Co-expression of Interleukin-17A molecular adjuvant and prophylactic Helicobacter pylori genetic vaccine could cause sterile immunity in Treg suppressed mice

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ABSTRACT

The increasing clinical significance of Helicobacter pylori (H. pylori) in human stomach cancer has led to global efforts to eradicate this pathogen. Recent studies have confirmed the importance of some cytokines such as Interleukin-18 (IL-18), Interleukin-8 (IL-8), Interleukin-17A (IL-17A) and Interleukin-22 (IL-22) in the pathogenesis of the so-called bacterium. This study was designed to compare the effects of Type 1T helper (Th1), Type 2 helper (Th2) cells, Regulatory T cells (Treg) and T helper 17 (Th17) modulatory effects on the efficacy of designed H. pylori vaccine by incorporating some molecular adjuvants in Treg competent and Treg suppressed groups. A bicistronic vector was used for simultaneous expression of codon-optimized Outer inflammatory protein a (OipA) gene and modified mice IL-17A, IL-17A, IL-22 and Foxp3 (forkhead box P3) cytokines from four cassettes. Immunization of mice groups was performed using produced plasmids intradermally. Specific IgG1 and IgG2 and IgA antibody titers produced in mice were confirmed by enzyme-linked immunosorbent assay (ELISA) in sera and intestine obtained four weeks after the last immunization. After being stimulated with a mixture of both anti-CD28 mAb and H. pylori lysate, frequencies of single Interferon-Gamma (IFN-γ), single IL-17 and dual IFN-γ/IL-17-secreting T-cells were documented using dual-color FluoroSpot. The kinetics of Th1, Th2 and Th17 in the immunized animals was determined by relative quantification of IL-17A, IL-22, IFN-γ, IL-8, IL-2 and IL-4 specific mRNAs. Four weeks after bacterial challenge, quantitative colony count in the isolated and homogenized stomachs was utilized to assess the level of protective immunity among all groups. The results of immunologic assays showed that the highest cell-mediated immunity cytokines were produced in IL-17 receiving group in addition, potent clearance of Helicobacter pylori infection was seen in this group as well.

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1. Introduction

Helicobacter pylori, the hallmark of gastric cancer, have become a challenging pathogen since its discovery in 1983 and confirming its oncogenicity in 1994 [1–5]. Current standard protocols for elimination of H. pylori suggested using chemotherapy in individuals with gastric symptoms. Nonetheless asymptomatic infections could still be assumed as a causative element in developing severe digestive diseases, including gastric cancer [6]. Significant rate of eradication failure and probability of re-infection are considered as the main problems of standard antibiotic therapy for the elimination of H. pylori infection [7]. In addition, H. pylori eradication is not always beneficial as it can be associated with an increased frequency of other disorders such as pediatric asthma, inflammatory bowel diseases and Barrett’s Esophagus.

Abbreviations: Treg, Regulatory T cells; IL-17A, Interleukin-17A; IL-22, Interleukin-22; IL-18, Interleukin-18; Foxp3, forkhead box P3; oipA, outer inflammatory protein A; H. pylori, Helicobacter pylori; Th1, Type 1 T helper; Th2, Type 2 T helper; Th17, Type 17 T helper

