British Microbiology Research Journal
4(5): 570-582, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Protection against *Helicobacter pylori* Infection in BALB/c Mice by Oral or Intramuscular Administration of Multicomponent Vaccine of rCagA +LPS +CpG

D. Esmaeili[1], A. Mobarez[2*], A. H. Salmanian[3] and A. Zavaran[4]

[1]Department of Bacteriology and Applied Microbiology research center, Baqiyatallah University and Tarbiat Modares University, Iran.
[2]Department of Bacteriology, Tarbiat Modares University, Iran.
[3]National institute Genetic Engineering and Biotechnology, Pakistan.
[4]Department of Immunology, Tarbiat Modares University, Iran.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

Received 6th June 2013
Accepted 22nd November 2013
Published 7th February 2014

ABSTRACT

Aims: In this research, the protective effects of this multicomponent vaccine were investigated using the BALB/c mice model.
Place and Duration of Study: This study was performed in Laboratory of Bacteriology, University of Tarbiat Modares, Tehran, IRAN, 2011 and 2012.
Methodology: BALB/c mice were immunized with different formulations three times orally followed by two times intramuscularly (i.m.) at 10-day intervals. The protective effects of two component vaccines plus CpG Adjutants were assessed after *H. pylori* ss1 challenge in different studies. The specific IgG antibodies titers in sera were studied by using ELISA, and Antigen specific IL-4, IL-10, IL-12, TGFβ and IFN-γ responses were measured in spleen of immunized mice after challenge using ELISA test. Clearance of *H. pylori* carried out according to standard protocol.
Results: In this study the IgG1/IgG2a ratio in the mice immunized with r*CagA* and r*CagA* plus CpG was <1. Analysis of lymphocyte proliferation of mice showed that one microgram r*CagA* increases lymphocytes proliferation excellent compared to control group. CpG

**Corresponding author: Email: mmmobarez@modares.ac.ir*

oligodeoxy nucleotides are known for their ability to induce entirely Th1-biased immune responses. Immunization of mice with *H. pylori* r*cagA*+LPS+CpG induced a strong local and systemic Th1 immune response. Only mice immunized with r*CagA*+LPS+CpG and LPS+CpG secreted significantly more IFNγ than others ($P<0.05$). Protection were correlated with an increase in *H. pylori*-specific interleukin-12 and IFN-γ and both immunoglobulin G1 (IgG1) and IgG2a serum titers following challenge.

Conclusion: Because of strong Th1 response, mice were protected from infection with *H. pylori* 6-fold reduction in the number of *H. pylori* in the gastric mucosa compared to no immunized mice. This study illustrates the crucial role of the immunization route and immunogenic candidate.

Keywords: Protection; H. pylori; Vaccine; rCagA; CpG; LPS.

1. INTRODUCTION

Recent therapies for *H. pylori* enable to eliminate *Helicobacter pylori* from gastric epithelium cells. Drug drawbacks include antibiotic resistance, infection recurrence, poor acceptance, patient compliance, Drug interferences, site effects, Normal flora destruction, and high expense [1]. Vaccination would be a useful in order to therapeutic and prophylactic purposes. Different studies about immunization strategies have been carried out in the mouse model, which result in reduced *H. pylori* colonization on challenge, but no formulations quietly induced sterile immunity [2].

The studies in transgenic murine models demonstrated that IL-12 and IFNϒ are critical for protective immunity [3,4]. Mouse models have extensively been used to analyze the efficacy and mechanisms of vaccination. Numerous studies have focused on immunization routes to achieve effective immunity. Though, it is not yet clear whether proper immunization routes can protect from H. pylori infection or from H. pylori-mediated diseases in humans [5,6] may be proved for human use in future [7]. CpG oligodeoxynucleotides are known for induce Th1-biased immune responses and Multicomponent vaccines are an important vaccine type with many advantages such as good safety, definite components, high efficacy and suitability for industrialized production [8]. Previous studies by animal experiments have demonstrated that vaccination with recombinant H. pylori subunit antigens with different adjuvants could induce appropriate immune protection against H. pylori infection [9-12]. However, immune protective effects are limited due to the insufficient immunity candidate and routes by vaccination with *H. pylori* antigens. The study by Rossi et al. demonstrated that bacterial colonization was decreased [12] in beagle dogs by i.m. vaccination with three recombinant H. pylori antigens (CagA, VacA, and NAP) [12]. The study indicates that the immunity against bacteria could be more effective by using a combination of different antigens participating in different aspects of the colonization and pathogenesis of infection. Previous work employing animals has illustrated that immunization with urease B (UreB), HspA or HpaA could protect against H. pylori challenge [13]. In recombinant vaccine design, adjuvants are commonly used to enhance the immune effect through different routes of immunity [5,14-18]. Recently, mucosal adjuvants have wide application in mucosal immunity against H. pylori infection. The cholera toxin (CT) and the heat-labile Escherichia coli enterotoxin (LT) are effective mucosal adjutants in the vaccination of H. pylori inactived whole cell (HWC) or recombinant antigens (19). However, both adjuvants are too toxic for use in human vaccines; therefore, CpG have been eveloped to decrease the toxicity and induce Th1 response [1,4].

