Protective Immunity Conferred by the C-Terminal Fragment of Recombinant *Pasteurella multocida* Toxin

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*Pasteurella multocida* serogroup D, producing *P. multocida* toxin (PMT), is a causative pathogen of progressive atrophic rhinitis (PAR) in swine. To evaluate the protective immunity and vaccination efficacy of the truncated form of PMT, a C-terminal form of recombinant PMT (designated PMT2.3; amino acid residues 505 to 1285 of PMT) was expressed in an *Escherichia coli* expression system, and the humoral and cellular immune responses to PMT2.3 were investigated. PMT2.3 vaccination in mice led to high levels of the anti-PMT antibody with a high neutralizing antibody titer. PMT2.3 also induced a cellular immune response to PMT, as demonstrated by the lymphocyte proliferation assay. Furthermore, strong protection against a homologous challenge with *P. multocida* was also observed in mice vaccinated with PMT2.3. In PMT2.3 vaccination in swine, high levels of serum antibody titers were observed in offspring from sows vaccinated with PMT2.3. Offspring from sows vaccinated with PMT2.3 or toxoid showed a good growth performance as depicted by mean body weight at the time of sacrifice, as well as in average daily gain in the postweaning period. Low levels of pathological lesions in turbinate atrophy and pneumonia were also observed in these offspring. Therefore, we consider PMT2.3—in the truncated and nontoxic recombinant PMT form—to be an attractive candidate for a subunit vaccine against PAR induced by *P. multocida* infection.

*P. multocida*, a Gram-negative bacterium, can infect a wide range of animal species. This pathogen is considered an etiological agent of numerous economically important diseases, including pneumonia and hemorrhagic septicemia in cattle, sheep, and goats, fowl cholera in chicken, and progressive atrophic rhinitis (PAR) in swine (7, 8, 30). Significant global economic losses in animal production caused by growth retardation and nutritional deficiencies are often due to respiratory diseases induced by *P. multocida* infections. Therefore, the protection of domestic animals by efficient vaccination has been considered the most important and attractive method for controlling these animal diseases (7, 16, 25).

Many *P. multocida* serogroup D strains produce *P. multocida* toxin (PMT), a dermonecrotic toxin, which is responsible for the clinical signs of PAR in swine. The signs of PAR usually appear by 8 to 12 weeks of age, and the disease progresses throughout the growing period. The most characteristic lesion is severe atrophy of the nasal turbinate bones accompanied by lateral deviation or shortening of the nose (6, 17, 18).

It has been reported that inoculation of both purified native and recombinant PMT without the pathogen can induce all major clinical signs of PAR in swine. The signs of PAR usually appear by 8 to 12 weeks of age, and the disease progresses throughout the growing period. The most characteristic lesion is severe atrophy of the nasal turbinate bones accompanied by lateral deviation or shortening of the nose (6, 17, 18).

It has been reported that inoculation of both purified native and recombinant PMT without the pathogen can induce all major clinical signs of PAR in experimentally challenged swine (12). Thus, PMT has been considered a suitable, effective molecule for vaccination (22). However, it has also been reported that native PMT is a poor immunogen and can be rendered more antigenic by the destruction of its native activity (29). Therefore, truncated and/or partial forms of PMT may serve as efficient immunogens to systemically stimulate a protective immune response without cytotoxic effects in animals.

It has been reported that nontoxic PMT derivatives with a short deletion could induce effective protection against *P. multocida* infection in swine (22). According to a recently published report by Seo et al., a shorter N-terminal fragment (residues 1 to 390) was found to be immunogenic and it induced effective protection (26, 27). However, our previous study suggested that the N-terminal region of PMT (residues 1 to 483) had relatively poor immunoreactivity to the antisera from mice immunized with PMT, as well as the antisera from infected swine. Additionally, protection against the homologous challenge could not be obtained by immunization with the N-terminal region of PMT. Furthermore, PMT2.3, which is a large portion of the C terminus corresponding to intracellular activity, showed high immunoreactivity to the antisera from infected swine in our previous study (15).

Therefore, in this study, we investigated the immune responses and protective immunity conferred by nontoxic PMT2.3 in mice. We then evaluated the practical efficacy of vaccination with the recombinant protein through passive transfer of maternal immunoglobulins in swine. The growth performances of their offspring were also observed.