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Considering the fact that both ignoring and/or eliminating the H. pylori infection might result in serious gastrointestinal disorders, it seems that prophylactic vaccination serves as an optimum procedure to eliminate all probable complications associated with H. pylori infection. Clarifying the types of immune responses that could have an impact on the reduction of the bacterial load is of great importance in the designing of an H. pylori vaccine candidate. A perfect and protective vaccine against H. pylori would induce Th1-biased immune responses to a large extent [9]. A number of DNA vaccine projects which have been conducted to shift the immune response from a Th2 to a Th1 type, have stressed the importance of adjuvants in modifying the type of immune responses. The critical role of regulatory T (Treg) cells in dampening immune responses against H. pylori is apparent [10]. Kao et al. consider that a suboptimal Th17 response could not cause complete eradication due to the fact that H. pylori makes changes the DC-polarized Th17/Treg balance toward a Treg-biased response, thus restraining the successful Th17 immunity against H. pylori infection [11]. Outer inflammatory protein, designated as OipA has an important role in the initiation of inflammatory response to H. pylori and in promoting duodenal ulcers as well as gastric cancer [12]. The ability to produce OipA antigen is significantly correlated with the clinical outcome of H. pylori infection and has been identified and proposed to be a promising vaccine candidate [13]. The objective of the current study was to design a bicistronic DNA vaccine by incorporating the codon-optimized oipA gene and to co-express it with mouse IL-17A, IL-18 and IL-22 molecular adjuvants. Besides, we assessed the effectiveness of the DNA vaccine against the H. pylori challenge in Treg suppressed mice.

2. Materials and methods

2.1. Construction of plasmids

The cloning process consisted of several steps to produce bicistronic plasmids containing the codon-optimized H. pylori oipA gene in combination with modified IL-18, IL-17A, IL-22 and foxp3. For efficient secretion of murine IL-18, the designed sequence was consisted of the Immunoglobulin kappa (IgK) in 5’ end. In addition, for increasing the half-life and effectiveness, we recombined the desired IL-18, IL17A, IL-22 and Foxp3 molecular adjuvants to a constant region of the Heavy immunoglobulin chain (FC2a) in the 3’ end. The construction of IL-18/oipA was described previously [14,15]. The construction of designed sequence of IL-17A and IL-22 was carried out in Bioneer Company in pBHA cloning vector (Bioneer, Korea). Subcloning of constructed genes into pIRE2-oipA construct was performed using double digestion, ligation, transformation and clonal selection. The expression and biological activity of cloned IL-17A and IL-22 was assessed by transfection of produced construct into KG-1 macrophage cell line and assessment of IL-6 in supernatants (CyTech Bioscience, Netherlands). Foxp3-Fc plasmid containing mouse truncated foxp3 gene (lacks nuclear localization signal) and IgG Fc fragment was gift from another research group. The procedure for expression and purification of Foxp3 recombinant protein in BL21-transformed Fox-pET cells was previously explained in detail [16].

2.2. Immunization schedule

Five weeks old BALB/c female mice (n = 160) were obtained from the animal facility center of Pasteur Institute of Iran and were housed in the animal laboratory containment facility of Guilan University of Medical Sciences, Faculty of Pharmacology. In conducting the current research, the researchers respected all the ethical issues concerning animal experiments. By the seventh week, the mice were randomized, caged into groups of ten and ear coded. For anti-Treg vaccination, 5 Groups out of 10 were injected Intramuscular (IM) in the quadriceps with 100 µg pIRES-FoxP3 DNA in 100 µl of endotoxin-free phosphate buffer saline (PBS) at day 0 or SC in the neck back with 100 µl recombiant protein (20 mg) + 100 µl incomplete Freund’s adjuvant at weeks 2 and 4 [17]. Subsequently different homologous DNA vaccine protocols were administered at weeks 6, 8 and 10. Five mice out of ten were used to evaluate immune responses and five were used to assess the bacterial challenge. Negative control groups were inoculated with pIRE2-eGFP plasmid not carrying H. pylori gene sequences. The experimental outline is exhibited in Table 1.