The aim of this study was immunization routes evaluation, immunogenes and adjuvant selection. Therefore, in this study, we evaluated the immune protection against *H. pylori* in BALB/c mice by intragastric (i.g.) or intramuscular (i.m.) administration of two *H. pylori* antigens (CagA and LPS) individually paired with adjuvant CpG.

In the present study, mice were vaccinated two times intragastric and three times by intramuscular with CpGs plus rCagA+LPS and the effect of this vaccination on immune responses and infection clearance was investigated.

2. MATERIALS AND METHODS

2.1 Synthetic Primers, Cloning and Construction of Recombinant Plasmids

Single primer pair was used to amplify *H. pylori* 26695 cagA gene target fragment based on GenBank. The primers had a *Bam* HI site incorporated into the 5′ end and a *Sac* I site at the 3′ end and their sequences as follows: F: 5′- *aaggatccactaacgaaaccattgacca*-3 and R: 5′-aagagctcactccctcaactctaacatt-3′ that enable amplified fragment to length of exactly 841bp. Recombinant CagA proteins were prepared in expression vector *E. coli* BL21 / pET-28a/cagA. Enzymatic digestion is done before using the plasmid to transform *E. coli* [19]. Recombinant CagA proteins were purified by column nickel.

2.2 Immunizations and Experimental Studies

Four groups of mice (*N*=10/group) were immunized three times orally and subsequently two times intramuscularly (i.m.) at 10 day intervals. Immunizations were performed with rCagA (5µg)+CpG (15µg) [Group 1], LPS (20µg)+CpG(15µg) [Group 2], rCagA(5 µg)+LPS(20µg) +CpG (15µg) [Group 3]. Antigen concentration in oral route was twofold i.m. immunizations were inoculated into the right thigh. Control animals (Group 4) received PBS using the same volume, route and schedule. Sera were collected 10 days after each immunization. Serum IgG1, IgG total and IgG2a antibodies specific to *H. pylori* antigens were measured by ELISA. 20 days after last immunization mice were challenged three times interval two days with 1×10^8 CFU *H. pylori* SS1. Antigen specific IL-4 and IFNϒ responses were measured in spleen of immunized mice after and post challenge and IL-10, IL-12, TGFβ after challenge using ELISA test.

The mice were sacrificed and used to assay naive splenocyte responses to antigen stimulation. Spleens were sterilely collected from all mice at the time sacrifice. Spleens were removed and ground through a screen mesh. RBC in splenocyte samples were lysed with "ACK (Ammonium-Chloride-Potassium) lysis buffer". Cell suspensions from all mice in each group were washed, centrifuged and solution passed through a 0.45 µm filter. All lymphocytes were then resuspended at 5×10^6 cells/ml in complete RPMI with 10% FCS [20,13].

2.3 Lymphocyte Proliferation Test

The mice were immunized five times with 10 µg of rCagA in 100µl sterile PBS with 10 days interval, three times by oral administration and twice by i.m. immunization. Six weeks after last immunization, spleens were removed and suspended in cold PBS (4ºC) under sterile condition from immunized and non-immunized mice; RBCs were lysed using NH4Cl buffer (A buffer solution is 0.24 M NH3 and 0.20 M NH4Cl). Cell suspension was prepared in complete RPMI 1640 (Gibco) and adjusted to 2.5×10^6 cells per milliliters. 100µl of cell suspension was added to each well of flat bottom 96 well plates and rCagA at concentration of 1µg/ml was

added to each one and as negative controls some wells were not added antigen. Volume of all wells adjusted to 200 µl and as positive we used PHA at final concentration of 5µg/ml. After incubation for 72 h at 37ºC in 5% CO2 humid incubator, cell proliferation was measured by using 3[4, 5-dimethylthiazol-2-ll]-2, 5-diphenyltetrazolium bromide; thiazolyl-blue (MTT) dye assay. Briefly, 20 µl MTT was added to each well and plates were further incubated at 37ºC for 4 h. Following incubation, the plates were centrifuged at 300 *g* for 10 min and then supernatant was aspirated carefully and formazan crystals were solubilized by adding 100 µl dimethyl sulfoxide into each well. The absorbance of each well was then determined at a wavelength of 540nm. Stimulation Index (SI) was calculated according to formula: SI=OD of the wells stimulated with antigen / OD of the wells containing only the cells without antigen stimulation [13,20].

2.4 Study of *H. pylori* Infection in Gastric Tissue

One to three (1/3) of antral tissue in each mice were taken and were analyzed for a quantitative of *H. pylori* infection .One tissue strip was placed in 0.5 ml of rapid urease solution. Mice gastric were placed in 200 µl of Brucella broth with 10% FCS, weighed and homogenized using a sterile ground –glass pounder. The serial dilutions of 0.1, 0.01, 0.001 and 0.0001 from gastric tissue cultured on Brucella agar(Nutrient medium) with 10% FCS in a 10% CO2 incubator for 5-7 days. *H. pylori* detection carried on gram staining, Urease rapid test, catalase (H2O2 3%) and oxidase disc. The scale of CFU per gram of stomach was counted.

