Protective Efficacy of DNA Vaccines Encoding Outer Membrane Protein A and OmpK36 of Klebsiella pneumoniae in Mice

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The immunogenicity of DNA vaccines expressing outer membrane proteins as antigens was evaluated in this study. DNA vaccines consisting of vector pVAX1 expressing either outer membrane protein A or OmpK36 were injected into mice by either the intradermal or the intramuscular route. Antibodies elicited were shown to be specifically reactive to OmpA and OmpK36 by immunoblotting. The immunoglobulin G (IgG) antibodies elicited by both vaccines included IgG1, IgG2a, IgG2b, and IgG3. Immunized mice exhibited a predominance of IgG1 over IgG2a, therefore indicating a stronger humoral response. Mice receiving either of the DNA vaccines produced high levels of interleukin-12 (IL-12) and IL-10 and low levels of gamma interferon, suggesting the induction of a mixed Th1 and Th2 response. Sera from DNA vaccine-immunized mice had significantly higher opsonic activity in opsonophagocytic assays than did sera from the control mice. The level of protection afforded by pOmpK36 DNA injected intradermally into mice was the highest. These results suggest that both OmpA and OmpK36 are excellent candidates for use in future studies of vaccination against infections caused by Klebsiella pneumoniae. This is the first study which established the efficacy of protection afforded by DNA vaccines based on outer membrane proteins against K. pneumoniae infections.

Klebsiella pneumoniae is an opportunistic pathogen capable of causing bacterial pneumonia and lung tissue destruction in patients with severe underlying conditions, such as diabetes mellitus and chronic pulmonary obstruction (24). K. pneumoniae has been reported to develop resistance to beta-lactam antibiotics by producing beta-lactamases, and to counter this resistance, more stable expanded-spectrum beta-lactams, such as cephalosporins, monobactams, and carbapenems, were introduced for treatment (27). However, beta-lactamase-producing K. pneumoniae strains which showed resistance to cephalosporins, fluoroquinolones, and carbapenems have been isolated (23).

The limited efficacy of antibiotics and the widespread resistance to antibiotics call for the use of other approaches to combat this pathogen, such as selective vaccination of patients at risk. Vaccine research on K. pneumoniae has focused on using purified capsular polysaccharide (CPS) preparations (5), core lipopolysaccharides (LPSs) (21), cytotoxin toxoid (29), and whole-cell lysates (16). However, a certain degree of risk is involved in using these purified bacterial components for systemic injection, due to adverse toxic reactions caused by improperly purified components. LPS contents higher than 300 ng may cause toxicity, such as erythema and pyrogenic symptoms (11). A CPS or LPS preparation requires extensive processing of K. pneumoniae strains. Moreover, there are 77 different CPS serotypes in K. pneumoniae, and hence, the capsular antigen vaccine developed in Europe was not optimal for use in Taiwan, as it did not contain the serotypes that were the most predominant there, serotypes K1, K54, and K57 (8). Jeannin et al. observed in 2002 that antigen-presenting cells (APCs) recognize and are activated by recombinant outer membrane protein A (OmpA) from K. pneumoniae (13). Tumor antigens coupled to OmpA are taken up by APCs and gain access to the major histocompatibility complex (MHC) class I pathway, triggering the initiation of protective antitumor cytotoxic responses in the absence of CD4+ T cell help and adjuvant. Thus, OmpA appears to have a new type of pathogen-associated molecular pattern (PAMP) usable in vaccines to elicit cytotoxic T lymphocytes (CTLs). When polysaccharides derived from Streptococcus pneumoniae were conjugated to the OmpA derived from K. pneumoniae, they showed greater immunogenicity than polysaccharides alone, and the humoral response generated was able to protect mice from bacterial challenge (18). However, purification of OmpA is a laborious and difficult procedure, as OmpA is insoluble due to its highly hydrophobic nature. Therefore, DNA vaccination based on outer membrane protein (OMP) antigens is an attractive option. Our previous study has identified OmpA and OmpK36 to be the highly immunogenic OMPs which were reactive with sera from patients infected with K. pneumoniae (17).