**MATERIALS AND METHODS**

**Bacterial strain, recombinant PMT2.3, and detoxified PMT.** The pathogenic *P. multocida* strain used in this study was isolated from swine suffering from severe PAR in South Korea. This strain was shown to be identical to strain P-934, which has been previously characterized as serogroup D and serotype 4 (13). The culture condition of bacteria was as described previously (15).

A 2.3-kb XhoI-PstI fragment encoding amino acids 505 to 1285 of PMT was cloned into pRSET C to generate a PMT2.3 clone for expression.
The cloning and construction of the expression vector for PMT2.3 were performed as described previously (15). The recombinant plasmid for PMT2.3 expression was transformed into *Escherichia coli* BL21(DE3) for overexpression. The culture conditions and procedures for purification of recombinant PMT2.3 were as described by Lee et al. (14, 15).

Crude extract of PMT was prepared from a *P. multocida* strain cultured in brain heart infusion (BHI) medium at 37°C for 24 h, and the procedures for purification were as described previously (4, 19). Purified PMT extract was detoxified by shaking with 0.3% (vol/vol) formalin for 48 h at 37°C, and detoxification was confirmed by investigating PMT-induced cytotoxic effects in Vero cells (4).

**Immunization and challenge in mice.** The animal experiments were performed under the control of the animal welfare committee of Seoul National University Institutional Animal Care and Use Committee (SNU1ACUC) in accordance with the laboratory’s animal ethics guidelines. At the end of the experiments, the animals were euthanized.

Fifty 6-week-old female BALB/c mice (Charles River Laboratories, MA) were randomly distributed into 5 groups of 10 mice each and immunized with phosphate-buffered saline (PBS) as a negative control or with the commercial vaccine for PAR (inactivated whole cells of *Bordetella bronchiseptica* and *P. multocida* with toxoid; Daesung Microbiological Labs, Co.), formalin-killed *P. multocida* isolate (bacterin), detoxified PMT (toxoid), or PMT2.3. Mice were subcutaneously injected with 50 μg of each antigen emulsified with an equal volume of complete Freund’s adjuvant (CFA) and incomplete Freund’s adjuvant (IFA) at the first immunization (day 0) and the second immunization (day 21), respectively. The commercial vaccine was administered at 1/20 of the stated swine dose as recommended by the manufacturer. The procedures and schedule for bleeding and challenge were as described previously (14). Briefly, on day 10 after immunization, blood was collected from the immunized mice by tail bleeding; the sera recovered by centrifugation were stored at −20°C for further assays. For the protection study, the immunized mice were intraperitoneally challenged with a 10–median 50% lethal dose (LD$_{50}$) of homologous *P. multocida* isolate (4.3 × 10$^4$ CFU) 2 weeks after the second immunization. The LD$_{50}$ was determined as described previously (14). Mortalities of the challenged mice were monitored for 4 days, and their survival rates were recorded.

**Immunization and challenge in swine.** In the swine experiments, 12 pregnant sows (crossbreed of Yorkshire and Landrace) used to produce three-way cross hybrid piglets (Yorkshire × Landrace × Duroc) were selected from commercial farms. They were randomly divided and immunized with PBS as a negative control (group A; 2 sows) or with the commercial vaccine for porcine respiratory disease (group B; 2 sows), toxoid (group C; 4 sows), or PMT2.3 (group D; 4 sows). Before the experiment, all sows were examined to determine whether they were preinfected with *P. multocida* and *B. bronchiseptica*. Pregnant sows were intramuscularly vaccinated twice at 5 and 2 weeks before parturition. One dose of the injection comprised 15 mg of PMT2.3 in 10% aluminum hydroxyl gel in group D and the equivalent amount of toxoid in group C. The administration of the commercial vaccine in group B was performed according to the manufacturer’s instructions.

Newborn piglets from sows were fed colostrum within 30 min after birth and kept nursing during the preweaning period (3 weeks), and then they were weaned at 4 weeks after birth. All offspring were intranasally challenged with the homologous strain of *P. multocida* (1.0 × 10$^8$ CFU) and *B. bronchiseptica* (1.0 × 10$^8$ CFU), which was isolated from specimens of Korean pigs with PAR and identified by biochemical tests and Vitek (Hazelwood, MO), an automatic bacterial identification system, at 3 weeks of age (10). Serum samples were collected from every piglet at 1, 3, 5, and 8 weeks after birth. The antibody titers were measured using an indirect enzyme-linked immunosorbent assay (ELISA) for PMT. The body weight of the piglets was measured at days 7, 21, and 148 after birth.

