert SL, Yamaguchi M, Chadwick DP, Herman EH, et al. Cardiotoxicity of human recombinant interleukin-2 in rats. A morphological study. Circulation 1993;87:1340e53.

32 Stobo JD. Phytohemagglutin and concanavalin A: probes for murine ‘T’ cell

activation and differentiation. Transplant Rev 1972;11:60e86.

33 Herbst MM, Prescott J, Palmer ADM, Schountz T. Sequence and expression analysis of deer mouse interferon-g, interleukin-10, tumor necrosis factor, and lymphotoxin-a. Cytokine 2002;17:203e 13.

34 Paetkau V, Mills G, Gerhardt S, Monticone V. Proliferation of murine thymic lymphocytes in vitro is mediated by the Concanavalin-A induced release of a lymphokine (costimulator). J Immunol 1976;117:1320e4.

35 Clipstone NA, Crabtree GR. Identification of calcineurin as a key signalling enzyme in T-lymphocyte activation. Nature 1992;25: 695e7.

36 Wiederrecht G, Lam E, Hung S, Martin M, Sigal N. The mechanism of action of FK-506 and cyclosporin A. Ann N Y Acad Sci 1993;30:9e19.

37 Lewis RS. Calcium signaling in T lymphocytes. Annu Rev Immunol 2001;19:497e521.

38 Sun X, Delbridge LM, Dusting GJ. Cardiodepressant effects of interferon-gamma and endotoxin reversed by inhibition of NO synthase 2 in rat myocardium. J Mol Cell Cardiol 1998;30:989e 97.

39 Takeshima H, Ikemoto T, Nishii M, Nishiyama N, Shimuta M, Sugitani Y, et al. eneration and characterization of mutant mice lacking ryanodine receptor type 3. J Biol Chem 1996;271:19649e 52.

40 Borish LC, Steinke JW. Cytokines and chemokines. J Allergy Clin Immunol 2003;111:460e75.

41 Sei Y, Gallagher KL, Daly JW. Multiple effects of caffeine on Ca2C release and influx in human B lymphocytes. Cell Calcium 2001;29:149e60.

42 Bezprozvanny I, Bezprozvannya S, Ehrlich BE. Caffeine-induced inhibition of inositol(1,4,5)-triphosphate-gated calcium channels from cerebellum. Mol Biol Cell 1994;5:97e103. 181 M. Ritter et al. / Cytokine 30 (2005) 177e181

Figure

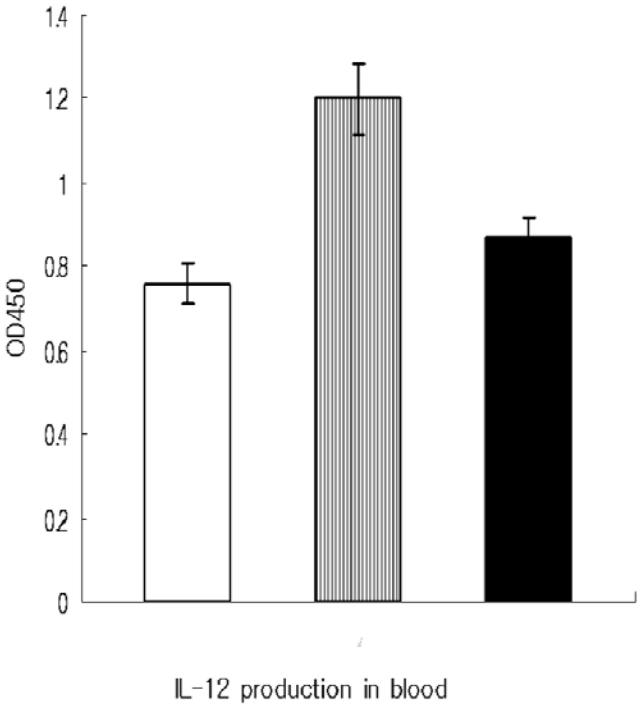

Fig 1. IL-12 expression in blood of mice inoculated with *S. typhimurium* harboring plasmid expressing interleukin-12. Mouse inoculate with a single dose of 10^3 c.f.u. *S. typhimurium* UK-1 harboring IL-12 plasmid (striped bars), non-treatment(white bars) or attenuated *S. typhimurium* UK-1 (black bars)