DNA immunization, which involves direct injection of plasmid DNA encoding the antigen into mouse tissues, has fostered a new generation of novel vaccine development (4). Production of both humoral and cellular immune responses against selected target antigens has been successfully demonstrated in a wide variety of animal models of viral and bacterial diseases (6). The DNA vaccine encoding the outer membrane protein F of Pseudomonas aeruginosa was shown

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to protect mice from *P. aeruginosa* chronic pulmonary infection (25). Strong protection was also observed with a model with PBP 2a DNA-vaccinated mice infected with methicillin-resistant *Staphylococcus aureus* (26).

The aim of this study is to construct a DNA vaccine suitable for the prevention of *K. pneumoniae* infections. Genes encoding vaccine candidate antigens such as OmpA and OmpK36 were individually cloned into a plasmid vector and expressed in mice. The immunogenicity and protective efficacy of the two DNA vaccines were evaluated by administration through two different routes in the murine model.

**MATERIALS AND METHODS**

**Construction of the OmpA and OmpK36 DNA vaccines.** The pVAX1 plasmid (Invitrogen, CA), which contains an immediate-early cytomegalovirus promoter to ensure efficient expression in a eukaryotic host, was used in this study. The two DNA vaccines, pOmpA and pOmpK36, were constructed as follows. The two genes, ompA and ompK36, were individually PCR amplified from the *K. pneumoniae* clinical isolate using specific primers (ompK36 sense [5′-GGTTGGAATCCATGAAAGTAAATGCT-3′], OmpK36 antisense [5′-GCTCGTGCTTGAAGACTGTGAAAC-3′], ompA sense [5′-CTGAGAACCTGTAAGATCCTGCCAAAGATAAC-3′], and ompA antisense [5′-ATACTACAATCTTAAAGCCTGCGGCTGAG-3′]) which contained specific restriction sites (underlined) at their respective 5′ and 3′ ends. The restriction endonuclease (RE)-digested PCR products and the pVAX1 vector were ligated with T4 DNA ligase (Life Technologies, Gaithersburg, MD). The two recombinant plasmids, pOmpK36 and pOmpA, were transformed into *Escherichia coli* DH5α. Transformants containing the pOmpK36 and pOmpA recombinant plasmids were confirmed by RE digestions and sequencing of the respective inserts.

**Purification of DNA vaccine.** Recombinant OmpK36, OmpA plasmids, and the pVAX1 plasmid in the *E. coli* DH5α host were extracted using an Endofree plasmid megakit (Qiagen), according to the manufacturer’s instruction. A total volume of 500 ml of the overnight culture of each strain was harvested for each round of plasmid extraction. The plasmids obtained at the end of the protocol were resuspended in 1 ml of phosphate-buffered saline (PBS). The amount of purified plasmid DNA was measured in a spectrophotometer by determination of the absorbance of 260 nm, and the final concentration was adjusted to 1 μg/ml in sterile PBS.

**In vitro expression of DNA vaccine constructs in eukaryotic cells.** Transient transfection of rhabdomyosarcoma (RD) cells was carried out to confirm protein expression by both the pOmpA and pOmpK36 DNA vaccine constructs in eukaryotic cells. RD cells were grown in OPTI-MEM (Gibco) with 10% fetal bovine serum and 100 μg/ml of streptomycin-penicillin at 37°C in 5% CO2, on 24-well plates. Transfection was carried out using Lipofectamine (Invitrogen). Briefly, RD cells were incubated with 2 μl of Lipofectamine diluted in OPTI-MEM containing 0.4 μg of either the pOmpK36 or pOmpA plasmid. The pVAX1 plasmid was used as the negative control. The transfection mixtures were added into each of the respective wells, and the plates were incubated for 48 h before protein extraction. Following 48 h of incubation, cell supernatants and lysates were analyzed by immunoblotting to detect the synthesis of OmpA and OmpK36 expressed from pOmpA and pOmpK36, respectively. Standard immunoblotting techniques were carried out using rabbit antiserum against either His-OmpA or His-OmpK36 diluted 1:100. To assay for transfection efficiency, a plasmid carrying the lacZ gene (pVAX1/lacZ) was also used. 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) staining for the detection of LacZ expression was performed, and stained cells were observed using a microscope.