2.3. Culture of H. pylori, bacterial challenge and stomach processing

In order to challenge the mice, four weeks after the last immunization, the mice were inoculated intragastrically at 2-day intervals with three doses of 10⁸ colony forming unit (CFU) of H. pylori SS1 bacterial suspension (kindly provided by Dr. Mohammadali Khanmirzaei, Stockholm University) in 0.1 mL Brucella broth, as described previously [18]. In order to reisolate the H. pylori from mice, the mice were then anesthetized by peritoneal injection of 1.43 mg/kg diazepam and 13 mg/kg ketamine 10% and homogenized stomachs were plated onto Columbia blood agar plates and the incubation lasted 5 days. The whole-cell sonicated (WCS) bacterial antigen was produced by inactivation of H. pylori SS1 bacterial cells using 4% formaldehyde for at least 26 h and subsequent steps of washing with PBS. After this process of inactivation, the sonication process was performed for resulted whole-cell H. pylori preparation. WCS antigen preparation was evaluated in terms of protein concentration. Finally, the accuracy of inactivation process was analyzed by culturing the inactivated bacteria onto Columbia blood agar plates as elaborated previously.

2.4. Antibody titration against OipA

One week after the last immunization and before bacterial challenge, blood samples (100 μl) were collected from the tail vein to measure the antibody responses by ELISA test (Nunc GmbH, Germany).
ELISA plates were coated with 100 μl of H. pylori OipA (50 μg/ml in 0.05 M carbonate buffer, pH 9.6) at 4°C overnight and were blocked with 5% BSA. 50 μl of serially diluted mouse serum (1:100 in PBS + BSA) was added to the wells and incubated at 37°C for 1 h and Horseradish Peroxidase (HRP)-conjugated anti-mouse IgG antibodies were added and incubated at 37°C for 1 h. The absorbance was measured at 450 nm using a microplate reader. To determine stomach mucosal IgA production, one-fourth of stomach tissue was homogenized in 1 ml PBS containing a protease inhibitor mixture (Roche, Germany) and 0.05 M ethylendiaminetetraacetic acid. After centrifugation, the 1:100 dilution of the supernatant were prepared and then added in ELISA plates pre-coated with 5 μg OipA protein. Finally, anti-mouse IgA (BD Pharmingen, Franklin Lakes, NJ, USA) was added to detect IgA anti-OipA antibodies.

2.5. IFN-γ/IL-17A dual FluoroSpot assay

IFN-γ/IL-17A Dual FluoroSpot assay kit (Mabtech, Sweden) was used to measure cells secreting either one or both pro-inflammatory cytokines IFN-γ and IL-17A, in the vaccinated groups according to manufacturer's instructions. In outline, 96-well FluoroSpot plates were pre-coated with 100 μl/well of capture antibody and kept at 4°C overnight. A total of 2.5 × 10^5 splenocytes were stimulated with a mixture of anti-CD28 mAb and 5 μg/ml of inactivated H. pylori and incubated in the CO2 incubator overnight. After 12 h, the splenocytes were eliminated from the wells and bound IFN-γ and IL-17A cytokines were incubated with both 100 μl/well of 1:200 diluted R4-6A2-BAM and 1 μg/ml of IL-17-II-biotin in 1 × PBS-0.1% BSA for 2 h. 100 μl of 1:200 diluted anti-BAM-Green and SA-Red were added to each well for 1 h followed by addition of 50 μl/well Fluorescence enhancer-II. Fluorescence-forming cells (FFCs) were counted under a FluoroSpot Reader and Analyzer (Mabtech, Sweden). The FluoroSpot results were represented as Fluorescence-Forming Units (FFU)/1 × 10^6 cells and were reported as fold of induction (FOI) compared to the mock control.

