Immunological Response to Parenteral Vaccination with Recombinant Hepatitis B Virus Surface Antigen Virus-Like Particles Expressing *Helicobacter pylori* KatA Epitopes in a Murine *H. pylori* Challenge Model

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Virus-like particles (VLPs) based on the small envelope protein of hepatitis B virus (HBsAg-S) are immunogenic at the B- and T-cell level. In this study, we inserted overlapping sequences encoding the carboxy terminus of the *Helicobacter pylori* katA gene product into HBsAg-S. The HBsAg-S–KatA fusion proteins were able to assemble into secretion-competent VLPs (VLP-KatA). The VLP-KatA proteins were able to induce KatA-specific antibodies in immunized mice. The mean total IgG antibody titers 41 days post-primary immunization with VLP-KatA (2.3 × 10^4) were significantly greater (P < 0.05) than those observed for vaccination with VLP alone (5.2 × 10^2). Measurement of IgG isotypes revealed responses to both IgG1 and IgG2a (mean titers, 9.0 × 10^4 and 2.6 × 10^4, respectively), with the IgG2a response to vaccination with VLP-KatA being significantly higher than that for mice immunized with KatA alone (P < 0.05). Following challenge of mice with *H. pylori*, a significantly reduced bacterial load in the gastric mucosa was observed (P < 0.05). This is the first report describing the use of VLPs as a delivery vehicle for *H. pylori* antigens.

Gastric colonization by *Helicobacter pylori* results in a chronic infection which may induce a strong but nonprotective immune response (10, 22, 24). While *H. pylori* is estimated to infect the stomach in more than half of the world’s population, less than 20% of patients develop serious disease, which suggests that there is a complex host/pathogen interaction. Infection with *H. pylori* also displays variable global demographics with respect to infection rates and associated gastric pathology. For example, the prevalence of *H. pylori* infection is estimated at >80% in the developing world, compared to <40% in the industrialized world (43). Similarly, gastric pathology associated with the bacterial infection also displays geographic variability. This is illustrated by the lower incidence of gastric cancer in African and South Asian populations, such as that experienced in East Asia, although the level of *H. pylori* infection in the former regions is higher (43). While antimicrobial chemotherapy remains the mainstay for eradicating *H. pylori* infections, a complexity of socioeconomic and logistical issues, together with complicated treatment regimens, compromises the effectiveness of such interventions in the developing world (10, 29).

While the occurrence of new *H. pylori* infections in adults is rare and is believed to be <0.5% per annum in the Western world, recrudescence of infection is becoming an increasing problem despite improvements in treatment regimens (4, 29, 33, 36, 43). Treatment failures may in part be due to poor patient compliance as a reflection of the complexity of chemotherapeutic regimens but may also be influenced by the emergence of antibiotic-resistant strains of the bacterium (12, 30, 34, 37, 42). Consequently, the development of effective vaccines against *H. pylori* remains an attractive option, although selection of appropriate antigens and optimal routes of vaccination remain to be fully defined (3, 7, 31). Since *H. pylori* is a gastric mucosa-associated bacterium, it seems intuitive that induction of a secretory IgA (sIgA) response may be an appropriate strategy. However, several reports have questioned the role of antibodies per se in the control of *H. pylori* infection, while others have suggested that the failure of the sIgA isotype may be a reflection of insufficient levels of IgA being secreted by the gastric mucosa (17, 28, 29). Gorrell and Robins-Browne demonstrated that *H. pylori*-reactive IgA could be readily detected in IgG-rich serum but was virtually absent in IgA-rich milk samples in a suckling mouse *H. pylori* challenge model (20). Their findings are consistent with the work of Abimiku and Dolby, which demonstrated that passive protection in sucking mice against intestinal infection by *Campylobacter jejuni* was largely due to IgG present in maternal milk (1). Furthermore, work by Ermak and others also demonstrated that parenteral vaccination route was as protective to mice against infection as the mucosal route (15). However, the role of specific Ig isotypes in *H. pylori* gastric immunity remains unclear; Todoraki, for example, reported that the use of a DNA vaccine encoding *H. pylori* heat shock protein A induced a predominantly IgG2a response, while use of heat shock protein B resulted in a predominantly IgG1 response. However, their work concluded that both vaccine regimens dramatically reduced gastric colonization in a mouse challenge model (57). Similarly, an apparent relationship between a
lower severity of *H. pylori* infections and patients with helminth infections seems to suggest that the role of IgE in attenuating *H. pylori* infectivity may need further investigation (16).