**Immunization.** Specific-pathogen-free (SPF) female BALB/c mice (Charles River Laboratories) were anesthetized by intraperitoneal (i.p.) injection of 100 μl of a cocktail containing metadomidine (1 mg/ml) and ketamine (100 mg/ml). Each mouse was immunized with 50 μl of DNA by either the intramuscular (i.m.) or the intradermal (i.d.) route. The mice were immunized at 2-week intervals for a total of four injections with either pOmpA or pOmpK36. Blood samples were taken 7 days after each injection. Ten mice were included per group. Mice from the control groups (groups 1 and 2) were injected with the parental vector pVAX1 intradermally and intramuscularly, respectively. Mice from groups 3 and 4 were injected i.m. with recombinant plasmids pOmpA and pOmpK36, respectively. Mice belonging to groups 5 and 6 were injected intradermally with the recombinant plasmids pOmpA and pOmpK36, respectively. Mice from group 7, which represented the negative control for the entire experiment, were injected with PBS.

**ELISAs.** Sera obtained from various groups of immunized and control mice were analyzed by enzyme-linked immunosorbent assay (ELISA) for reactivity against purified recombinant proteins OmpA and OmpK36. Each experiment was carried out twice. The wells of microtiter plates (Immulon 4; Dynatech) were coated with 10 μg/ml of recombinant protein OmpA or OmpK36. Antigens suspended in 0.1 M NaHCO3 coating buffer (pH 9.6) were added (50 μl) to each well of 96-well assay plates. The plates were left overnight at 4°C for efficient coating of the well with the antigens. On the following day, the plates were washed with wash buffer solution (1 × PBS, 0.05% Tween 20) using an ELISA washer (ELISA plate washer; Tecan, NC). Thereafter, the plates were blocked with 5% bovine serum albumin in 1% PBS-0.05% Tween 20 solution at room temperature for 1 h. After repeated washings, dilutions of test serum from each group comprising 10 mice were incubated in triplicate in the antigen-coated microtiter plate at 37°C for 1 h. A preimmune serum sample was used as a negative control. Bound immunoglobulin G (IgG) was detected by diluted (1: 1,000) alkaline phosphatase-conjugated goat anti-mouse IgG (Southern Biotechnology Associates Inc., Birmingham, AL) using p-nitrophenylphosphate (Sigma-Aldrich) as the substrate. Endpoint measurements for specific antibodies elicited in response to pOmpA or pOmpK36 or to pVAX1 as the negative control were measured by reading the absorbance (optical density at 405 nm [OD405]) in a microtiter plate reader (Microplate imaging system ELISA plate reader). The positive cut-off value was established at 1.5 times the optical density of the serum sample obtained from a mouse which received pVAX1 and which served as the negative control.

To define the type of immune response, the isotype profile of the antisera from the final bleed was identified by ELISA. Isotype-specific biotinylated anti-IgG secondary antibodies, such as anti-mouse IgG1, IgG2a, IgG2b, and IgG3 (mouse immunoglobulin screening/isotyping kit; Zymed Laboratories Inc., CA), were used to probe for the primary mouse antibodies bound to coating antigens, such as the purified recombinant outer membrane proteins A and OmpK36. Incubation with streptavidin-horseradish peroxidase followed by the addition of the 4-nitrophenylphosphate (Sigma-Aldrich) substrate resulted in a chromogenic development, which was quantitated by determination of the OD405 in the microtiter plate reader (Microplate imaging system ELISA plate reader).