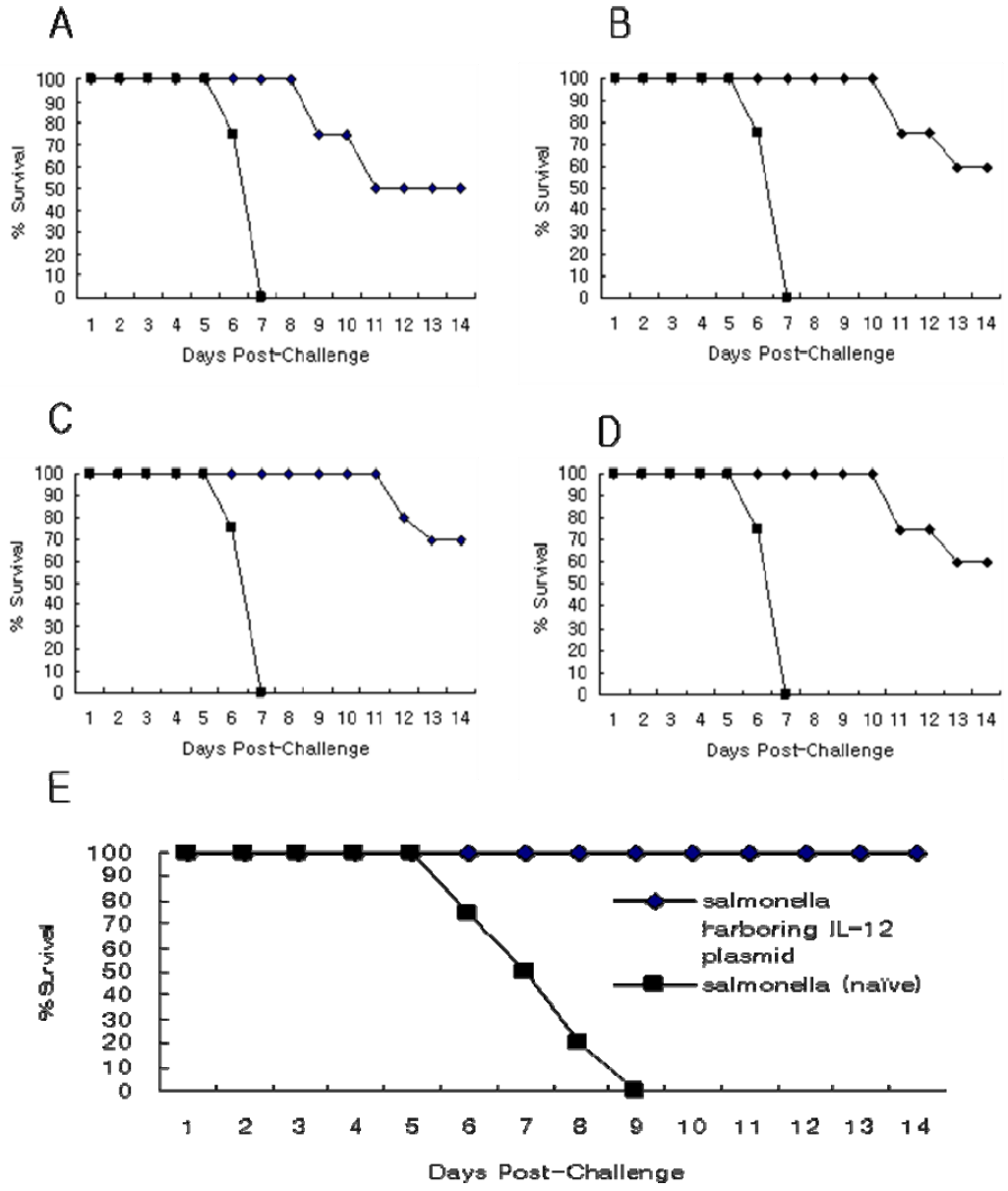

Fig 2. Challenge of mice with virulent *S. typhimurium* UK-1 with or without plasmid expressing interleukin-12. Survival curve comparing BALB/c mice after orally inoculation with A. 10^7 B. 10^6 C. 10^5 D. 10^4 E. 10^3 c.f.u. *S. typhimurium*