The role of T-cell responses in formulating vaccine targets against *H. pylori* also remains to be fully elucidated. Several studies suggest that induction of a Th2 response may be a key mediator in an effective immune response. These observations are consistent with reports that *H. pylori* infection induces a Th1-mediated inflammatory response which seems less antagonistic to infection (29, 31, 55, 61). These observations are also consistent with findings that have demonstrated that *H. pylori* infection can activate macrophages and elicit secretion of proinflammatory cytokines, such as interleukin 1β (IL-1β), IL-6, IL-8, and IL-23, which in turn results in further recruitment and activation of neutrophils, macrophages, and T cells (11, 13, 14, 31, 53, 54, 57). Furthermore, *H. pylori* derivatives, such as neutrophil-activating protein (HPNAP) and peptidyl prolyl cis-trans isomerase (HP0175), support the induction of a polarized Th1 response, eliciting further secretion of proinflammatory cytokines, such as gamma interferon (IFN-γ), tumor necrosis factor alpha (TNF-α), and IL-1β (31, 52). Since IL-1β is a known potent inhibitor of gastric acid secretion, there is an increased risk of *H. pylori* infection in patients that have genetic polymorphisms in the IL-1 gene cluster. Some genetic polymorphisms are known to result in raised levels of IL-1β expression, leading to reduced gastric acid production and increased inflammatory responses. IL-1β gene cluster polymorphisms, such as IL-1B*31C, IL-1B*511T, and IL-1RN*2/*2, also have an increased association with the development of gastric cancer (13, 29, 32, 52). Conversely, there is increasing evidence that induction of a Th2 polarized T-cell response may enhance clearance of the bacterial infection. For example, *H. pylori*-infected patients with a concurrent helminth infection (which is recognized to induce a Th2-type response) have been reported to have a significant reduction in *H. pylori*-associated diseases (16, 62). A plausible explanation of this relationship could be that helminth infections elicit secretion of IL-4, a cytokine which induces sequential Ig class switching to IgG1 and IgE (47, 48, 63), whereas in classical infections, where in polarized Th1 cells, IL-12 induces an inflammatory pathway eliciting IFN-γ and class switching to an IgG2 isotype (47, 55). However, several studies have also shown that the Th1-promoting cytokine IL-12 is an important mediator in promoting acute-phase protection against infection, while other studies have shown that both Th1 and Th2 responses remain important in longer-term (memory-cell-mediated) protection (17, 55).

A further impediment to the development of effective vaccines, and in particular mucosal vaccines, is the limited range of adjuvants licensed for human use (44, 58). In this context, virus-like particles (VLPs), such as particles composed of hepatitis B virus (HBV) surface protein HBsAg-S have been used as carriers to stimulate both humoral and cell-mediated responses (5, 38, 40, 45, 49, 59). HBsAg-S is of particular interest in that not only can it be used as a carrier to stimulate an effective cell-mediated immunity (CMI) and humoral response for foreign epitopes, but the protein is also the most common molecule used in HBV vaccines, and consequently, recombinant derivatives have the potential to be exploited for dual-vaccination regimens. The current HBV vaccines are recognized to be safe, effective, long lasting, and cost efficient, which makes the protein an attractive platform for delivering foreign epitopes (18, 27, 39, 51, 63). In brief, HBV encodes three envelope proteins: HBsAg-S, HBsAg-M, and HBsAg-L, where HBsAg-M and HBsAg-L are amino-terminal extensions of HBsAg-S. HBsAg-S is a hemiglycosylated protein which self-assembles in the endoplasmic reticulum membrane even in the absence of additional viral proteins or nucleic acid. The VLP contains an external highly immunogenic region known as the major antigenic site, or “a determinant,” and has been used by several groups as a platform to deliver foreign epitopes (8, 39, 59, 60). Cloning in this site is attractive since it enhances the immunologic recognition of foreign peptides by presenting them in a surface orientation.