**Opossumphagocytic killing assay.** The opossumphagocytic killing assay mixture contained 100 μl of heat-inactivated mouse serum (neat), 100 μl of bacterial cell preparation (106 CFU), 100 μl of rabbit serum as a complement source, and 700 μl of a human peripheral blood polymorphonuclear leukocyte (PMN) preparation containing approximately 106 cells. For the control reaction, Hanks’ balanced salt solution was added in place of the mouse serum. The reaction mixture was incubated with agitation at 37°C for 1 h. After the reaction, an aliquot was removed and diluted with 0.9% saline, and the diluted aliquot was spread on nutrient agar plates in triplicate. The plates were incubated at 37°C overnight, and the number of colonies grown was counted on the next day. The mean number of colonies was calculated for each reaction mixture. The percent phagocytosis was calculated.

**Challenge studies.** For protection studies, 10 mice from groups 1 to 7 were challenged i.p. at 2 weeks after the last immunization with 107 CFU (the lethal dose [LD50] of *K. pneumoniae*) in 100 μl of 0.9% saline. Mice (n = 10) injected with pVAX1 were used as the negative-control group. Eight days after the challenge, the surviving mice were evaluated for the protection efficacy of the respective DNA vaccine. Every day the animals were observed for signs of severe illness, such as weight loss, change in coat appearance, and lack of movement. On day 14 all remaining mice were terminated to collect blood for opossumphagocytic assay.

**Statistical analysis.** The nonparametric Mann-Whitney test was used for comparison between groups in all in vitro experiments. P values of ≤0.05 were considered significant. Survival estimates and median survival times were determined using the method of Kaplan-Meier. One-way analysis of variance (ANOVA) was used to evaluate the antibody response.

**RESULTS**

**In vitro expression of DNA vaccine in eukaryotic cells.** A positive control for the transfection protocol was carried out using a pLAcZ reporter plasmid. RD cells which were transfected with the pLAcZ plasmid using the transfection protocol stained positive for β-galactosidase intracellularly using X-Gal.
as the substrate (data not shown), thus confirming that transfection of the RD cells was successful.

The ability of recombinant plasmids pOmpA and pOmpK36 to express their respective antigens was evaluated in RD cells. Following transfection of RD cells, cell lysates were subjected to immunoblotting with the antibodies against the two separate recombinant antigens produced in rabbits. Anti-OmpA and anti-OmpK36 antibodies were able to recognize the proteins expressed by RD cells transfected with the appropriate plasmid (Fig. 1). This confirmed that the plasmids could drive functional expression of the cloned bacterial genes in eukaryotic cells. When RD cells were transfected with the pVAX1 control plasmid, no protein bands were observed by Western blotting (data not shown).

**Immune response in mice following DNA vaccination.** Western blot analysis confirmed that the sera obtained from mice vaccinated with either the pOmpA or the pOmpK36 DNA showed specific protein bands (OmpA or OmpK36) when they were probed with the respective recombinant outer membrane protein (Fig. 2). Antibodies from the pOmpA-immunized mice were immunoreactive against the OmpA of *K. pneumoniae* (Fig. 2, lanes 2 and 3). Similarly, antibodies from pOmpK36-immunized mice were found to be highly reactive against the OmpK36 of *K. pneumoniae* (Fig. 2, lanes 4 and 5). When preimmune sera or sera from the group of mice which received only the pVAX1 plasmid DNA or PBS were used in the Western blot, no immunoreactive protein bands were observed (Fig. 2, lanes 6 and 7).

**Analysis of total IgG response to DNA vaccination.** Sera recovered from various groups of DNA vaccine-immunized and control group mice were analyzed by ELISA for reactivity against their specific recombinant outer membrane proteins, OmpA and OmpK36. All the immunized mice (10 per group) showed significant production of antibodies compared to that by their control groups ($P < 0.0002$). The route of DNA vaccine administration had a significant impact on the total IgG production (Fig. 3). Mice that received either of the DNA vaccines, pOmpA or pOmpK36, by the intradermal route had significant ($P < 0.0001$) levels of IgG compared to those in the mice that received either of the vaccines by the intramuscular route. With both the pOmpA and the pOmpK36 DNA vaccines, significant increases in IgG titers in sera from mice were observed after each successive booster, given at the 2nd, 4th, and 6th weeks (data not shown) and the 8th week (Fig. 3). None of the mice injected with PBS or with the pVAX1 vector intramuscularly or intradermally showed any significant IgG levels.

