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FULL PAPER

Protective effects of intranasal vaccination with plasmid encoding pseudorabies virus glycoprotein B in mice

Ayato Takada, Katsunori Okazaki, and Hiroshi Kida*

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Abstract

Intranasal administration of plasmid DNA encoding glycoprotein B of pseudorabies virus into mice induced both serum and secretory antibody responses. These mice resisted intranasal challenge with lethal dose of the virus, but did not intraperitoneal challenge. On the other hand, intramuscular injection of the plasmid induced less secretory and higher serum antibody responses than those of intranasally vaccinated mice. None of them was protected from virus challenge. The present results suggest that administration of plasmid DNA encoding glycoprotein B by respiratory mucosal route generates local secretory antibodies which serve to protect animals from pseudorabies virus infection.

Key words: DNA vaccine, intranasal vaccination, mucosal immunity, pseudorabies virus

Introduction

Pseudorabies virus (PrV), also known as Aujeszky's disease virus, belongs to the family *Herpesviridae*. Like other alpha-herpesviruses, PrV causes latent infection in its natural host, pigs[24]. PrV in the latent state may be reactivated by environmental or physical stress of the host, resulting in the spread of the virus to other animals[6]. Parenteral vaccination of pigs, dogs, and cattle with live or inactivated PrV induced serum antibodies to the virus, sensitized lymphocytes, and consequently suppressed the severity of disease, but conferred only partial protection against PrV challenge[1,2,15,20].

As is the case of other secondary hosts for PrV, the mouse is a terminal host, and unable to survive acute virus replication. The mouse, thus, has been used as a good model animal to evaluate the protective effect of vaccination against PrV infection. Previously, we showed that intranasal vaccination of mice with inactivated PrV virions induced secretory IgA and IgG antibody responses specific to glycoprotein B (gB), resulting in complete protection of the animals against intranasal challenge with lethal doses of the virus[26]. The gB is one of the major glycoproteins of PrV, consisting of a complex of three glycoproteins which are covalently linked by disulfide bonds[8,14,17], and shares homology with glycoprotein B of herpes simplex virus 1[23]. It has a major role for the virus entry into

Laboratory of Microbiology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University.

* Corresponding author: Dr. Hiroshi Kida, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan, Tel: (+81-11) 706-5207, Fax: (+81-11) 709-7259, e-mail: kida@vetmed.hokudai.ac.jp

cells[21,22]. We, therefore, demonstrated that intranasal vaccination with purified gB conferred the protective immunity into mice[25].

Recently, a number of works has shown that immunization using a plasmid DNA expressing viral proteins (DNA vaccine) is a novel and new method to give animals protective immunity by eliciting variable antibody and /or cytotoxic T-cell responses. However, those studies mainly focused on the systemic immune responses induced by parenteral vaccination such as intramuscular injection and gene gun delivery system. On the contrary, only a limited information is available on the DNA vaccination by mucosal routes. In the present study, the efficacy of intranasal and intramuscular vaccinations with plasmid DNA encoding PrV-gB was compared for the potential to induce secretory antibody responses and hence to confer protective immunity against PrV infection on mice.

Materials and Methods

Virus and cell culture

PrV strain YS-81 and CPK cells (porcine kidney cells) were kindly provided by Dr. M. Shimizu of the National Institute of Animal Health (Tsukuba, Japan). CPK cells were cultured in Eagle's minimal essential medium (EMEM) supplemented with 10% calf serum. PrV was inoculated to CPK cell monolayers at a multiplicity of 0.01 PFU /cell. After adsorption for 1 hr, EMEM was added and the cultures were incubated at 37℃ for 2 days. From the culture fluids harvested, the virus was purified by differential centrifugation and sedimentation through a 10–50% sucrose gradient[12].