In this study, we explored the effect of parenteral vaccination with recombinant HBsAg-S–*H. pylori* KatA VLPs on the humoral response and gastric colonization by *H. pylori* in a mouse challenge model. A further goal was to determine if the HBsAg-S VLP template could be utilized to deliver bacterial epitopes in a secretion-competent manner.

**MATERIALS AND METHODS**

**H. pylori** culture. *H. pylori* strain 26695 was cultured from frozen stocks on Columbia agar base (CAB) (Oxoid Ltd., England) containing 7% (wt/vol) defibrinated horse blood and Skirrow's selective supplement (10 mg liter⁻¹ vancomycin, 5 mg liter⁻¹ trimethoprim lactate, 2,500 IU polymyxin B, and 5 mg liter⁻¹ amphotericin B) and incubated for 48 h at 37°C in air containing 10% CO₂ and 95% relative humidity. CAB cultures were confirmed as *H. pylori* by observing characteristic motility using phase-contrast microscopy, characteristic morphology by Gram staining, positive urease, and catalase activity. *H. pylori* isolated on solid medium was used to prepare liquid cultures in brain heart infusion (BHI) (Oxoid) broth containing Skirrow's selective supplement and 5% (vol/vol) horse serum. Broth cultures were incubated under microaerophilic conditions for 48 h at 37°C.

**Isolation of H. pylori genomic DNA and PCR amplification of the katA gene.** *H. pylori* genomic DNA was isolated and purified from freshly grown stocks using a commercial DNA isolation kit, following the manufacturer's instructions (Bio-Rad Laboratories Pty. Ltd., Regents Park, Australia). DNA was reconstituted in TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 7.6]). Aliquots of genomic DNA were analyzed for purity by conventional gel electrophoresis in a 1% agarose gel. The DNA preparation was stored at −20°C until use.

**H. pylori katA DNA** was amplified by PCR from nucleotide 1114 to nucleotide 1515 (Fig. 1) using published DNA sequences (NCBI accession no. 2314010). Custom primers for amplification of *katA* were designed and synthesized commercially (Gewerks, Adelaide, Australia). The forward and reverse primer sequences used were 5'-CAC CAT GCA AAA CGG ATA CATA CG-3' and 5'-CTT TTT TTT TTG TTT GTC GTG CAT GTC-3', respectively. PCR amplicons were purified using a commercial kit in accordance with the manufacturer's instructions (Roche Products Pty. Ltd., Castle Hill, Australia) and tested for purity by conventional electrophoresis in a 1% agarose gel. The synthesized DNA sequence encoding the KatA carboxyl terminus was cloned into the commercial prokaryotic expression vector pET101/D-TOPO (Invitrogen Pty. Ltd., Melbourne, Australia). The recombinant plasmid was transformed into *Escherichia coli* strain TOP 10 (Invitrogen) for amplification and then isolated, and the correct sequence orientation was confirmed by sequencing. Recombinant pDNA was then transformed into BL21 Star(DE3) *E. coli* (Invitrogen) for expression of the recombinant KatA (recKatA) protein. The 6×His-tagged recKatA protein was purified by gravity-flow affinity chromatography using Ni-nitrioltriacetic acid (NTA) agarose (C/N 30210; Qiagen, Doncaster, Australia), following the manufacturer's instructions, dialyzed, and quantified using a standard Bradford assay. recKatA expression was confirmed by Western blotting using a commercial anti-His antibody that had been coupled to alkaline phosphatase ( Pierce Ltd., Rockford, IL).