2.6. RNA extraction and cytokine assay by real-time PCR

A semi-quantitative real-time RT-PCR method was applied to assess the relative cytokine expression the splenocytes in response to vaccine administration. A total of 10^6 cells/ml RPMI were stimulated with 5 μg/ml of H. pylori SS1 as described above. After stimulation the cells were harvested, suspended in lysis buffer and kept at ~80°C for later mRNA extraction. To isolate mRNA of the stimulated splenocytes, the frozen cells were thawed and the process was continued according to the manufacturer's guidelines of the RNA extraction using RNeasy plus mini kit (Qiagen, Germany). cDNA synthesis was carried out using QuantiTect Reverse Transcription Kit (Qiagen, Germany). Extracted RNAs were treated with DNase I according to the manufacturer's specifications to remove any contaminating genomic DNA. The resultant cDNA was used in a real-time PCR assay in order to quantify Th1 and Th2 and Th17 cytokine mRNA levels in vaccinated animals. Secretion of cytokines including IL-17A, IFN-γ, IL-18, IL-22, IL-4 and IL-2 were assessed applying specific primers (Table 2). The β-actin mRNA was used as a housekeeping gene (as a positive control) to make the achieved data normal, and master mix, containing no template, was used as a negative control for PCR. Primers for all target sequences were designed using AlleleID software (PREMIER Biosoft, USA). Real-time PCR was carried out with a StepOnePlus™ Real-Time PCR System (ABI, USA) and Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific, USA). Melting curve analysis to distinguish the PCR products was carried out after amplification using the conditions recommended in the SYBR green kit manual to diminish the likelihood of nonspecific amplification, or to reduce the chance of formation of primer-dimer. Finally, the amount of target, normalized to β-actin and relative to the calibrator, was calculated by 2^{-ΔΔct}. Thus, all the experimental samples are expressed as an n-fold change relative to the calibrator.

2.7. Statistical analysis

One way ANOVA and the Student-Newman-Keuls test were performed using SigmaPlot (Systat Software, San Jose, CA, USA) to compare statistically significant differences among groups. Differences between groups were considered statistically significant at P < 0.05. All the data were expressed as the mean ± SD. The comparative analyses were done by one-way analysis of variance (ANOVA). The Tukey-Kramer's correction was also applied, when required for multiple comparisons. The statistics were analyzed using GraphPad InStat software and the statistical significance level was defined as P < 0.05.

3. Results

3.1. Confirmation of cloning procedures and biological activity of molecular adjuvants

Our findings obtained from enzymatic digestion and sequencing of the constructed plasmids revealed successful cloning of the oipA, IL-18, IL-17A, IL-22 and foxp3 in bicistronic expression cassettes. In order to scrutinize the expression of OipA and molecular adjuvants in eukaryotic cells, BHK-21 and HEK293T cells were transfected simultaneously with the IL-18/oipA, IL-17/oipA, IL-22/oipA and pRES-Foxp3-Fc plasmids. A plasmid construct for the expression of the enhanced Green Fluorescent Protein (eGFP) (pRES2-EGFP) was also used as transfection control. The successful expression of cloned genes was confirmed by immunofluorescence microscopy and Real-time PCR assay. Moreover, the Foxp3-Fc expression was confirmed by SDS-PAGE followed by western blot analysis. Additionally, the presence of fusion protein was shown by specific antibody after purification. To measure the bioactivity of IL-18/IgFc, IL-17A/IgFc and IL-22/IgFc the specificity of this cytokine in activating immune cells and secretion of IFN-γ and IL-6 in the supernatant of the KG-1 cells indicated interleukin levels of 5 pg/ml, 1.8 pg/ml and 1.9 pg/ml, respectively (Fig. 1).

3.2. Antibody responses in immunized groups

The oipA receiving group (group I) produced significant IgG isotypes compared to the mock controls (P < 0.001), more shifting to IgG1 subtype. However, in Treg suppressed groups, the level of IgG2a antibody was higher than that of oipA group. The highest IgG2a antibody response was seen in Treg suppressed oipA/IL-18 group indicating the role of IL-18 molecular adjuvant in isotype switching toward Th1 antibody and the function of Treg in diminishing the antibody response. There was no significant difference in antibody response between groups receiving oipA and oipA along with IL-17 or IL-22 groups. However, a significant elevation in IgG2a antibody level was confirmed in Treg suppressed groups in comparison to Treg competent groups. The ratios of IgG2a/IgG1 were significantly greater than that of the mock control and other groups, which was an indication of a strong Th1 immune response. These results confirmed the ability of oipA co-expressed with interleukins in inducing a Th1-biased immune response.