3. RESULTS AND DISCUSSION

H. pylori specific antibody responses and Th1/Th2 cytokine pattern in serum immunized mice Serum IgG1, IgG2a and total IgG antibodies specific to *H. pylori* rCagA and LPS were measured by ELISA. The IgG2a/IgG1 ratio in the mice immunized group1, 2, 3 were >1 that indicate Th1 type response. Total IgG in grup1 significantly was more than other (Fig. 1).

To study antigen specific Th1/Th2 profile responses IL-4 and IFN-γ secreting splenocytes were measured (Figs 2, 3). In immunized mice, group1 (rCagA + CpG), 2 (LPS +CpG) and 3 (rCagA+LPS+CpG) promoted IFN-γ scale but group2 was observe that IL-4 also increased (Figs 2, 3).

3.1 IL-12, IL-10, TGF-β Cytokine Pattern

IL-12 production was unregulated in immunized mice groups 1, 2, 3 post challenge, that indicating Th1 immune response. IL-10 amount increased in Group2 (LPS +CpG) post challenge. The responses seen in this study indicate that the presence of *H. pylori antigens* in a vaccine activates innate responses and promotes antigen specific Th1 biased adaptive responses. The results suggest that formulation contain LPS promoted a Th1 response and with acceleration of IL-10 induced Th1/Th2 balance. This ratio in group of LPS+CpG is more than others 1 that indicate LPS in formulation of vaccine induce TGFβ response and balance Th1/Th2 (Figs 4, 5, 6).

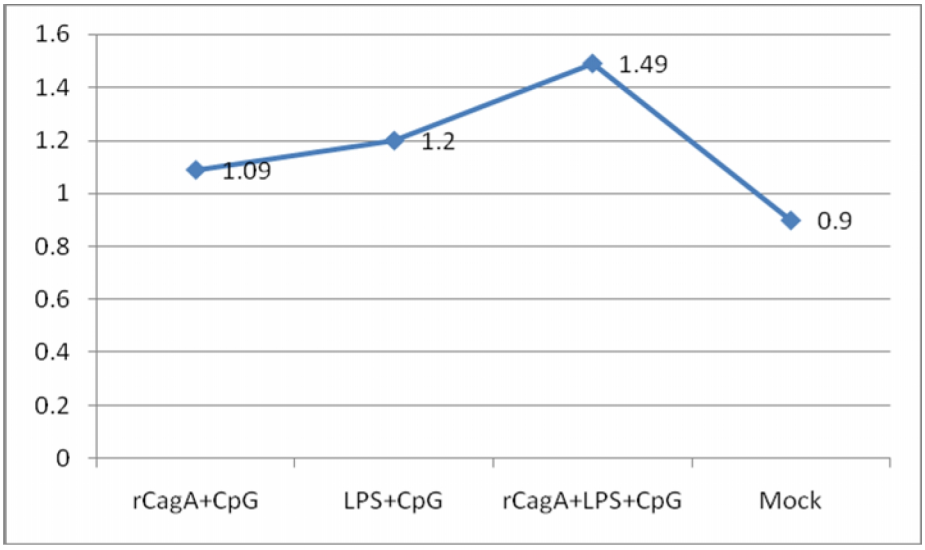

Fig. 1. The ratio of IgG2α/IgG1 in immunized groups

This ratio is more than 1 that indicates formulation of vaccine induces Th1 immune response.

Formulation of rCagA+LPS+CpG enable induces immune response more than others.

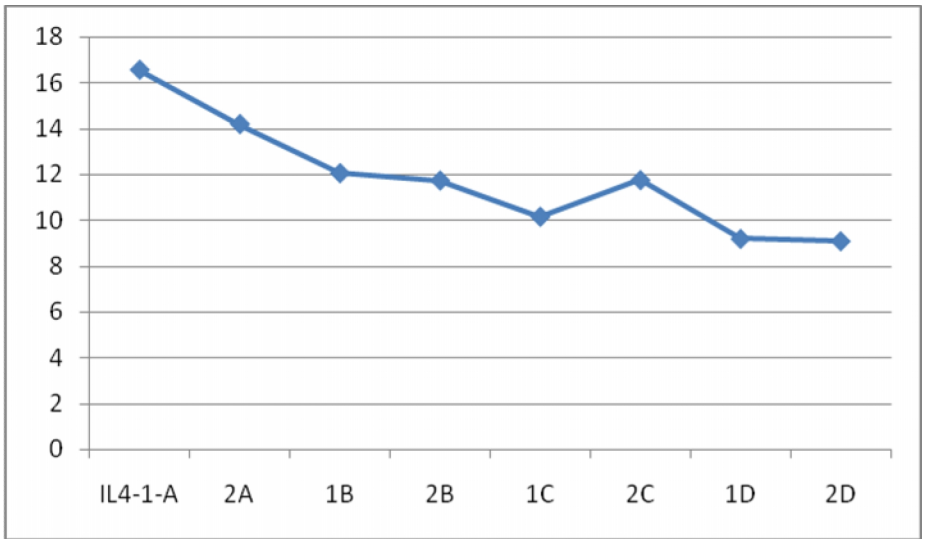

Fig. 2. The ratio of IL-4 in immunized groups after and post challenge

The results indicate that in Group rcagA+CpG(A) IL-4 titer post challenge is decrease but in group LPS+CpG (B) IL-4 titer alittle decrease also in group rCagA+LPS+CpG IL-4 titer elevated that indicate LPS enable balance reponse of Th1/Th2