Construction of H. pylori KatA carboxyl terminus VLPs. A summary of the research protocol used in preparing recombinant HBsAg-S Kat VLPs is shown in Fig. 1. Six sequentially overlapping DNA sequences (Fig. 2 and Table 1) encoding the H. pylori KatA carboxyl terminus were synthesized using PCR. Each katA (C-terminus-encoding) fragment was inserted into the pGEM-T-Easy vector and grown in JM 109 cells for sequence verification. Each of the cDNA fragments, specific for the katA-encoded C-terminal domain, were ligated into the unique AgeI restriction site of the pcDNA3-HBsAg expression vector (38). Sequence integrity and orientation were confirmed by DNA sequencing. The pcDNA3-HBsAg-katA constructs were amplified, purified using a maxiprep kit with standard procedures, and transfected into HuH-7 cells by the Ca3(PO4)2 method as previously described (38).

Purification of wt H. pylori KatA. Wild-type (wt) KatA was prepared as previously described (22). In brief, colonies of H. pylori were harvested from CAB plates and resuspended in 0.1 M phosphate-buffered saline (pH 7.2). Cells were pelleted by centrifugation and disrupted by sonication. Cellular material was removed by centrifugation (5 min at 10,000 × g). The supernatant was passed through a 0.22-μM filter, and the filtrate was loaded on a K26/100 gel filtration column of Sephacryl S-300 HR (Pharmacia Biotec Inc., Piscataway NJ) and eluted with sodium phosphate buffer at a flow rate of 1 ml min⁻¹. Catalase-positive fractions were determined by oxygen reduction activity in 3% H2O2. The concentration of protein in the eluants was determined using a commercial Bradford assay (Pierce) by applying bovine serum albumin as a standard.

Animal experimentation. All animal experimentation was approved by the University of Southern Queensland and the Queensland Institute of Medical Research Animal Ethics Committees and was performed in accordance with the guidelines set by the National Health and Medical Research Council.

Raising of H. pylori KatA antibody. Antibody to the H. pylori recombinant KatA and wt KatA proteins was raised in rabbits using standard procedures. In brief, equal volumes of protein suspension (0.5 to 1.0 mg ml⁻¹ in phosphate buffer) were mixed with incomplete Freund’s adjuvant, followed by the injection of 0.5 ml of the suspension into the quadriceps muscle of each hind leg of adult New Zealand White rabbits. Three booster doses of 0.1 mg ml⁻¹ protein in 0.3 ml of phosphate buffer alone at 7-day intervals were administered intravenously (i.v.) using the marginal ear vein. Seven days after the final vaccination, arterial blood was harvested and serum was separated using standard procedures. Antibody titers to the recKatA and wt KatA pro-
teins were determined by indirect enzyme-linked immunosorbent assay (ELISA) as previously described (23).

Confirmation of recombinant VLP-KatA expression. Several methods were applied to verify the expression of recombinant HBsAg proteins and the formation of VLPs. To identify secretion-competent VLPs, cell culture supernatants were overlaid on a 20% sucrose cushion and centrifuged, and the pellets were resuspended in 1 ml of Dulbecco’s modified Eagle’s medium (Invitrogen/Gibco). The recombinant VLP-KatA (here referred to as VLP-KatA) particles were identified by electron microscopy (Analytical Electron Microscopy Facility, Queensland University of Technology, Brisbane, Australia) and by an HBsAg-specific chemiluminescence immunoassay (Abbott Prism HBsAg; performed at the Red Cross Blood Bank, Brisbane, Australia). The concentration of HBsAg was estimated against an HBsAg standard curve using a commercial vaccine as the known standard (Engerix-B; GlaxoSmithKline Biologicals, Rixensart, Belgium).