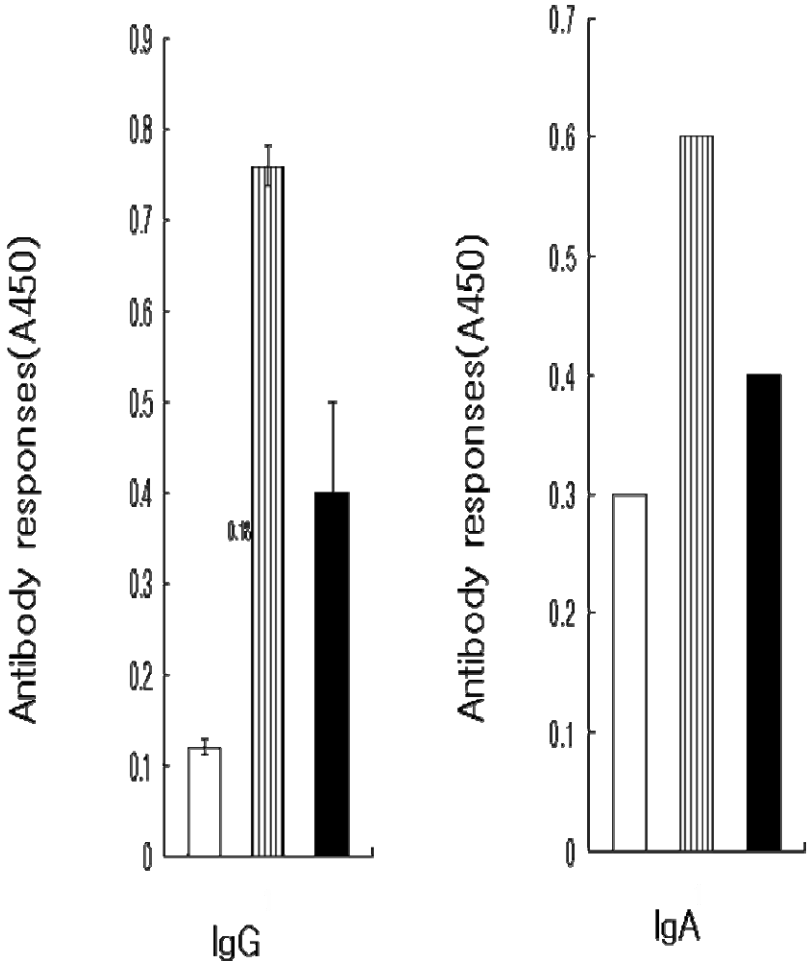

Fig. 3. antibody production in mice inoculated with *S. typhimurium* UK-1 harboring plasmid expressing interleukin-12. A, serum anti-*salmonella* IgG antibody responses in mice orally immunized with a single dose of 10^3 c.f.u. *S. typhimurium* UK-1 harboring IL-12 plasmid (striped bars), non-treatment(white bars) or attenuated *S. typhimurium* UK-1 (black bars) B, serum anti-*salmonella* IgA antibody responses in mice

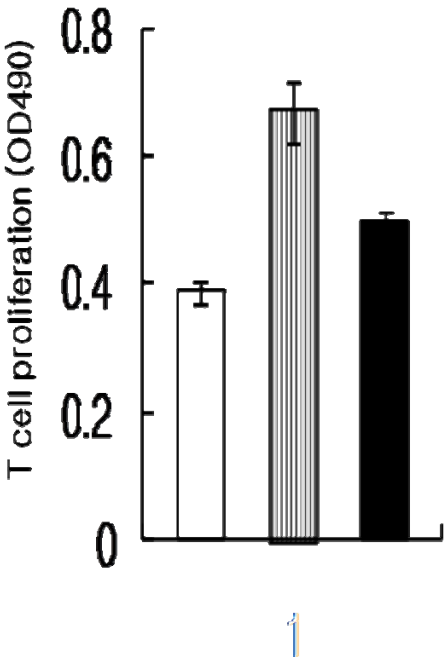

Fig 4 T cell proliferation in mouse spleen cells after immunized with *S. typhimurium* UK-1 harboring plasmid expressing interleukin-12. CD4 T cells from mouse after orally inoculation with 10^3 c.f.u. *S. typhimurium* UK-1 harboring IL-12 plasmid (striped bars), non-treatment(white bars) or attenuated *S. typhimurium* UK-1(black bars) B, serum anti-*salmonella* IgA antibody responses in mice