Construction of plasmids

Genomic DNA of PrV was prepared from purified virions as described previously[19]. A *Kpn*I-C fragment containing PrV gB gene was cloned into the *Kpn*I site of plasmid pGEM-4Z (Promega), which was designated pGKC. To subclone a *Ppu*MI-*Nco*I fragment containing the whole gB gene into pGEM-4Z, the construction was performed as follows (Fig. 1) : Since the gB gene possess the *Ppu*MI site, the *Ppu*MI-*Nco*I fragment was constructed from two following plasmids. The plasmid pGKC was digested with *Nhe*I and blunt-ended with large fragment of DNA polymerase I (Klenow fragment). To give the *Nhe*I site of the gB gene, a 10-mer *Bam*HI or *Eco*RI linker was inserted into the blunt-ended site, and each plasmid was digested with *Bam*HI or *Eco*RI, respectively, and ligated by T4 DNA ligase. The resulting plasmids were designated pGBH1 and pGBT, respectively. To give an *Eco*RI site upstream from the gB gene in pGBH1, the *Eco*RI linker was inserted into the *Ppu*MI site end-filled with Klenow fragment. The plasmid was designated pGBH2. The *Eco*RI-*Nhe*I fragment from pGBH2 was inserted into the *Eco*RI and *Nhe*I sites of pGBT. The plasmid was designated pGB1. To remove the *Nco*I and the *Nco*I-*Bam*HI fragments in the pGB1, the plasmid was digested with *Nco*I and the *Bam*HI linker was inserted into the *Nco*I site end-filled with Klenow fragment. The plasmid was digested with *Bam*HI, and then ligated by T4 DNA ligase. The resulting plasmid was designated pGB2. The *Xba*I linker was inserted into the *Eco*RI site of pGB2, end-filled with Klenow fragment. The *Xba*I fragment containing the gB gene was transferred into eukaryotic expression vector, pEF-BOS[16] containing a human elongation factor 1 α promoter. The resulting plasmid was designated pEF-BOSgB. The pEF-BOS and pEF-BOSgB were grown in *Escheria coli* HB101 and purified on cesium chloride density gradients by standard protocols. Cos-1 cells (a gift from Dr. K.C.Gupta, Rush University, Chicago, USA) were transfected with pEF-BOSgB by using Lipofectin reagent (Gibco BRL). Expression of the gB gene was monitored by indirect immunofluorescence assay using gB-specific monoclonal antibodies 19 /425).