At day 148 after birth, the offspring were euthanized and snouts of these animals were transversely sectioned at the level of the first premolar tooth. The degree of nasal turbinate atrophy of each was graded and recorded as 0 to 4 as follows: 0, no atrophy; 1, mild atrophy with less than half of the turbinate scroll bone missing; 2, moderate atrophy with half or more of the turbinate scroll bone missing; 3, severe atrophy in which the turbinate scroll is straight and only a small portion remains; and 4, complete atrophy with no turbinate scroll bone remaining (24). The degree of pneumonia was scored from 0 (normal) to 100 (complete pneumonia) based on the visual percentage of lung lesions.

**Indirect ELISA for determination of anti-PMT antibody.** Anti-PMT antibodies in the sera were titrated by indirect ELISA. PMT was used as an antigen, and the procedures were as described previously (15). In case of titration of antibody in the offspring, the relative antibody concentration was calculated by the following equation: [OD value of experiment − OD value of negative control]/OD value of negative control (where OD is the optical density). The value was presented as a percentage.

**ELISPOT assay for anti-PMT antibody secretion cells.** Lymphocytes were isolated from the spleens of immunized mice. A 96-well plate was coated with PMT (0.1 μg/ml) and lymphocytes (5 × 10$^5$ cells per well) were used. Biotin-conjugated anti-mouse IgG, horseradish peroxidase (HRP)-streptavidin conjugate, and the substrate DAB (3,3′-diaminobenzidine; Sigma-Aldrich Co.) were used for the enzyme-linked immunosorbent spot (ELISPOT) assay. General procedures and spot counting were as described elsewhere (16, 31).

**Serum neutralization assay.** The titers of the neutralizing antibody were measured based on the ability of the antibody to inhibit PMT-induced cytotoxic effects in Vero cells (21). Sera from immunized mice were serially diluted with Dulbecco’s modified Eagle’s medium (DMEM) and transferred to microplates (50 μl/well), followed by the addition of an equal volume of DMEM containing a 4-fold minimal toxic dose (MTD) of PMT. The plates were incubated at 37°C for 1 h, and 100 μl of Vero cells (1 × 10$^5$ cells) were added to each well. The plates were then incubated at 37°C in 5% CO$_2$ for another 5 to 7 days. The endpoint titer was measured as the highest dilution of sera that could neutralize 4-fold MTD of PMT.

**Lymphocyte proliferation assay.** For the measurement of the cell-mediated immune response specific to PMT, a lymphocyte proliferation assay was performed in vitro. Mononuclear cells from the immunized mice were isolated and seeded onto 96-well plates (5 × 10$^5$ cells per well). PMT (10 μg/ml) was added to each well, and the plate was incubated at 37°C in 5% CO$_2$ for 72 h. After the addition of 1 μCi of $[^3]$H thymidine ($[^3]$HdTdr) per well, the cells were incubated for an additional 18 h and then harvested onto filter strips for determination of incorporation. The results were presented as counts per minute (cpm) corrected for background proliferation in the absence of PMT (3).

**Statistical analysis.** The statistical significance of variation among different groups was determined by one-way analysis of variation (ANOVA). Student’s two-tailed t test was used to estimate the variance parity between the experimental and control groups. Differences were considered statistically significant at P values of <0.05.

**RESULTS**

**Humoral immune response in mice.** The titers of the anti-PMT antibody, following the immunization of mice, were measured by indirect ELISA using PMT as an antigen. The IgG levels in mice immunized with the commercial vaccine, bacterin, toxoid, and PMT2.3 increased after the second immunization and after the challenge (Fig. 1A). Both toxoid-immunized mice and PMT2.3-immunized mice showed significant differences of IgG levels between immunization and postchallenge ($P < 0.05$). PMT2.3-immunized mice showed relatively higher levels of antibody production than the other groups for both immunization and postchallenge.

The numbers of anti-PMT antibody secretion cells isolated from the spleens of immunized mice were measured by ELISPOT assay (Fig. 1B). Although a few anti-PMT antibody-secreting cells were detected in all experimental groups before immunization,
the number of cells increased after the second immunization in all groups except in the controls, and a statistically significant difference ($P < 0.05$) was observed between the PBS control and each experimental group. After homologous challenge, a greater number of cells were detected in all the experimental groups compared to the postimmunization data, and the highest increase in the cell numbers was observed significantly in the PMT2.3 group compared to the other groups ($P < 0.05$).