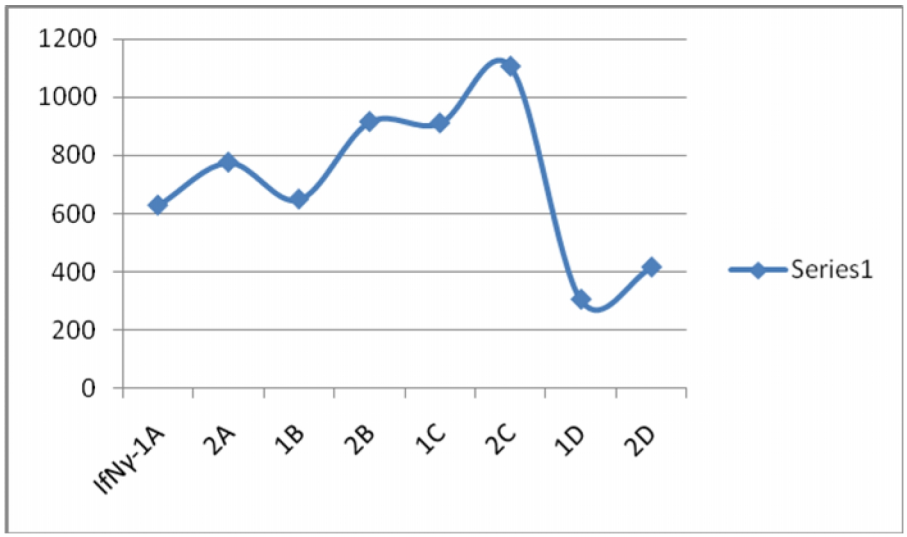

Fig. 3. The ratio IFNɣ in immunized groups pre and post challenge
The results indicate that IFNɣ titer in group rcagA+CpG(A), LPS+ CpG(B) and rCagA+LPS+CpG(C) ratio of IFNɣ titer elevated but in group rCagA+LPS+CpG other than of others this titer elevate more than other.

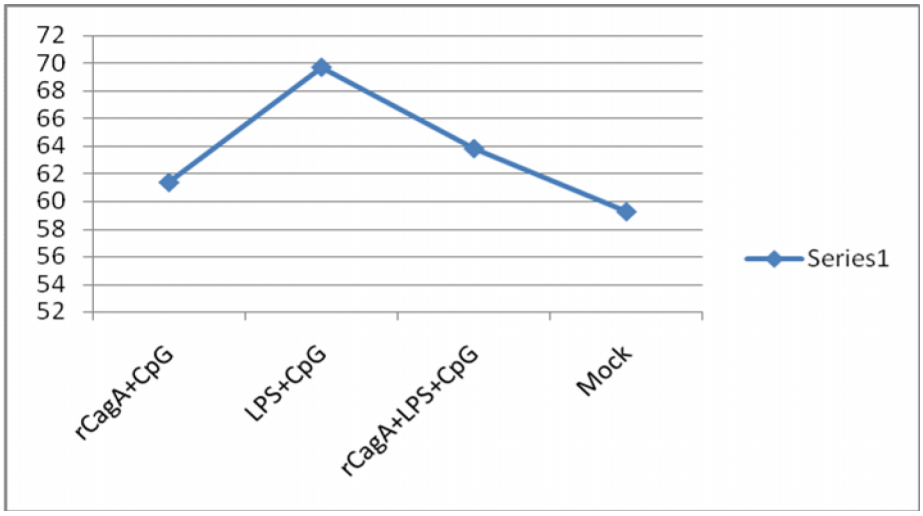

Fig. 4. The ratio of TGFβ post challenge in immunized groups
This ratio in group of LPS+CpG is more than others 1 that indicate LPS in formulation of vaccine induce TGFβ response and balance Th1/Th2.