**Antibody isotype profiles elicited by DNA vaccines.** Mice vaccinated with the pOmpA or the pOmpK36 plasmid DNA

![FIG. 1. Expression of ompA and ompK36 genes in eukaryotic cells. Anti-OmpA and anti-OmpK36 antibodies were able to recognize the OmpA and OmpK36 proteins expressed by the transfected RD cells by immunoblot analysis. Lanes 1, lysed RD cells that had been transfected with pOmpA plasmid (A) or pOmpK36 plasmid (B); lanes M, molecular mass markers.](https://example.com/figure1)

![FIG. 2. Western blot analysis of sera raised by DNA vaccination with either pOmpA or pOmpK36. This is a composite of multiple blots. Lane 1, molecular mass marker; lane 2, sera obtained from mice immunized with the pOmpA DNA vaccine intradermally; lane 3, sera obtained from mice immunized with the pOmpA DNA vaccine intramuscularly; lane 4, sera obtained from mice immunized with the pOmpK36 DNA vaccine intradermally; lane 5, sera obtained from mice immunized with the pOmpK36 DNA vaccine intramuscularly; lanes 6 and 7, sera obtained from mice immunized with the pVAX vector by the intradermal and intramuscular routes, respectively. Bands corresponding to the OmpA (35-kDa) and OmpK36 (36-kDa) proteins are indicated.](https://example.com/figure2)
developed specific antibodies belonging to isotypes IgG1, IgG2a, IgG2b, and IgG3. The subclass distribution of serum IgG1 and IgG2a antibodies was examined during the course of immunization and was used as an indicator of the type of immune response induced (Th1 versus Th2). Very high levels of IgG1-specific antibodies and significant levels of IgG2a-specific antibodies were induced in mice immunized intradermally or intramuscularly with either pOmpA or pOmpK36 (Fig. 4). There were also significant increases in the levels of serum IgG2b- and IgG3-specific antibodies in all the mice receiving pOmpA or pOmpK36 compared to those in the group of mice receiving the pVAX1 vector by either route \((P < 0.05)\). Mice receiving the pOmpA DNA intramuscularly had higher IgG2a levels \((P < 0.02)\) than those receiving the plasmid DNA intradermally, reflecting a shift toward a Th1 response compared to that in the group of mice receiving pVAX1 either intramuscularly or intradermally. No significant difference in IgG2a levels was observed when mice were injected with pOmpK36 either intradermally or intramuscularly (Fig. 4). Both the IgG1 and IgG2a isotype levels increased with each of the boosting regimens. The data are consistent with induction of both a Th1 and a Th2 response by either of the DNA vaccines.

Cytokine production in response to DNA vaccines. Sera from mice immunized with either the pOmpA or the pOmpK36 DNA vaccines were analyzed for the cytokines gamma interferon (IFN-\(\gamma\)), interleukin-12 (IL-12), tumor necrosis factor alpha (TNF-\(\alpha\)), IL-2, IL-5, and IL-10. Mice immunized with both DNA vaccines intradermally produced the highest levels of IL-12, TNF-\(\alpha\), and IL-5. Sera obtained from mice immunized with either of the DNA vaccines (pOmpA or pOmpK36) intramuscularly had higher levels of IFN-\(\gamma\) cytokines than sera obtained from mice receiving the DNA vaccine intradermally. However, the levels of IL-12, IFN-\(\gamma\), TNF-\(\alpha\), IL-2, IL-5, and IL-10 were elevated in mice receiving the DNA vaccine compared to the levels in mice receiving only the pVAX1 DNA by either route \((P < 0.05)\) (Fig. 5). Overall, mice immunized with DNA vaccine by the intradermal route had significant levels of proinflammatory cytokines such as IL-12 and TNF-\(\alpha\). In comparison, mice vaccinated by the intramuscular route had significant levels of IFN-\(\gamma\) \((P = 0.0001)\), a cytokine produced by T cells. Comparison of the responses to the two DNA vaccines showed that the pOmpK36 DNA vaccine induced higher levels of IL-12, IL-5, and TNF-\(\alpha\) cytokines than the pOmpA DNA vaccine (Fig. 5). The results showed that immunization with the ompA and ompK36 DNA vaccines induced a mixed Th1 and Th2 response. However, further evidence for the roles of these cytokines in protection need to be analyzed.