**Cellular immune response in mice.** $[^3]$H]TdR incorporation in the lymphocytes cultured in the presence of PMT was observed in each experimental group after immunization, except in the PBS group (Fig. 2); however, a relatively lower increase in the proliferation was noted in the toxoid-immunized group. Among the experimental groups, a statistical difference was observed only between the bacterin and toxoid groups ($P < 0.05$). After the pathogen challenge, greater proliferation of PMT-specific lymphocytes was detected in all experimental groups. However, the proliferation of lymphocytes in the commercial vaccine and toxoid groups was relatively lower than in the others. A statistically significant difference in lymphocyte proliferation after the challenge was observed between the commercial vaccine group and the bacterin group ($P < 0.05$).

**Protective immunity conferred by PMT2.3 in mice.** The titer of the PMT-specific neutralizing antibody was also measured by its ability to inhibit the cytopathic effects induced by PMT in Vero cells (21). The mean titers of the neutralizing antibody in mice immunized with PMT2.3 (1:240) were higher than in those immunized with the commercial vaccine (1:40), bacterin (1:80), and toxoid (1:120) after the pathogen challenge (Table 1). In the protection study, the highest survival rate (90%) was observed in the PMT2.3-immunized mice, followed by the bacterin (80%), toxoid (70%), and commercial vaccine (50%) groups. The survival rate of the negative control (PBS group) was 0. The survival rates of all the experimental groups were statistically significant compared to the negative controls ($P < 0.05$); however, no significant differences were observed in the survival rates among the groups.

**Serum antibody titer in offspring piglets.** The numbers of offspring used in this study were 20 for PBS (group A), 16 for the commercial vaccine (group B), 35 for toxoid (group C), and 39 for PMT2.3 (group D). The serum antibody titers were measured using indirect ELISA, and the relative antibody concentration was calculated (Fig. 3). Relative antibody concentration values were high at the beginning of colostrum feeding and decreased with the increasing age of the piglets. Only the PMT2.3 group showed a significantly high level of relative antibody concentration in the

### TABLE 1 Evaluation of protection conferred on immunized mice against *P. multocida* challenge

| Group | Type of vaccine | Mean titer of neutralizing antibody | Survival rate (%)$^a$ |
|-------|----------------|------------------------------------|-----------------------|
| 1     | PBS            | 1:4                                | 0                     |
| 2     | Commercial vaccine | 1:40                           | 50$^*$                |
| 3     | Bacterin       | 1:80                               | 80$^*$                |
| 4     | Toxoid         | 1:120                              | 70$^*$                |
| 5     | PMT2.3         | 1:240                              | 90$^*$                |

$^a$ The survival rate was calculated as the ratio of living mice to total mice challenged in a group 4 days after the challenge. Statistical significance ($P < 0.05$) compared with negative control (group 1) is indicated with an asterisk.
The antibody titers were measured from serum samples collected from every piglet at 1, 3, 5, 8, and 12 weeks after birth. The challenge with virulent *P. multocida* (1.0 × 10^6 CFU) and *B. bronchiseptica* (1.0 × 10^6 CFU) was performed at 3 weeks of age. Relative antibody concentration was calculated by the following equation: \( \frac{\text{OD value of experiment} - \text{OD value of negative control}}{\text{OD value of negative control}} \). Statistical significance between the PMT2.3 group and other groups is indicated with an asterisk \((P < 0.05)\).

**Growth and disease status of offspring.** The initial mean body weight of the offspring in each group was not statistically different (Table 2). The mean body weight of the offspring from the commercial vaccine group (7.11 kg) was significantly higher than that of the other groups \((P < 0.05)\) at the beginning of weaning (21 days). At day 148, the offspring from the toxoid and PMT2.3 groups showed significantly higher mean body weight than the other offspring. Similar patterns were observed in the average daily gain (ADG). Offspring from the commercial vaccine group had significantly higher ADG than the other offspring in the preweaning period, and offspring in the toxoid and PMT2.3 groups had significantly higher ADG than the others in the postweaning period \((P < 0.05)\).