To demonstrate the integrity of the VLP-KatA proteins, concentrated protein samples were analyzed by polyacrylamide gel electrophoresis followed by Coomassie blue staining and immunoblot analysis. The commercially available vaccine (Engerix-B; GlaxoSmithKline Biologicals, Rixensart, Belgium). To further identify putative B-cell epitopes, KatA peptide sequences were commercially synthesized in overlapping 15mer residues to cover the complete KatA carboxyl-terminus domain (Pepset, Mimotopes Pty. Ltd., Clayton, Australia). The peptides were screened by polyclonal rabbit anti-KatA antiserum. Reacting residues were compared to the KatA sequences inserted into the VLPs for homology.

Mouse immunization and challenge protocol. C57BL/6 mice (female, 4 to 6 weeks old; n = 10) were immunized subcutaneously at the tail base on day 0 with a single antigen preparation containing 5 μg (each) of VLP-KatA constructs no. 1, no. 4, and no. 5, with hydrogel as an adjuvant. Mice were boosted on days 21 and 28. Controls received wt VLP-alhydrogel, KatA-alhydrogel, and adjuvant alone. Two weeks after the final immunization, mice were challenged with 10^7 CFU of viable H. pylori strain SS1 orogastrically on two consecutive days.

H. pylori bacterial load determination. The procedure used to determine the bacterial load in the gastric mucosa of mice following challenge with live H. pylori strain SS1 was as previously described (22). In brief, at 41 days post-primary immunization, mice were killed by CO₂ asphyxiation, and the stomach of each mouse was removed and bisected along the lesser and greater curves. One-half was placed in 10% neutralized buffered formalin for histology, and the other half was weighed and homogenized in 0.1 M phosphate-buffered physiological saline (pH 7.4). The homogenate was serially diluted in physiological saline and cultured on CAB as described above. Five-micrometer histological sections of murine gastric tissue were stained with Giemsa stain to determine the bacterial load by using a positive/negative grading scale.

Statistical analysis. Statistical analyses of serum ELISAs for total IgG and IgG isotype comparisons following vaccination with respective immunogens were performed using the Mann-Whitney and Kruskal–Wallis tests. Statistical analysis of murine H. pylori challenge data was performed using 1-way analysis of variance (ANOVA) followed by Bonferroni’s multiple-comparison test. F and t values were provided for all quantitative data. Differences between groups were considered statistically significant at P values of <0.05. All statistical analyses were performed using the Instat 3 statistical analysis software program (GraphPad Software Inc., San Diego, CA).

RESULTS

Expression of recombinant VLPs containing epitopes of H. pylori KatA. To demonstrate secretion competence and functionality of the modified HBsAg proteins, constructs 1 to 6 were transfected into HuH-7 cells, and the cell culture supernatant of transfected cells was harvested 5 days posttransfection. Each construct expressed a different C-terminal KatA sequence in the “a” determinant region of HBsAg–S. The recombinant VLPs were par-
tially purified from approximately 4 ml of cell culture supernatant by ultracentrifugation, and the pellet was resuspended and screened with an HBsAg–VLP-specific chemiluminescence assay (Abbott Prism). The monoclonal anti-HBsAg-S antibody identified HBsAg-S activity in the cell culture supernatant of HuH-7 cells transfected with the VLP-KatA constructs (no. 1, 4, and 5). The presence of HBsAg-S activity in the cell culture supernatant strongly indicated that secretion-competent modified HBsAg-S VLPs were present (Table 2). Western blot analysis with anti-wt KatA sera showed that KatA–HBsAg-S fusion proteins could be detected in cell culture supernatants of cells transfected with constructs 1, 4, and 5 (Fig. 3). This is consistent with the measurement of anti-HBsAg-S activity, indicating that fusion proteins are expressed. Constructs 2, 3, and 6 did not express secretion-competent HBsAg-S VLPs, since the anti-HBsAg-S and anti-KatA assays failed to detect these proteins, a result which may be due to the misfolding or unstable expression of the modified HBsAg-S proteins (39). The Western blot analysis also showed that the HBsAg-S fusion proteins of constructs 1 and 4 were present in larger amounts than the HBsAg-S fusion proteins of construct 5.