Analysis of protection elicited by DNA vaccine in mice. To investigate the efficacy of DNA vaccination against \(K.\) pneumoniae, mice immunized with the pOmpA or pOmpK36 vaccine or pVAX1 DNA were challenged with \(10^6\) CFU (the LD) of an ESBL-producing \(K.\) pneumoniae clinical isolate. The levels of infection were evaluated by measuring the numbers of CFU in the blood of mice surviving the challenge. Protection experiments demonstrated that mice given DNA vaccine pOmpA \((P = 0.0081)\) or pOmpK36 \((P = 0.0032)\) by the intradermal route had a significantly higher degree of protection than did mice from the control groups receiving PBS or the pVAX1 vector. All the 10 mice from the two negative-control groups used in this study died after being challenged with \(10^8\) CFU of \(K.\) pneumoniae (Fig. 6). Mice receiving the two DNA vaccines by the i.m. route had significantly lower levels of protection than those receiving the vaccines intradermally. However, the levels of protection were significantly higher than those observed with the two negative-control groups (Fig. 6). These results indicated that the pOmpK36 DNA vaccine given by the i.d. route afforded 100% protection against \(K.\) pneumoniae infection.

Opsonophagocytic killing assay. An opsonophagocytic uptake assay using mouse immune sera was performed. The antibodies raised in mice immunized with the pOmpK36 DNA vaccine intradermally were capable of promoting 95.2% phagocytic killing of ESBL-producing \(K.\) pneumoniae. A lower phagocytic killing rate, at 82.4%, was observed in sera obtained from mice receiving pOmpK36 intramuscularly. Similarly, the antibodies raised in mice immunized with the pOmpA vaccine intradermally were capable of promoting higher phagocytic killing of ESBL-producing \(K.\) pneumoniae, at
78.5%, than antibodies raised in mice receiving pOmpA intramuscularly (62%). The sera obtained from the pVAX1-immunized mice did not show much higher phagocytic activity against *K. pneumoniae* than the control sera from mice injected with PBS alone (Fig. 7).

**DISCUSSION**

Interest in vaccine development has been rekindled over recent years due to the failure to treat infections caused by multiresistant bacteria. New technological developments such as immunoproteomics provide the platform for identifying appropriate immunogenic proteins for new vaccine design. Recombinant technology has enabled researchers to develop molecular vaccines which have been shown to elicit both cellular and humoral responses (7, 20).

We have shown that both OmpA and OmpK36 are strongly immunogenic (17), and in this investigation, the gene encoding each of the antigens was cloned in a eukaryotic plasmid which was administered as a DNA vaccine. Our results confirmed that recombinant pVAX1 plasmids containing either the *ompA* or the *ompK36* gene were able to express the respective protein in the mammalian cells. OmpA and OmpK36 antibodies were detected in the sera of animals vaccinated with the pOmpA or the pOmpK36 plasmids.

The IgG subclass of antibodies produced against the DNA vaccine was determined to be predominantly of the IgG1 isotype, which is indicative of a Th2 response. Antibodies are particularly effective against extracellular microbes and in neutralizing microbial toxins through several mechanisms, which differ depending on the particular Ig isotype. For example, IgG binding to specific antigenic determinants (epitopes) present in the infectious organisms leads to opsonization and increased phagocytosis by macrophages and neutrophils, which in turn leads to killing of the microbes. Furthermore, antibody binding to microbes might also result in bacterial cell lysis through a...
mechanism known as antibody-dependent cell-mediated cytotoxicity (ADCC). In ADCC, recognition of pathogens coated with IgG via Fc receptors present on the effector cells (e.g., neutrophils, macrophages, and NK cells) results in destruction of the microbes by release of lytic proteins stored in the granules of the effector cells (31). Binding of human IgG1, IgG3, and IgM to microbes also activates the complement pathway, which leads to lysis of the pathogenic organisms and promotes opsonization and enhanced phagocytosis.