The turbinate atrophy and pneumonia scores of offspring were measured at day 148 (Table 3). The mean value of the turbinate atrophy score for each experimental group was significantly lower than that of the negative PBS controls, and the lowest score was noted in the PMT2.3 group. Furthermore, the mean value of the pneumonia scores of each experimental group was significantly lower than that of the PBS group. No statistically significant difference was observed among the experimental groups for mean turbinate atrophy scores and pneumonia scores.

**TABLE 2 Growth status of the offspring at preweaning and postweaning**

| Sow group | Type of vaccine in sows | Mean body wt (kg) at: | Avg daily gain (g) | 6 weeks | 12 weeks | Postweaning | 
|-----------|-------------------------|----------------------|-------------------|---------|----------|-------------| 
| A PBS     |                         | 2.8 6.49 92          | 264 673           |         |          |             | 
| B Commercial vaccine | 2.9 7.11** 99  | 301*** 724          |                   |         |          |             | 
| C Toxoid  |                         | 2.6 6.38 126**      | 270 942****       |         |          |             | 
| D PMT2.3  |                         | 2.9 6.58 125**      | 263 932****       |         |          |             | 

\( ^a \) Significant difference \((P < 0.05)\) of the mean body weight among groups is indicated with one asterisk (at 21 days) or double asterisks (at 148 days). Significant difference \((P < 0.05)\) of the mean value of ADG among groups is indicated with triple (preweaning) or quadruple (postweaning) asterisks.

**DISCUSSION**

The 146.5-kDa PMT is encoded by the toxA gene of the *P. multocida* serogroup D strain (22). PMT is an essential virulence factor associated with PAR, and inoculation of PMT alone can reproduce the clinical signs of PAR (12). The molecular mechanisms of the virulence of PMT remained poorly understood; however, its association with the activation of the proliferation of osteoclasts and the inhibition of osteoblasts has been suggested (6, 18). PMT is also a putative mitogen that induces DNA synthesis and proliferation of fibroblasts and osteoblasts at very low concentrations (18) (23). PMT has many cellular effects, including rearrangements of the actin cytoskeleton (32).

To prevent or control PAR, a formalin-killed pathogen as a bacterin and a purified formalin-inactivated PMT as a toxoid have been used widely as vaccines (5). However, because of the small doses and low concentrations of native PMT (less than 1% of total proteins) in *P. multocida*, large-scale cultures of the bacteria are required for the production of the toxoid for vaccination. In addition, it has been demonstrated that native PMT is poorly immunogenic in vivo and has difficulty initiating PMT-specific immune responses for protection (29). Therefore, the development of nontoxic and highly immunogenic PMT derivatives as vaccine candidates has been investigated. In our study, PMT2.3 was produced from *E. coli* as a large amount of nontoxic PMT derivatives, which were described previously (15). This high expression of recombinant PMT2.3 can provide sufficient quantities of recombinant PMT derivatives.

It has been reported that PMT can inhibit the humoral immune response and reduce the antibody levels in mice and swine (1, 9). In our studies, we observed a relatively higher antibody titer and an increase in the number of antibody-secreting cells in mice immunized with PMT2.3 than those in mice immunized with toxoid or bacterin (Fig. 1). The C-terminal region of PMT has been reported to be the catalytic domain (11, 20). The C-terminal fragment of PMT has the essential residue required for the intracellular activity of PMT; antibodies against the C-terminal fragment inhibit the mitogenic effect of PMT (2, 23). In our studies, antibodies raised against PMT2.3 could efficiently neutralize the cytotoxic activity of PMT toward Vero cells in a neutralization assay (Table 1). Therefore, it is said that that PMT2.3 can induce a high level of protective antibodies and reduce the clinical and pathological effects of PAR. Survival rates were also in accordance with the results of the neutralization antibody titer in each experimental group. Interestingly, in our study, the toxoid itself induced a relatively high PMT-specific antibody titer compared to previous results that demonstrated poor anti-PMT production.
following native PMT administration (28, 29). This result may be explained by the difference in experimental design or methods applied in administration.

In cellular immunity, a significant increase in lymphocyte proliferation was detected in all immunized groups after immunization (Fig. 2); however, a relatively lower increase in lymphocyte proliferation was also observed in the toxoid-immunized group. After the homologous challenge, the level of proliferation was just a little higher than that after immunization in all the experimental groups. This relatively low proliferation of lymphocytes implies low contribution to the activation of the immune system by cell-mediated responses.