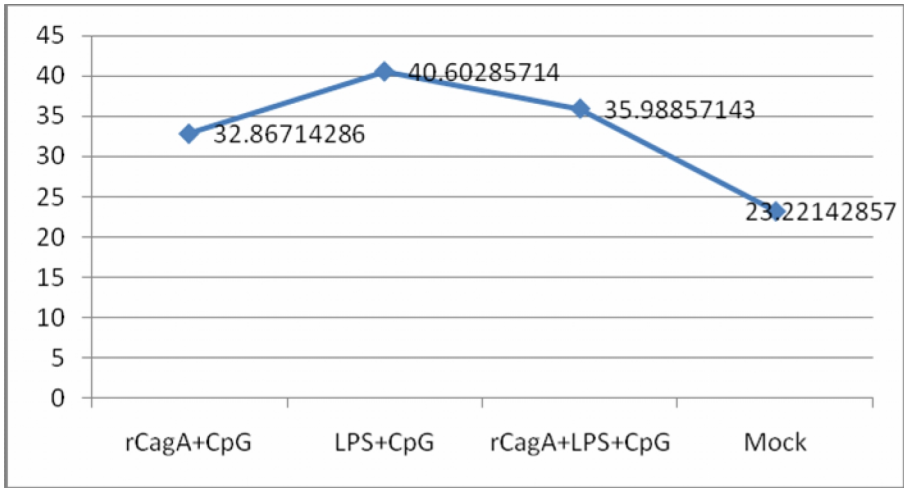

Fig. 5. The ratio of IL12 titer in immunized groups post challenge
Result indicate that in goups rCagA+CpG, LPS+CpG and rCagA+LPS+CpG IL-12 titer have elevate but in group LPS+CpG enable induce immune response more than others.

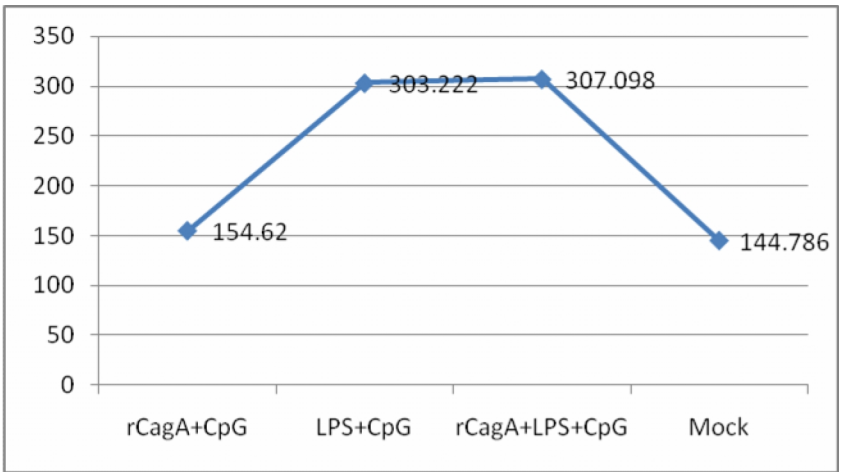

Fig. 6. The ratio of IL-10 titer in immunized groups post challenge
The results indicate that in Groups of LPS+CpG and rCagA+LPS+CpG ratio of IL-10 elevate becaue LPS enable also induce balanceTh1/Th2 by induction of IL-10

3.2 Study of Synergistic Effect rCagA and *H. pylori* O2 Serotype

In this research was shown that mixture LPS + rCagA increased antigen specific antibody and cytokine responses. In mice immunized with LPS and rCagA, the immune response elevated which indicated synergistic effect of this antigen on stimulating of immune response against *H. pylori* infection.

3.3 Vaccination reduces *H. pylori* colonization

40 days after the challenge, mice were sacrificed and gastric samples were evaluated for microbiological tests. The results obtained by urease and culture indicated that rCagA+LPS+CpG reduced bacteria colonization (Table 1, 2).

Table 1. Clearance of *H. pylori* in mice peptic post challenge

Group	A	B	C	D
Number	3000	600	100	10^7

Table 2. Evaluation of inflammation pre and post challeng

Grade of inflammation		Group
Post challenge	Pre challenge	
1	1	r*CagA*+CpG
0-1	1	LPS+CpG
0-1	1	r*CagA*+LPS+CpG
2	1	Mock (PBS)

3.4 Statistical analysis

Comparison of responses between and into groups of mice with ANOVA test and multiple groups were done with tukey test. Two-way analysis of variance and LSD, using SPSS V13 statistical software, compared the levels of proliferative responses to antigen. Probability values below 0.05 were considered statistically significant.

4. CONCLUSION

In the previous studies, many *H. pylori* recombinant antigens such as UreB, HspA, vacA, NAP, catalase, Omp and HpaA were identified as candidate protective antigens that were used to design for protection against *H. pylori*. However, the protective immunity induced by *H. pylori* vaccines usually had limited effectiveness [21-26]. It is probable that the effective immunity against bacteria could be achieved by combining different antigens participating in different aspects of the pathogenesis of the infection and immunization routes [12,27-29]. The CagA and LPS are an important virulence factors and the main protective antigen, and it has been used in vaccination trials to prevent *H. pylori* infection in mice model. In the present study, we utilized the rCagA subunit as the basic vaccine component to serotype O2 *H. pylori* LPS and analyzed their protective efficiency [28,29,1,4,5].

Moreover, the protective effects of two-antigen vaccines were greater than effects of single. Our results revealed that Systemic and mucosal immunization with appropriate antigen and adjuvant could prevent *H. pylori* infection in mice thus the site of immunization and candidate antigens are important [28,30,12]. The Use of simultaneous LPS and rCagA along with CpG have synergistic effects and promotes a Th1 immune response (IFN-Ɣ , IL-12 , IgG2a) that aid in protection and clearance of *H. pylori* infection. We illustrated that although rCagA+CpG solely promote Th2 response but also only Th1- intensing vaccine was protective. Th1 immune responses are characterized by increase in IFN- Ɣ relative to IL-4 and increase of IgG2a than to IgG1. Protection was associated with high levels of IFN- Ɣ and IgG2a. It has been previously suggested that protection against *H. pylori* is associated with the development of Th1 accompanied to IFN- Ɣ and IL-12 high levels [11,12,10,3,1].