The cytokine profiles of the immunized mice in the present study indicated that the ompA or the ompK36 DNA vaccine administered either intradermally or intramuscularly elicited mixed Th1 and Th2 responses. A Th1 response was shown by increased production of IgG2a as well as elevated levels of IL-12, IL-2, and IFN-γ cytokine production in comparison with those in mice receiving only pVAX1. The antibodies elicited from DNA immunization with pOmpK36 intradermally provided 100% protection, as immunized mice were able to survive a lethal challenge, while mice receiving the pVAX1 vector alone did not ($P < 0.0032$).

The highly immunogenic protein OmpK36 is a porin protein which serves as a diffusion barrier to extracellular solutes and which interacts with the bacterial environment. The OmpK36 porin is generally recognized to be highly conserved among strains (2). An example of a related protein which was used as a successful DNA vaccine is the Pseudomonas aeruginosa outer membrane protein F (OprF), which has been well tested in mice (25). The role of K. pneumoniae porins in pathogenesis has not yet been studied in detail. The porin complex contains two distinct proteins, K35 and K36, according to their molecular masses of 35,000 and 36,000 Da, respectively (10). It has been shown that porins can form a complex with C1q, the first component of the classical pathway of the complement system, and that they can activate the complement cascade, a property observed neither with other OMPs nor with rough LPS (1, 3).

All of the enterobacterial porins tested to date have been shown to bind C1q (19). The formation of porin-C1q complexes activates the complement classical pathway. Together with an activated complement alternative pathway, the result is the effective elimination of serum-sensitive K. pneumoniae strains (3). Since this interaction is antibody independent and K. pneumoniae is an important pathogen for immunocompromised hosts and infants, this may represent a relevant mechanism for the elimination of serum-sensitive strains and for the protection of susceptible hosts.

Gram-negative bacteria express OMPs such as OmpA that activate innate cells via Toll-like receptors (TLRs) (30). OmpA is one of the major OMPs that is highly conserved among the Enterobacteriaceae family and is involved in bacterial virulence and growth (15, 22). It contributes to the ability of Gram-negative bacteria to invade mammalian cells, as OmpA-deficient Escherichia coli exhibits attenuated virulence (32). Unlike soluble proteins that induce a weak immune response when administered in the absence of adjuvant, OmpA from several species could induce specific humoral and cytotoxic responses in the absence of adjuvant (14). OmpA from K. pneumoniae has potent carrier properties, and it has been incorporated in the design of anti-infectious vaccines suitable for nasal immunization (9, 26). In 2000, Jeannin et al. analyzed the interactions between OmpA and the antigen-presenting cells (APCs), and their data indicate that OmpA interacts with dendritic cells.
(DCs) and macrophages, thereby suggesting that the immune system has acquired the ability to recognize this new type of PAMP (12). OmpA also favors antigen internalization and cross-presentation by APCs and therefore appears to be a new protein that is able to induce CTLs in the absence of CD4+ T cell help and adjuvant. OmpA-mediated antigen cross-presentation is of great interest for antigen delivery and CTL induction. In this investigation, both the ompA and ompK36 DNA vaccines were administered to mice in the absence of any adjuvant. The strong humoral and cellular immune responses observed could be aided by Omps that were readily taken up by dendritic cells which upon maturation secrete IL-12.

In conclusion, we have shown that vaccination with plasmid DNA containing the ompK36 or the ompA gene leads to production of humoral antibodies and also promotes a Th1 cell-mediated immune response. The IgG response was predominantly of the IgG1 isotype, and IgG2a was produced at a lower level. The opossumphagocytic antibodies elicited by pOmpK36 injected intradermally into mice provided the highest level of protection against challenge by a lethal dosage of K. pneumoniae. On the basis of these findings, genetic immunization with OmpA and OmpK36 has the potential to be developed as a DNA vaccine to protect humans against infections caused by K. pneumoniae.

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