The Western blot analysis also revealed that the fusion proteins of constructs 4 and 5 had a size of approximately 35 kDa, while the protein expressed by construct 1 had a size of approximately 43 kDa. The high molecular mass of KatA HBsAg-S construct 1 is possibly due to the generation of a novel glycosylation site. Anal-
yses of HBsAg-S variant proteins have shown that certain mutations can generate novel N-glycosylation sites (19).

VLP formation was confirmed by electron microscopy for constructs 1, 4, and 5. While an increased molecular mass of construct 1 was observed by Western blotting, electron microscopy revealed VLPs for construct 1 to be similar in size to VLPs for construct 4 (Fig. 4).

Murine challenge experiments. To demonstrate the immunogenicity of VLP-KatA, mice were immunized three times with a mixture of recombinant VLP-KatAs (constructs 1, 4, and 5) subcutaneously in the presence of alhydrogel as an adjuvant. Immune responses were measured by ELISA using a mixture of peptides corresponding to the inserted KatA sequences used in constructs 1, 4, and 5. KatA serum IgG antibody responses were detected in mice immunized with a mixture of VLP-KatA constructs, with KatA, and with VLP alone (Fig. 5). The mean IgG antibody titer \(2.3 \times 10^9\) detected at day 41 post-primary immunization with VLP-KatA was significantly greater than observed for vaccination with VLP alone \(5.2 \times 10^2\) \(F = 30.10, df (5,54); t = 9.514, df (54), with \(P < 0.05\). The relatively low VLP titer was likely to be a nonspecific response. Differences in titers between VLP-KatA and VLP alone were not significant \(P < 0.05\) at days 21 and 33 post-primary inoculation (following boosting at 21 and 28 days). Measurement of the IgG isotype at 41 days post-primary inoculation with VLP-KatA and KatA alone showed responses for both IgG1 and IgG2a (Fig. 6). The mean IgG2a titers for mice vaccinated with

![FIG 4](https://example.com/fig4.png)

**FIG 4** Electron micrographs illustrate secretion-competent recombinant HBsAg-KatA VLPs prepared as described in Material and Methods. Panel A shows VLPs from construct 4, which are composed of HBsAg-S subunits of the expected 38-kDa size. Panel B illustrates VLPs derived from construct 1, which are comprised of HBsAg-S subunits apparently larger in size than the expected 43 kDa (see Fig. 3).

![FIG 5](https://example.com/fig5.png)

**FIG 5** KatA-specific IgG antibody response in serum of mice following subcutaneous vaccination with recombinant HBsAg-KatA constructs (VLP-KatA) or recombinant HBsAg (VLP) alone using alhydrogel as an adjuvant. Serum IgG titers were determined by ELISA and measured at days 21, 33, and 41 post-primary vaccination. Results are shown as average antibody titers detected post-primary vaccination, with the standard error of the mean represented as a bar. The rise in VLP-KatA IgG titers 41 days postvaccination in serum from mice vaccinated with VLP-KatA was significantly greater than that of the titer observed for mice vaccinated with VLP alone \(P < 0.05\).

![FIG 6](https://example.com/fig6.png)

**FIG 6** KatA-specific IgG1 and IgG2a antibody responses in serum of mice following subcutaneous vaccination with recombinant HBsAg-KatA constructs (VLP-KatA) or KatA alone (KatA) using alhydrogel as an adjuvant. Results are shown as average antibody titers detected at 41 days post-primary vaccination, with the standard error of the mean represented as a bar. The increase in the IgG2a antibody titer induced by VLP-KatA compared to results for vaccination with KatA alone was significant \(P < 0.05\). The increase in the IgG1 titer induced by KatA alone was significantly greater than that induced with VLP-Kat \(P < 0.05\).
FIG 7 Bacterial numbers of *H. pylori* viewed per microscopic field in Giemsa-stained stomach tissue sections of recombinant HBsAg-KatA (VLP-KatA)-vaccinated mice challenged with the *H. pylori* SS1 strain, as described in Materials and Methods. Controls were recombinant HBsAg alone (VLP), KatA, and alhydrogel adjuvant alone. Bacterial load is expressed as bacterial load per microscopic field examined. The reduction in the bacterial load following vaccination with VLP-KatA compared to that with adjuvant alone was significant (*) [F value, 4.883, with df (3,36); VLP-KatA vs. alhydrogel has a t value of 3.748 with df 36 and a P value of <0.005).