<table>
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<th>mRNAs</th>
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<th>Reverse</th>
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<td>AAGGGTGAGGATCGTCTG</td>
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<td>CGATTGGGAACTGCGGCTG</td>
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<tr>
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<td>β-actin</td>
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<td>CAATAGTGATAGCCGTGCGT</td>
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Table 2
Primer sequences used for cytokine real-time PCR analysis.
To assess mucosal immunity, the gastric IgA production against OipA was analyzed for each group. The immunized groups showed significantly augmented mucosal IgA levels compared to the mock control group (p < 0.0001). These findings indicated that co-expression of oipA and molecular adjuvants including IL-17, IL-22, and IL-18 elicited systemic and mucosal humoral immune responses in vaccinated groups. The highest secretory IgA titers were seen in Treg−IL-17/oipA group (no significant as compared to the Treg−IL-18/oipA and Treg−IL-22/oipA groups). IgA titers were low in the Treg− and pIRES2-eGFP mock controls, and all of these showed no statistically significant difference (P > 0.05) (Fig. 2).

3.3. Protection against infection of the stomach with Helicobacter pylori SS1

Mice immunized with oipA had significant drops in bacterial load compared to the mock control groups, indicating high immunogenicity of this codon optimized antigen (P < 0.001). As shown in Fig. 3, the sterile immunity was seen among Treg suppressed mice that received oipA in combination with IL-17, and it indicated that IL-17 has a crucial role in eliciting protective immunity against H. pylori. In the groups in which oipA was administered with either IL-22, the mice had an almost 4-log decrease in bacterial load compared to that of the mock controls (P < 0.001). Interestingly, the Treg suppressed group injected with oipA/IL-18 group showed lower protection rate, compared to Treg suppressed oipA group indicating the inverse effect of IL-18 in diminishing the H. pylori. In the Treg suppressed and mock control groups, H. pylori remained at high density in the mouse stomachs as compared to immunized groups (p < 0.001).

3.4. Dual-color FluoroSpot results

All immunized groups had higher IFN-γ and IL-17 levels than those of Foxp3 and mock control group (p < 0.00001). The strongest IL-17 level was seen in Treg suppressed oipA/IL-17 and oipA/IL-22 groups. The Treg suppressed oipA/IL-18 administration has led to significantly higher frequencies of single IFN-γ-secreting T-cells in comparison to other groups. The higher proportions of dual IFN-γ/IL-17 secreting T cells were observed in groups with Treg suppression and oipA/IL-17 and oipA/IL-22 administration as compared to other groups. There were no significant differences between the Treg− and the mock control groups. After stimulated with anti-CD28 mAb and H. pylori lysate, the higher proportion of dual IFN-γ/IL-17 secreting T cells were observed in groups with Treg suppression and oipA/IL-17 and oipA/IL-22 administration (Fig. 4).