Mice immunized with LPS+rCagA+CpG produced significantly more IFN- Ɣ, IL-12 and IgG2a than other (P<0.05). Three groups of immunized mice produced IFN- Ɣ, IL-12 and IgG2a that indicate all formulation enable induce Th1 immunity. Splenocytes from mice immunized with LPS+CpG and rCagA+LPS+CpG produced more IL-10 than mock group (P<0.05). Our results suggest that probably both Th1 and Th2 immune responses may be effective against *H. pylori* infection. Immunization and challenge studies with rCagA+LPS+CpG indicate 6log reduction in the bacterial brunt.

Previous studies by animal experiments have demonstrated that vaccination with recombinant *H. pylori* subunit antigens with different adjutants could induce appropriate immune protection against *H. pylori* infection [9,-12,31,32]. However, immune protective effects is limited due to the insufficient immunity candidate and routes by vaccination *H .pylori* antigens. The study by Rossi et al. demonstrated that bacterial colonization was decreased [12] in beagle dogs by i.m. vaccination with three recombinant *H. pylori* antigens (CagA, VacA, and NAP) [12]. The study indicates that the immunity against bacteria could be more effective by using a combination of different antigens participating in different aspects of the colonization and pathogenesis of infection. Previous work employing animals has illustrated that immunization with Urease B (UreB), HspA, or HpaA could protect against *H. pylori* challenge [13,15, 6,9].

We observed that Th1 response led to a 6-fold decrease in bacterial density. Our results show that systemic immunization with CpG and *rCagA+LPS* in a murine model of *H. pylori* gastritis is able to elicit strong local and systemic Th1/Th2 responses to *H. pylori* and enable confer protection.

This study demonstrates the ability to induce long-term immunity against *H. pylori*, provides correlates of protection and illustrates the crucial role of the immunization route. The recent studies have suggested that Th1 immunity is required for protection.

In this study 32CagA fragment of N-terminal was selected and In this study the IgG1/IgG2a ratio in the mice immunized with rCagA and rCagA plus CpG was <1 and also IFN- Ɣ and IL-12 promoted indicating a Th1 type response. These data suggest that immunization with rCagA promoted a Th1 immune response that indicating proper design for humoral, cellular responses induction. *H. pylori* CagA serves as an appropriate antigen for the sensitive and specific detection of H. pylori-specific serum immunoglobulin by ELISA. Most recombinants of CagA previously expressed were at its carbonic end and the antigenicity of its amino end has not been reported. Since the 5′-end of cagA had no usable restriction endonuclease sites and also absence of EPIYA motif and contain sequence with properties of conserved regions, Immune response induce we design this fragment with DNA star program and used PCR to amplify the 5′-end fragment of cagA.

Effective immunizations against *H. pylori* will possible affected treatment in next future. In conclusion, we recommended multicomponent vaccine contain of rCagA32 KD and serotype O2 LPS for vaccine formulation against *H. pylori* infection [30].

Because of strong Th1 response, mice were protected from infection with *H. pylori* 6-fold reduction in the number of *H. pylori* in the gastric mucosa compared to non immunized mice (Table 1). This research demonstrate that this formulation decrease inflammation grade (Table 2).

Flach et al., 2010 showed that an increase in the mRNA level of IL-12p40, tumour necrosis factor alpha (TNF-α), IFN-γ, and IL-17 was found to be strongly correlated with reduced bacterial numbers in the stomachs of mice after challenge with live *H. pylori* bacteria (33). Besides, Akhiani et al. Sayi et al. and Shi et al., 2010 showed in studies with interferon-gamma-knockout mice that this cytokine is responsible for conferring protection against *H. pylori* infection in immunized animals [4,34,35]. The formulation (rCagA+LPS+CpG) have induced a high production of cytokines IFN-γ and IL-12 (compared to the control group) which are important in controlling infection. This justifies the large difference in CFU and evaluating inflammation between the groups.

Protection against Helicobacter pylori infection following immunization is IL-12-dependent and mediated by Th1 cells. The CD4+ T cell-mediated IFN-γ response to *Helicobacter pylori* infection is essential for clearance and determines gastric cancer risk [36,37]. The CpG-oligodeoxynucleotide is a potent adjuvant of vaccine against Helicobacter pylori, and T helper 1 type response and interferon-γ correlate with the protection. IL-4 showed a small production before and after the challenge, in other words, a small difference compared to the control group, indicating that the vaccine formulation (rCagA + LPS + CpG) induces a Th1 response.

In addition, after challenge, was observed an increased expression of the anti-inflammatory cytokines as IL-10 and TGF-β in immunized protected mice, which possibly occur as a consequence of or an attempt to balance the proinflammatory response induced and already established (because before the challenge had elevated production of IFN- Y) which inversely correlated with *H. pylori* colonization (observed in CFU assay).