VLP-KatA (3.65 x 10^3) were significantly higher than those for mice immunized with KatA alone (1.76 x 10^3) [F = 39.84, df (3,36); t = 4.818, df (36), with P < 0.05]. The IgG1 mean titers for mice immunized with KatA alone (5.96 x 10^3) were significantly higher than those for VLP-KatA vaccines (4.5 x 10^3) [F = 39.84, df (3,36); t = 3.716, with P < 0.05].

Following completion of the immunization schedule, mice were challenged with 10^7 CFU of *H. pylori* with the bacterial load in the stomach tissue determined 21 days postchallenge. Microscopic examination of stomach tissues revealed that mice vaccinated with VLP-KatAs (Fig. 7) had a significantly reduced bacterial load compared to that of the mice immunized with adjuvant alone [F = 4.883, df (3,36); t = 3.478, df (36), with P < 0.05]. While there was an apparent reduction in bacterial gastric colonization in mice vaccinated with KatA and VLP alone compared to results for immunization with the adjuvant, this was not significant at the P < 0.05 level. In addition to a decrease in the bacterial burden as determined by microscopic examinations, there was also a significant decrease in the bacterial CFU/g stomach tissue derived from VLP-KatA-vaccinated mice. Mice vaccinated with VLP-KatA with alhydrogel adjuvant had a mean of 2.2 x 10^3 CFU/g tissue, whereas in control mice immunized with adjuvant alone the bacterial load was 1.4 x 10^3 CFU/g tissue [F = 4.883, df (3,36); t = 3.748, df (36), with P < 0.05].

DISCUSSION

In this study, we examined the effect of parenteral vaccination with a recombinant HBsAg VLP expressing *H. pylori* KatA epitopes on the humoral response and gastric colonization in a mouse *H. pylori* challenge model. We have previously described the importance of catalase expression by *H. pylori* in establishing chronic infection in the face of an aggressive inflammatory response (22, 23). The KatA gene is expressed to protect the bacterium from oxidative damage following oxidative burst from phagocytic cells sequestered to the site of infection (22, 41, 46, 56). While *H. pylori* catalase appears to be located in the bacterial cytosol and possibly in the periplasmic space as well, vaccination with wild-type KatA is still able to elicit an effective and protective immune response, an observation that has been reported for a number bacterial species other than *H. pylori* (23, 46). In this study, we used a 130-amino-acid (aa) carboxyl-terminal sequence of the KatA protein which is unique to *H. pylori*. This approach, we believe, would significantly reduce the risk of cross-reactivity and the generation of immune complexes with either human or other bacterial catalase proteins (22, 23, 46).

However, most monomeric antigens, such as synthetic peptides, recombinant proteins, and viral subunit vaccines, are poorly immunogenic and generally require the use of adjuvants to induce an appropriate immune response (1a, 44). In this context, HBsAg VLPs, because of their spatial structure, are generally able to induce a more effective immune response than denatured particles or soluble proteins (9, 26). In addition, since HBsAg VLPs are able to self-assemble into particles, they can be produced in large quantities and due to their particulate structure are easily enriched and purified. HBsAg VLPs have been reported to elicit both epitope-specific B- and/or T-cell responses and have the potential to be administered without an adjuvant (38).