3.5. Th1 and Th2 and Th17 cytokine profile by Real-time PCR

Significantly, higher levels of Th1, Th2 and Th17 cytokine expression were observed in the immunized groups, compared with the control groups (Fig. 5a–f). However, Th17 cytokine induction was higher in Treg suppressed animals vaccinated with oipA/IL-17 constructs than the Treg completed animals vaccinated with oipA/IL-17 vaccine (p < 0.05). IL-12 simulation by the antigen increased in all the mice compared to control groups, however, this increase was not considerably remarkable in oipA group (p > 0.05). The level of IFN-γ in Treg suppressed oipA/IL-18 and oipA/IL-18 groups were significantly higher than that of the other groups (p > 0.05). Two groups could induce the highest levels of IL-22: Treg suppressed oipA/IL-18 (P < 0.05) and Treg suppressed oipA/IL-22 (P < 0.05). Only Treg competent groups could elicit significantly higher levels of IL-4 compared to the other groups (P < 0.01). IL-8 level of Treg− oipA
In the present report, we evaluated the immune responses induced by IL-18. Some early studies of IL-18 as a genetic adjuvant have reported its role in enhancing Th1 immune responses. In this study, we investigated the role of IL-18 in combination with DNA vaccine constructs including IL-18, IL-8, IL-17A and IL-22 in pathogenesis and anti-microbial responses of H. pylori in human and animal models [19]. Research has also shown that co-administration of cytokine proteins or cytokine gene-encoded plasmids enhance comprehensive humoral and cellular immune responses [20]. In this study, we investigated the role of oipA gene in combination with IL-18, IL-17A and IL-22 molecular adjuvants in gastric epithelial cell defense against H. pylori. Our findings suggested that administration of IL-17A molecular adjuvant and oipA gene could bring about sterile immunity in Treg suppressed mice. In order to increase the secretion of IL-18 molecular adjuvant and reciprocal immune response, the designed plasmid has contained the kappa chain of immunoglobulin upstream of the adjuvant sequence. For increasing the half-life and effectiveness, we recombinated the desired adjuvants to a constant region of the chain Heavy immunoglobulin expresses FCε2a downstream of the adjuvant sequence. For increasing the secretion and efficiency of this adjuvant. IL-18 administration in Treg suppressed groups enhanced the efficacy of the oipA DNA vaccine by shifting the immune response to a Th1 type. In addition, controversial studies have been performed on the protective or harmful consequences of IL-17 in infectious diseases, in particular respiratory and digestive diseases [27]. Th17 cells indicated the discovery of a new subtype of T CD4 secreting IL-17A, IL-17F, IL-22, and IL-21 cells [28]. While several studies have provided evidence that IL-17A contributes to innate immune cell recruitment, IL-17A and IL-22 are also associated with anti-microbial responses and control of bacterial colonization in several models [18]. The roles of IL-17A and IL-22 in control of H. pylori proliferation have not been fully elucidated. Studies in IL-17RA/-/- mice suggested that IL-17A signaling was required for control of bacterial burden. Studies in IL-21/-/- mice demonstrated that IL-21 was required for activation of Th1 and Th17 responses and, therefore, demonstrates that IL-21 is required for the control of bacterial colonization [29]. However, the contribution of IL-22 to bacterial colonization has not been elucidated.

Recent studies have shown that Tregs suppress the immune response to H. pylori infection. Studies in mice infected with H. pylori have indicated that depletion of Tregs could bring about increased gastric inflammation and reduced colonization of H. pylori infection [30]. These findings suggested that Tregs had an important role in the persistence of H. pylori colonization in gastric mucosa. Furthermore, the number of Tregs and the expression of IL-10 and TGFβ1 were significantly higher in infected patients who developed gastritis than those with PUD, and this firmly revealed that Treg cell responses in infected patients with gastritis were capable of modulating Th1 and Th17 cell responses that possibly contributed to the persistence of H. pylori infection [31].

4. Discussion

Some studies confirmed the potential role of some cytokines including IL-18, IL-8, IL-17A and IL-22 in pathogenesis and anti-microbial responses of H. pylori in human and animal models [19]. Research has also shown that co-administration of cytokine proteins or cytokine gene-encoded plasmids enhance comprehensive humoral and cellular immune responses [20]. In this study, we investigated the role of oipA gene in combination with IL-18, IL-17A and IL-22 molecular adjuvants in gastric epithelial cell defense against H. pylori. Our findings suggested that administration of IL-17A molecular adjuvant and oipA gene could bring about sterile immunity in Treg suppressed mice. In order to increase the secretion of IL-18 molecular adjuvant and reciprocal immune response, the designed plasmid has contained the kappa chain of immunoglobulin upstream of the adjuvant sequence. For increasing the half-life and effectiveness, we recombinated the desired adjuvants to a constant region of the chain Heavy immunoglobulin expresses FCε2a downstream of the adjuvant sequence. For increasing the secretion and efficiency of this adjuvant. IL-18 administration in Treg suppressed groups enhanced the efficacy of the oipA DNA vaccine by shifting the immune response to a Th1 type.