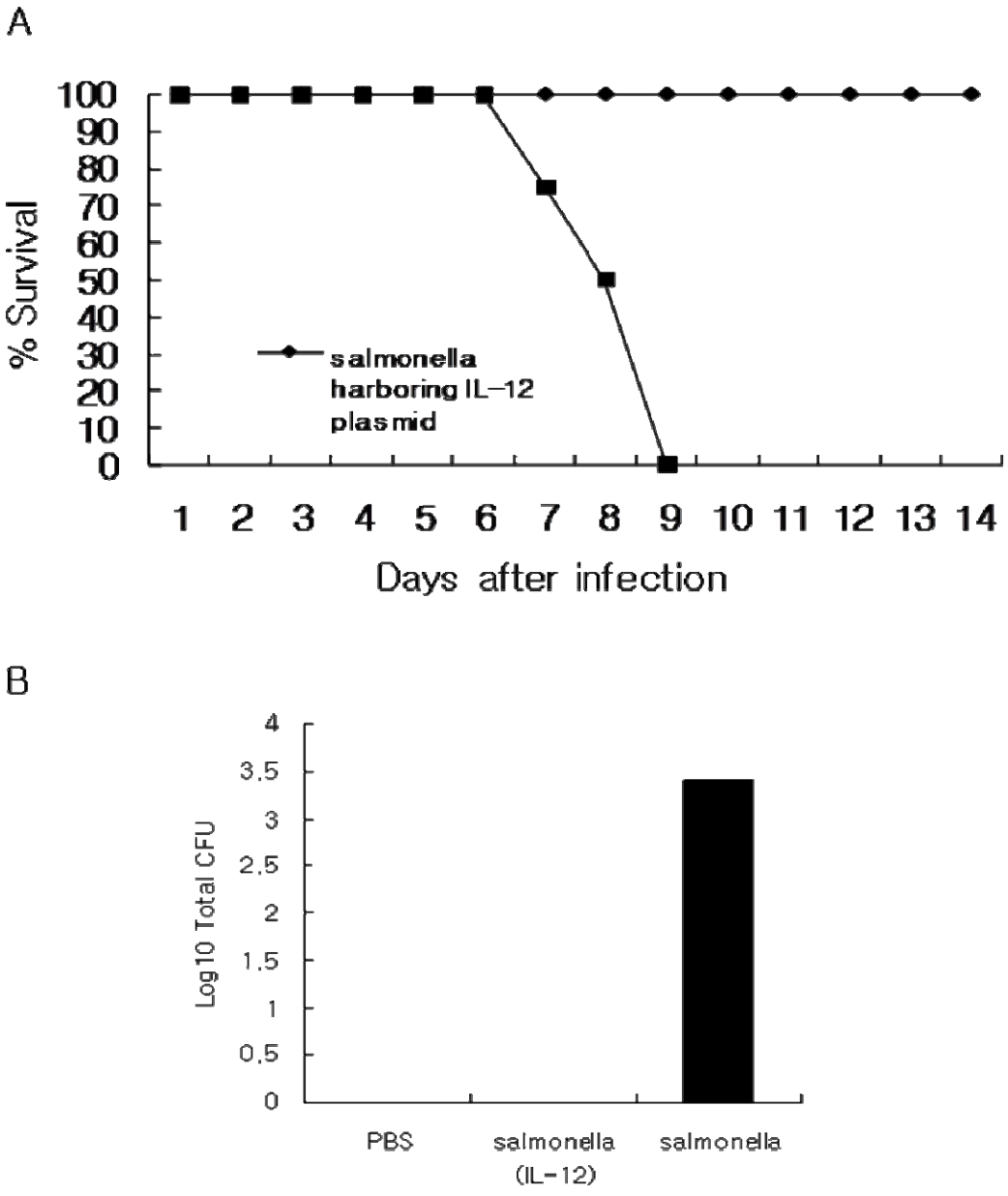

Fig 5. Protection of mice against lethal *S. typhimurium* UK-1 challenge by immunized with lethal *S. typhimurium* UK-1 harboring plasmid expressing interleukin-12. A. recovery of c.f.u of *S. typhimurium* from spleens B, survival curve after oral challenge with 10^3 c.f.u. *lethal S. typhimurium*