Animal colostrum is important for the maintenance of early protection against PAR in piglets, and passive transfer of maternal immunoglobulin from sows to their offspring should be considered for development of the vaccine against PAR in piglets. In this study, we investigated the efficacy of protection against PAR in newborn piglets by passive transfer of maternal PMT-specific antibodies through colostrum from sows vaccinated with PMT2.3. The level of serum antibodies in offspring reflected the relative antibody concentration (Fig. 3). The antibody titers in offspring tended to reach relatively high levels in the newborns and then decreased gradually. In the case of PMT2.3, it appears that effective antibody transfer from sows to their offspring can be achieved by maternal vaccination using PMT2.3 in general because of the significantly high value of the relative antibody concentration in the preweaning period (1 to 3 weeks after birth), although we did not quantify PMT-specific antibodies in the colostrum directly. However, the results of growth status and disease score were not coincident with these antibody titer results (Tables 2 and 3); both the PMT2.3 and toxoid groups showed similar levels of influence on growth and clinical signs. Thus, it is likely that PMT2.3 can induce the protection of offspring by passive transfer of maternal immunoglobulins as much as toxoid can in this experimental condition.

In conclusion, PMT2.3 was efficiently produced in an E. coli expression system in easy and large quantities, and it could induce high levels of the neutralization antibody and a specific cellular immune response against PMT. It conferred a relatively high level of protective immunity in mice and effective protection in swine by passive transfer of maternal antibodies through colostrum. Therefore, we consider nontoxic PMT2.3 to be an effective candidate for the development of a subunit vaccine against PAR induced by P. multocida infection in swine.