In addition, controversial studies have been performed on the protective or harmful consequences of IL-17 in infectious diseases, in particular respiratory and digestive diseases [27]. Th17 cells indicated the discovery of a new subtype of T CD4 secreting IL-17A, IL-17F, IL-22, and IL-21 cells [28]. While several studies have provided evidence that IL-17A contributes to innate immune cell recruitment, IL-17A and IL-22 are also associated with anti-microbial responses and control of bacterial colonization in several models [18]. The roles of IL-17A and IL-22 in control of H. pylori proliferation have not been fully elucidated. Studies in IL-17RA/-/- mice suggested that IL-17A signaling was required for control of bacterial burden. Studies in IL-21/-/- mice demonstrated that IL-21 was required for activation of Th1 and Th17 responses and, therefore, demonstrates that IL-21 is required for the control of bacterial colonization [29]. However, the contribution of IL-22 to bacterial colonization has not been elucidated. Recent studies have shown that Tregs suppress the immune response to H. pylori infection. Studies in mice infected with H. pylori have indicated that depletion of Tregs could bring about increased gastric inflammation and reduced colonization of H. pylori infection [30]. These findings suggested that Tregs had an important role in the persistence of H. pylori colonization in gastric mucosa. Furthermore, the number of Tregs and the expression of IL-10 and TGFβ1 were significantly higher in infected patients who developed gastritis than those with PUD, and this firmly revealed that Treg cell responses in infected patients with gastritis were capable of modulating Th1 and Th17 cell responses that possibly contributed to the persistence of H. pylori infection [31].

The application of animal models helped the authors to reveal that administration of IL-17A and IL-22 with H. pylori oipA skewed the response toward Th17 differentiation by IL-17 and IL-22 secretion mechanism, rather than induction of a strong Th1 and Th2 activation. Besides, functional experiments of Treg suppression confirmed the enhancement of the H. pylori-specific Th17 response. This was correlated with the eradication of H. pylori and validated the principal role of Th17 immunity in bacterial clearance. Our data demonstrated that administration of oipA/IL-17 in Treg suppressed animals improved protection against H. pylori challenge and could achieve sterile immunity. These
data demonstrated that suppression of Treg subset and administration of oipA DNA vaccine along with IL-17 and IL-22 molecular adjuvants improved protection against *H. pylori* challenge and could achieve sterile immunity. Th1, Th2 and Th17 cell responses in the vaccinated animals were analyzed by Real-time PCR. The authors demonstrated that the secretion of IL-17 and IL-22 was induced upon immunization with oipA/IL-17 in Treg suppressed mice and considered an essential role for Th-17-derived IL-17 and IL-22 in skewing T cell differentiation away from Treg and toward Th17 responses. Our findings suggested that mice immunized with IL-18/oipA showed remarkable increases in IL-12 and IFN-γ secretion with no significant changes in IL-4 secretion, representing elicitation of a Th1-biased immune responses. Irrespective of the different immune responses stimulated by a multitude of combined vaccination protocols, all inoculated mice reduced bacteria loads.
following *H. pylori* challenge. Furthermore, Treg suppressed mice administered with IL-17A/oipA attained complete bacterial clearance.

To the extent of our knowledge, the current study for the first time revealed that the administration of IL-17A as a molecular adjuvant in a DNA vaccine protocol could shift immune responses and improve the effectiveness of DNA vaccine. Although similar studies will be challenging in human from the point of view of suppressing regulatory T cells, present study provides valuable guidance in lymphocyte subunit modulation and its role in developing an effective prophylactic vaccine to prevent *H. pylori* infection.

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**Author contributions**

Conceived and designed the experiments: MS. Performed the experiments: MN MS MT MM NNM. Analyzed the data: FNC. Wrote the manuscript: MS FNC EHA. Received the grant: MS. Reviewing and editing the article: AM BST.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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