In mice immunized with LPS and rCagA, immune responses elevated extremely which indicated synergistic effects of these antigens on stimulating of immune response against *H.pylori* infection.

In conclusion, we have demonstrated that *H. pylori* multicomponent vaccines comprising rCagA can confer more effective protection against *H. pylori* infection in BALB/c administered both intragastrically and intramuscularly.

ACKNOWLEDGEMENTS

We are so thankful of Dr. Graham (University of Washington, Seattle, Washington, Baylor College of Medicine, and Houston, Texas. USA), Dr. Kuster (Laboratory of Gastroenterology Rotterdam, Netherlands) for their warmly helps and advices.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Taylor JM, Ziman ME, Huff JL, Moroski NM, Vajdy M, Solnick JV. Helicobacter pylori lipopolysaccharide promotes a Th1 type immune response in immunized mice vaccine. 2006;24:4987-4994.

2. Kleanthous H, Tibbitts TJ, Gray HL. Sterilizing immunity against experimental *Helicobacter pylori* infection is challenge-strain dependent. Vaccine. 2001;19:4883-4895.
3. Akhiani AA, Pappo J, Kabok Z Schön K, Gao W, Franzén LE, Lycke N . Protection against *Helicobacter pylori* infection following immunization is IL-12-dependent and mediated by Th1 cells. J Immunol. 2002;169:6977-6984.
4. Shi T, Liu WZ, Shi GY, Xiao SD. The role of Th1-type response and gamma-interferon in the protection induced by *Helicobacter pylori* vaccine. Zhonghua Yi Xue Za Zhi. 2003;83:1321-1325.
5. Aebischer T, Schmitt A, Walduck AK, Meyer TF. *Helicobacter pylori* vaccine development: facing the challenge. Int J Med Microbiol. 2005;295:343-353.
6. Harris DP, Serrano HC, Venegas EA .Vaccines under development: *Helicobacter pylori*]. Rev Chilena Infectol. 2006;23:249-256.
7. Hoffelner H, Rieder G, Haas R. *Helicobacter pylori* vaccine development optimisation of strategies and importance of challenging strain and animal model. Int J Med Microbiol. 2008;298:151-159.
8. Jiang Z, Pu D, Huang AL, Tao XH, Wang PL. Construction, expression and antigenic study of bivalent vaccine candidate with 26,000 OMP and heat short protein A of human *Helicobacter pylori*. Zhonghua Yi Xue Za Zhi. 2003;83:862-867.
9. Zhao YX, Qi JH, Zhang H, Duan GC, Xi YL .Construction, expression and purification of UreB-Omp11 fusion protein of *Helicobacter pylori* and its immunocompetence. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi. 2007;23:906-910.
10. Zavala-Spinetti L, Breslin MB, Correa H, Begue RE. Development and evaluation of a DNA vaccine based on *Helicobacter pylori* urease B: failure to prevent experimental infection in the mouse model. Helicobacter. 2006;11:517-522.
11. Malfertheiner P, Schultze V, Rosenkranz B, Kaufmann SH, Ulrichs T, Novicki D, Norelli F, Contorni M, Peppoloni S, Berti D, Tornese D, Ganju J, Palla E, Rappuoli R, Scharschmidt BF, Del Giudice G. Safety and immunogenicity of an intramuscular *Helicobacter pylori* vaccine in noninfected volunteers: a phase I study. Gastroenterology. 2008;135:787-795.
12. Rossi G, Ruggiero P, Peppoloni S,Pancotto L, Fortuna D, Lauretti L, Volpini G, Mancianti S, Corazza M, Taccini E,Di pisa F,Rappuoli R, Del Giudice G . Therapeutic vaccination against *Helicobacter pylori* in the beagle dog experimental model: safety, immunogenicity, and efficacy. Infect Immun. 2004;72:3252-3259.
13. Nystrom J, Svennerholm AM. Oral immunization with HpaA affords therapeutic protective immunity against H. pylori that is reflected by specific mucosal immune responses. Vaccine. 2007;25:2591-2598.
14. Blanchard TG, Eisenberg JC, Matsumoto Y. Clearance of *Helicobacter pylori* infection through immunization: the site of T cell activation contributes to vaccine efficacy. Vaccine. 2004;22:888-897.
15. Rudnicka W, Covacci A, Wadstrom T, Chmiela M. A recombinant fragment of *Helicobacter pylori* CagA affects proliferation of human cells. J Physiol Pharmacol. 1998;49:111-119.
16. Solnick JV, Canfield DR, Hansen LM, Torabian SZ. Immunization with recombinant *Helicobacter pylori* urease in specific-pathogen-free rhesus monkeys (Macaca mulatta). Infect Immun. 2000;68:2560-2565.
17. Guy B, Hessler C, Fourage S .Systemic immunization with urease protects mice against *Helicobacter pylori* infection. Vaccine. 2001;16:850-856.
18. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning: A Laboratory Manual. Second Edition. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press. 1989;1:626.
19. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods. 1983;65:55-63.