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REFERENCES

1. Bagley KC, Abdelwahab SF, Tuskan RG, Lewis GK. 2005. Pasteurella multocida toxin activates human monocyte-derived and murine bone marrow-derived dendritic cells in vitro but suppresses antibody production in vivo. Infect. Immun. 73:413–421.
2. Busch C, Orth J, Djouder N, Aktories K. 2001. Biological activity of a C-terminal fragment of Pasteurella multocida toxin. Infect. Immun. 69:3628–3634.
3. Chen X, Li M, Le X, Ma W, Zhou B. 2004. Recombinant hepatitis B core antigen carrying preS1 epitopes induce immune response against chronic HBV infection. Vaccine 22:439–446.
4. Chrisp CE, Foged NT. 1991. Induction of pneumonia in rabbits by use of a purified protein toxin from Pasteurella multocida. Am. J. Vet. Res. 52:56–61.
5. Confer AW. 1993. Immunogenes of Pasteurella. Vet. Microbiol. 37:353–368.
6. Felix R, Fleisch H, Frandsen PL. 1992. Effect of Pasteurella multocida toxin on bone resorption in vitro. Infect. Immun. 60:4984–4988.
7. Foged NT, Nielsen JP, Jorsal SE. 1989. Protection against progressive atrophic rhinitis by vaccination with Pasteurella multocida toxin purified by monoclonal antibodies. Vet. Rec. 125:7–11.
8. Harper M, Boyce JD, Adler B. 2006. Pasteurella multocida pathogenesis: 125 years after Pasteur. FEMS Microbiol. Lett. 265:1–10.
9. Jordan RW, et al. 2003. Modulation of the humoral immune response of swine and mice mediated by toxigenic Pasteurella multocida. FEMS Immunol. Med. Microbiol. 39:51–59.
10. Kang ML, et al. 2007. Pluronic F127 enhances the effect as an adjuvant of chitosan microspheres in the intranasal delivery of Bordetella bronchiseptica antigens containing dermonecrototoxin. Vaccine 25:4602–4610.
11. Kitadokoro K, et al. 2007. Crystal structures reveal a thiol protease-like catalytic triad in the C-terminal region of Pasteurella multocida toxin. Proc. Natl. Acad. Sci. U. S. A. 104:5139–5144.
12. Lax AJ, Chanter N. 1990. Cloning of the toxin gene from Pasteurella multocida and its role in atrophic rhinitis. J. Gen. Microbiol. 136:81–87.
13. Lee J, Kang S, Park SI, Woo HJ, Kwon M. 2004. Molecular cloning and characterization of the gene for outer membrane protein H in a Pasteurella multocida (D4) isolate from pigs with atrophic rhinitis symptoms in Korea. J. Microbiol. Biotechnol. 14:1343–1349.
14. Lee J, Kim YB, Kwon M. 2007. Outer membrane protein H for protective immunity against Pasteurella multocida. J. Microbiol. 45:179–184.
15. Lee J, Woo HJ. 2010. Antigenicity of partial fragments of recombinant Pasteurella multocida toxin. J. Microbiol. Biotechnol. 20:1756–1763.
16. Liao CM, et al. 2006. Immunogenicity and efficacy of three recombinant subunit Pasteurella multocida toxin vaccines against progressive atrophic rhinitis in pigs. Vaccine 24:27–35.
17. Magyar T, King VI, Kovacs F. 2002. Evaluation of vaccines for atrophic rhinitis—a comparison of three challenge models. Vaccine 20:1797–1802.
18. Mullan PB, Lax AJ. 1998. Pasteurella multocida toxin stimulates bone resorption by osteoclasts via interaction with osteoblasts. Calcif. Tissue Int. 63:340–345.
19. Nakai T, Sawata A, Tsujii M, Samejima Y, Kume K. 1984. Purification of dermonecrotic toxin from a sonic extract of Pasteurella multocida SP-72 serotype D. Infect. Immun. 66:439–443.
20. Orth JH, et al. 2009. Pasteurella multocida toxin activation of heterotrimeric G proteins by deamidation. Proc. Natl. Acad. Sci. U. S. A. 106:7179–7184.
21. Penningtons AM, Storm PK. 1984. A test in Vero cell monolayers for toxin production by strains of Pasteurella multocida isolated from pigs suspected of having atrophic rhinitis. Vet. Microbiol. 9:503–508.
22. Petersen SK, et al. 1991. Recombinant derivatives of Pasteurella multocida toxin: candidates for a vaccine against progressive atrophic rhinitis. Infect. Immun. 59:1387–1393.
23. Pullinger GD, Sowdhaminii R, Lax AJ. 2001. Localization of functional domains of the mitogenic toxin of Pasteurella multocida. Infect. Immun. 69:7839–7850.
24. Register KB, Ducey TF, Brockmeier SL, Dyer DW. 2001. Reduced virulence of a Bordetella bronchiseptica siderophage mutant in neonatal swine. Infect. Immun. 69:2137–2143.
25. Sakano T, Okada M, Taneda A, Mukai T, Sato S. 1997. Effect of Bordetella bronchiseptica and serotype D Pasteurella multocida bacterin-toxoid on the occurrence of atrophic rhinitis after experimental infection with B. bronchiseptica and toxigenic type A P. multocida. J. Vet. Med. Sci. 59:55–57.
26. Seo J, Lee S, Pyo H, Lee J, Kim T. 2010. Protective potential of an attenuated Pasteurella multocida, which expresses only the N-terminal truncated fragment of P. multocida toxin. Can. J. Vet. Res. 74:25–29.
27. Seo J, Pyo H, Lee S, Lee J, Kim T. 2009. Expression of 4 truncated fragments of Pasteurella multocida toxin and their immunogenicity. Can. J. Vet. Res. 73:184–189.
28. van Diemen PM, de Jong MF, de Vries Reilingh G, van der Hel P, Schrama JW. 1994. Intranasal administration of Pasteurella multocida toxin in a challenge-exposure model used to induce subclinical signs of atrophic rhinitis in pigs. Am. J. Vet. Res. 55:49–54.
29. van Diemen PM, de Vries Reilingh G, Parmentier HK. 1994. Immune responses of piglets to Pasteurella multocida toxin and toxoid. Vet. Immunol. Immunopathol. 41:307–321.
30. Vasfi Marandi M, Mittal KR. 1997. Role of outer membrane protein H (OmpH)- and OmpA-specific monoclonal antibodies from hybridoma tumors in protection of mice against Pasteurella multocida. Infect. Immun. 65:4502–4508.
31. Yao XY, Yuan MM, Li DJ. 2006. Mucosal inoculation of Lactobacillus expressing hCGbeta induces an anti-hCGbeta antibody response in mice of different strains. Methods 38:124–132.
32. Zywietz A, Gohla A, Schmelz M, Schultz G, Offermanns S. 2001. Pleiotropic effects of Pasteurella multocida toxin are mediated by Gq-dependent and -independent mechanisms. Involvement of Gq but not G11. J. Biol. Chem. 276:3840–3845.