Mutational analysis of HBsAg assembly has demonstrated that insertion of foreign sequences is compatible with HBsAg VLP formation (35, 38). To enhance expression of our katA carboxyl-terminus-encoding DNA, the sequences were inserted into a unique restriction site of the small envelope protein of HBV (HBsAg-S) cDNA that encodes an exposed area of HBsAg-S, known as the major antigenic site or ‘‘a’’ determinant region. The purpose was to ensure surface orientation of the inserted putative B-cell epitopes. However, the procedure did not account for the spatial requirements in the native protein, and consequently the secretion efficiency could not be fully predicted and an observed reduction in HBsAg antigenicity was not unexpected (8, 9, 38). HBsAg is known to elicit a strong humoral response when used as a vaccine against hepatitis B virus infection in human subjects (25, 26, 39). Our data for IgG1 and IgG2a levels suggest that both Th1 and Th2 responses are elicited following immunization of mice with VLP-KatA constructs. We recognize that a Th1 bias may have been augmented by the incorporation of the alhydrogel adjuvant in the vaccine formulation. We were also able to demonstrate that the induced humoral response appears to confer a significant degree of protective immunity against *H. pylori* challenge, as shown by reduced gastric colonization following infection with the bacterium. Our findings are consistent with earlier reports that suggest induction of an IgG response is important in determining immunity to *H. pylori* infection (61). Because *H. pylori* is a mucosa-associated microbe, it could be anticipated that slgA would play a more significant role in immunity against infection with the bacterium. However, Gorrell and Robins-Browne, in a recent passive-immunity study, were able to demonstrate that the slgA concentration in gastric fluids was low and most likely was derived from swallowed saliva (20). Furthermore, they observed an equivalence between IgG and slgA levels in stomach contents, which suggested that the IgA accumulated in the stomach by leakage across the gastric mucosa rather than by active secretion. While the suboptimal levels of slgA may be mediated by the bacterium. However, Gorrell and Robins-Browne, in a recent passive-immunity study, were able to demonstrate that the slgA concentration in gastric fluids was low and most likely was derived from swallowed saliva (20). Furthermore, they observed an equivalence between IgG and slgA levels in stomach contents, which suggested that the IgA accumulated in the stomach by leakage across the gastric mucosa rather than by active secretion. While the suboptimal levels of slgA may be mediated by the bacterium. However, Gorrell and Robins-Browne, in a recent passive-immunity study, were able to demonstrate that the slgA concentration in gastric fluids was low and most likely was derived from swallowed saliva (20). Furthermore, they observed an equivalence between IgG and slgA levels in stomach contents, which suggested that the IgA accumulated in the stomach by leakage across the gastric mucosa rather than by active secretion. While the suboptimal levels of slgA may be mediated by the bacterium.

Our data also suggest that the VLP-Kat vaccination induces a Th1 polarization which is reflected by an increased IgG2a response, an observation that could be anticipated because of the use of alum hydroxide as an adjuvant and because native particulate...
VLPs, such as HBsAg, are known to elicit both humoral and cell-mediated immune responses (8, 38). While this study was undertaken using an acute challenge model of infection, an important issue relating to longevity of protection against infection remains to be resolved. For example, it has been demonstrated that in long-term protection, IL-12 may be an important correlate. Similarly, while the role of antibodies in protection against H. pylori infection remains contentious, there appears to be evidence that in long-term protection both Th1 and Th2 responses may play an important role (35).

In this proof-of-concept study, our aim was to demonstrate that bacterial peptides incorporated into the HBsAg carrier VLP expressed secretion-competent modified HBsAg proteins (VLP-KatA) and that vaccination with the VLP-KatA proteins was able to induce KatA-specific antibodies and protection against infection in immunized mice. In this study, we transfected HuH7 cells because of prior experience and the work of others reporting the generation of secretion-competent chimeric HBsAG VLPs with this cell line (39, 50). We conclude that this is the first report of the use of VLPs as a delivery vehicle for H. pylori KatA antigenic sequences. Finally, HBsAg presents as a safe delivery vehicle and warrants further studies of its potential use as a template for bacterial vaccine candidates.

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