Vaccination and protection tests

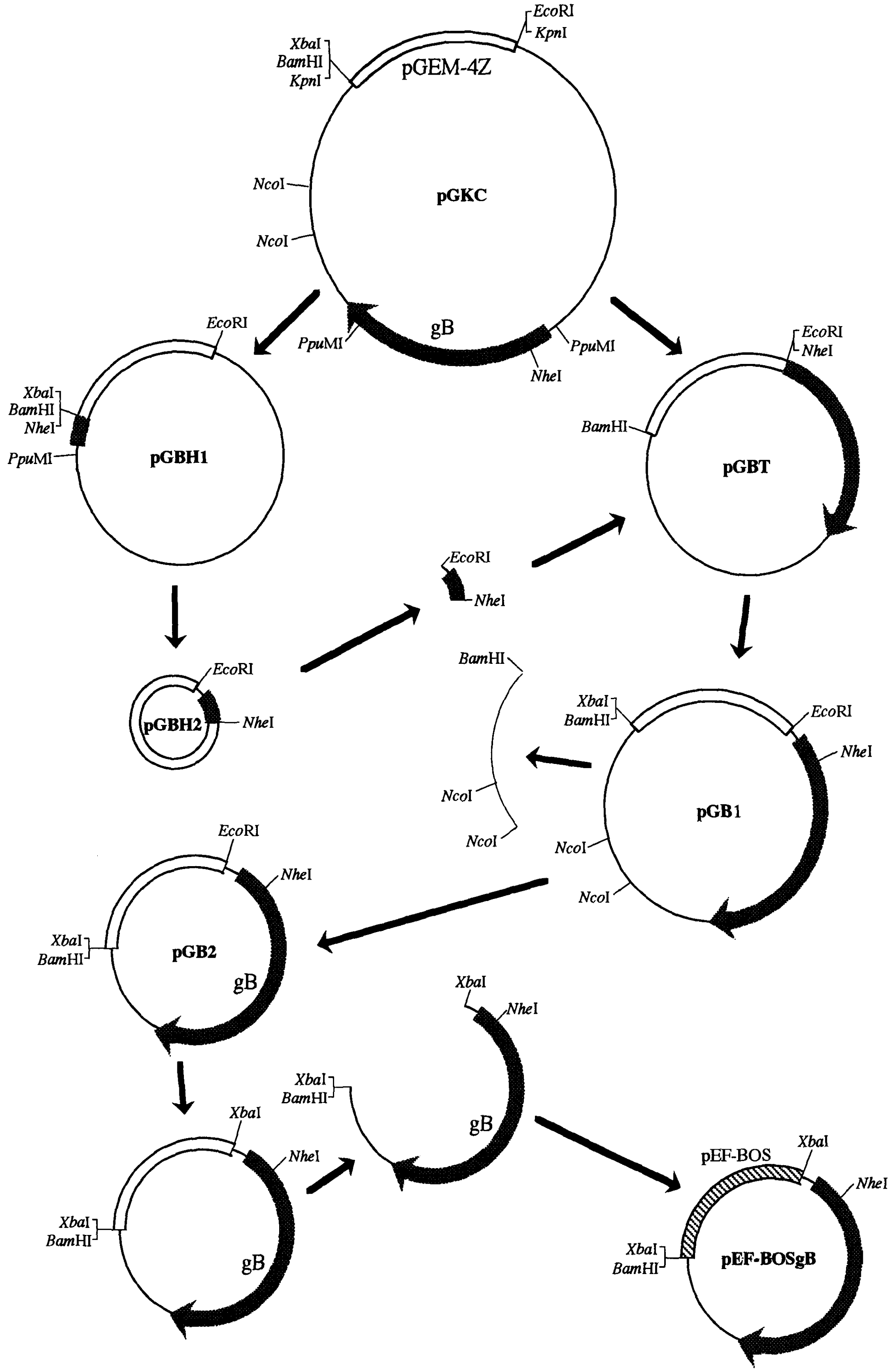


Fig. 1. Construction of the plasmid expressing PrVgB. The gB gene was cloned from *Kpn*I-C fragment as described in the text. The restriction sites in the plasmids, used for the construction, were indicated.

Vaccines were prepared as follows ; purified plasmid 100 μg in 25 μl of Dulbecco's Modified Eagle's medium (DME) was mixed with an equal volume of Lipofectin reagent. For intramuscular injection, 150 μl of DME was added to the mixture. For intranasal vaccination, 5 μg of cholera toxin B subunit (CTB) (Sigma Chemical Co.) was added to the 50 μl mixture as an adjuvant. Fifteen 6-week-old female ddY mice (Shizuoka Laboratory Animal Center) were anesthetized by an intraperitoneal injection with sodium pentobarbital (0.5–0.75 mg) and then vaccinated by intranasal administration or intramuscular injection with plasmid pEF-BOS gB or pEF-BOS vaccines. The vaccination was done three times at 2-week intervals. Ten days after the last vaccination, 5 mice of each group were sacrificed, and the sera, the nasal and tracheal-lung washes were collected as described by Nedrud *et al.*[18]. The remaining 10 mice were challenged by intranasal administration (5 mice) or intraperitoneal injection (5 mice) with 10 LD_{50} of live virus in 5 μl or 100 μl of PBS, respectively, under anesthesia.

Antibody assays

Neutralization test was done by plaque reduction assay using CPK cells with pooled mouse sera. The sera were incubated at room temperature with 100–200 PFU of PrV in the presence of 5 % guinea pig serum as a source of complement or of heat-inactivated serum (56℃, 30min.). Anti-PrV IgA and IgG antibodies were measured by enzyme-linked immunosorbent assay (ELISA)[11]. Disrupted viral antigen was prepared from the purified virus. Rabbit anti-mouse IgA and goat anti-mouse IgG conjugated to horseradish peroxidase were purchased from Zymed Laboratories and Bio-Rad Laboratories, respectively.

Results

Serum IgG antibody response of DNA-vaccinated mice

Sera of 5 mice from each group were pooled and tested for antibodies specific to PrV by ELISA and neutralization tests (Figs. 2 and 3, respectively). Low level of virus specific IgG antibodies was detected in the sera of mice vaccinated intranasally with pEF-BOSgB. In the sera of mice vaccinated intramuscularly with pEF-BOSgB, significantly high level of IgG antibodies was detected. IgG antibodies were detected also in the sera of mice vaccinated with pEF-BOS intramuscularly, but the level was lower than those of mice vaccinated with pEF-BOSgB. In the presence of complement, the sera from mice vaccinated intramuscularly or intranasally with pEF-BOSgB gave 55 % or 22% reduction of plaques, respectively, at the dilution of 1:2, while little neutralizing activity was found in the absence of complement. Neither samples from mice vaccinated intramuscularly nor intranasally with pEF-BOS neutralized virus infectivity, irrespective of the presence of the complement.