20. Denizot F, Lang R. Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J Immunol Methods. 1986;89:271–277.
21. Ermak TH, Giannasca PJ, Nichols R . Immunization of mice with urease vaccine affords protection against *Helicobacter pylori* infection in the absence of antibodies and is mediated by MHC class II-restricted responses. J Exp Med. 1998;188:2277-2288.
22. Xu C, Li ZS, Du YQ . Construction of a recombinant attenuated *Salmonella typhimurium* DNA vaccine carrying Helicobacter pylori hpaA. World J Gastroenterol. 2005;11:114-117.
23. Koesling J, Lucas B, Develioglou L, Aebischer T, Meyer TF. Vaccination of mice with live recombinant *Salmonella typhimurium* aroA against *H. pylori*: parameters associated with prophylactic and therapeutic vaccine efficacy. Vaccine. 2001;20:413-420.
24. DiPetrillo MD, Tibbetts T, Kleanthous H, Killeen KP, Hohmann EL. Safety and immunogenicity of phoP/phoQ-deleted Salmonella typhi expressing *Helicobacter pylori* urease in adult volunteers. Vaccine. 1999;18:449-459.
25. Miyashita M, Joh T, Watanabe K. Immune responses in mice to intranasal and intracutaneous administration of a DNA vaccine encoding Helicobacter pylori-catalase. Vaccine. 2002;20:2336-2342.
26. Manente L, Perna A, Buommino E,Altucci L,Citro G, Baldi A,Iaquinto G, Tufano M.A,De Luca A . The *Helicobacter pylori's* protein VacA has direct effects on the regulation of cell cycle and apoptosis in gastric epithelial cells. J Cell Physiol. 2008;214:582-587.
27. Bergman MP, Engering A, Smits HH, van Vliet SJ, van Bodegraven AA, Wirth HP, Kapsenberg ml, Vandenbroucke-Grauls CM, van Kooyk Y and Appelmelk BJ.Helicobacter pylori modulates the T helper cell 1/T helper cell 2 balance through phase-variable interaction between lipopolysaccharide and DC-SIGN. J Exp Med. 2004;200: 979–990.
28. Sommer F, Wilken H, Faller G, Lohoff M. Systemic Th1 Immunization of Mice against Helicobacter pylori infection with CpG oligonucleotides as Adjuvants does not protect from infection but enhances gastritis. Infection and Immunity. 2004;72(2):1029–1035.
29. Jennifer M. Taylor, Melanie E. Ziman, Julie Fong, Jay V. Solnick, Michael Vajdy. Possible Correlates of Long-Term Protection against Helicobacter pylori following Systemic or Combinations of Mucosal and Systemic Immunizations, Infection and Immunity. 2007;75(7):3462-3469.
30. Esmaeili D, Mohabati Mobarez A, Hatef Salmanian A, Zavaran HA, Hosseini doust R. Immune Responses of Conserved 32 KD fragment from N-terminal of H. pylori cagA gene. Journal of pure & Applied Microbiology . 2009;4(1):117-123.
31. Ihan A, Pinchuk IV, Beswick EJ. Inflammation, immunity, and vaccines for Helicobacter pylori infection. Helicobacter. 2012;1:16-21.
32. Sundeep A, Steven J C. Vaccination as a Method of Preventing Helicobacter pylori–Associated Gastric Cancer. 2013;14(8):1890-1891.
33. Flash C.F, Ostberg A.K, Nilsson A.T, Malefyt R.D.M, Raghavan S. Proinflammatory Cytokine Gene Expression in the Stomach Correlates with Vaccine-Induced Protection against Helicobacter pylori Infection in Mice: an Important Role for Interleukin-17 during the Effector Phase;Infect Immun. 2012;79(2):879–886.
34. Sayi A., Kohler E., Hitzler I., Arnold I., Schwendener R., Rehrauer H., Muller A . The $CD4^+$ T cell-mediated IFN-gamma response to *Helicobacter* infection is essential for clearance and determines gastric cancer risk. J. Immunol. 2009*;*1827085–7101.

35. Shi Y, Liu XF, Zhuang Y, Zhang JY, Liu T, Yin Z, Wu C, Mao XH, Jia KR, Wang FJ, Guo H, Flavell RA, Zhao Z, Liu KY, Xiao B, Guo Y, Zhang WJ, Zhou WY, Guo G, Zou QM. *Helicobacter pylori*-induced Th17 responses modulate Th1 cell responses, benefit bacterial growth, and contribute to pathology in mice.J. Immunol. 2010;1845121–5129.
36. Hai-Bo Li, Jin-Yong Z, Ya-Fei H, Li C, Bin L, Kai-Yun L, Wu-CY, Zhuo Z, Quan-M Z, Chao Wu. Systemic immunization with an epitope-based vaccine elicits a Th1-biased response and provides protection against Helicobacter pylori in mice. Vaccine. 2012;31(1):120-126.
37. Mori J, Vranac T, Smrekar B, Cernilec M, Serbec VČ, Horvat S, Ihan A, Benčina M, Jerala R. Chimeric flagellin as the self-adjuvanting antigen for the activation of immune response against Helicobacter pylori. Vaccine. 2012;31;30(40):5856-63.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid= 399&id=8&aid=3569