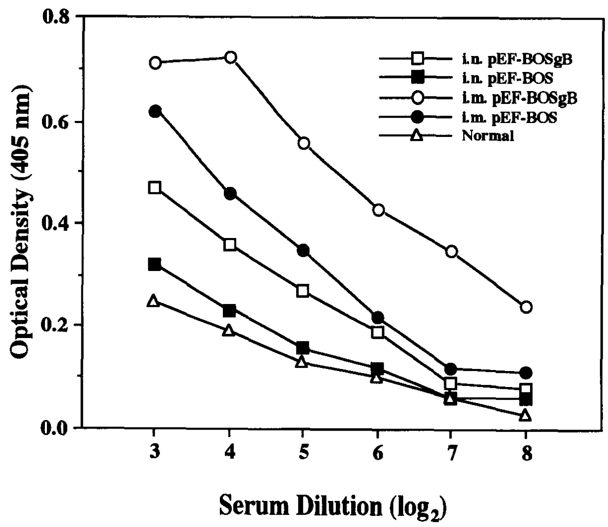


Fig. 2. ELISA of the sera from mice vaccinated intranasally (i.n.) or intramuscularly (i.m.) with pEF-BOSgB or pEF-BOS. Mice were vaccinated intranasally (squares) or intramuscularly (circles) with pEF-BOSgB (open) or pEF-BOS (solid). Sera of 5 mice from each group were pooled and tested. Normal ddY mouse serum was also tested (open triangle).

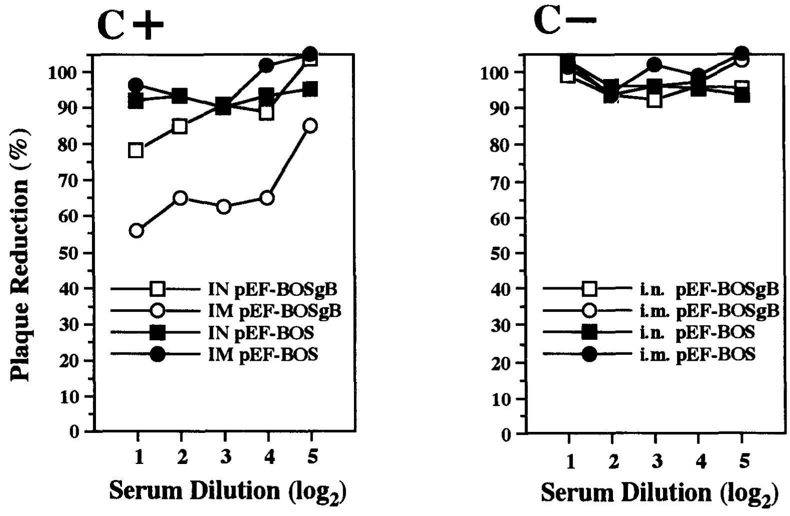


Fig. 3. Neutralizing activity of the sera from mice vaccinated with pEF-BOSgB or pEF-BOS. Neutralization tests were done by plaque reduction assay with or without addition of complement (C+ or C-, respectively). The number of plaques formed with normal serum gave 100 % at each dilution.

Secretory antibody response of DNA-vaccinated mice

IgA and IgG antibodies in the secretion of the respiratory tracts of the vaccinated mice were examined (Fig. 4). In the nasal washes of 2 of the 5 mice vaccinated intranasally with pEF-BOSgB, IgA antibodies were detected at low but obviously higher level than those of mice vaccinated with pEF-BOS. No difference of IgA levels in the trachea-lung washes was found between these two groups. IgG antibody levels in the nasal and trachea-lung washes of these mice also showed similar pattern. On the other hand, IgA antibody levels in both samples from mice vaccinated intramuscularly with pEF-BOSgB were not higher than background levels given by samples from mice vaccinated with the control plasmid pEF-BOS. IgG antibodies were scarcely detected in the nasal and trachea-lung washes of intramuscularly vaccinated mice, with one exception. This markedly high level of IgG antibodies might be derived from the serum.

Protective effects of intranasal or intramuscular vaccination with plasmid DNA

To evaluate protective effects of intranasal and intramuscular vaccination with pEF-BOSgB, mice were challenged with lethal dose of live PrV intranasally or intraperitoneally. Survival rates of the mice are shown in Fig. 5. Forty percents of mice (2 /5) vaccinated intranasally with pEF-BOSgB survived after the intranasal challenge. One mice of this group resisted the challenge as shown by prolonged survival periods (132 hrs), while all of the mice vaccinated with pEF-BOS intranasally were died within 72 hrs. None of these mice vaccinated intranasally survived after the intraperitoneal challenge. On the other hand, mice vaccinated intramuscularly with pEF-BOSgB did not resisted both intranasal and intraperitoneal challenges. Irrespective of the route of vaccination, mice vaccinated with control plasmid (pEF-BOS) died within 3 or 4 days after intranasal or intraperitoneal challenge, respectively.

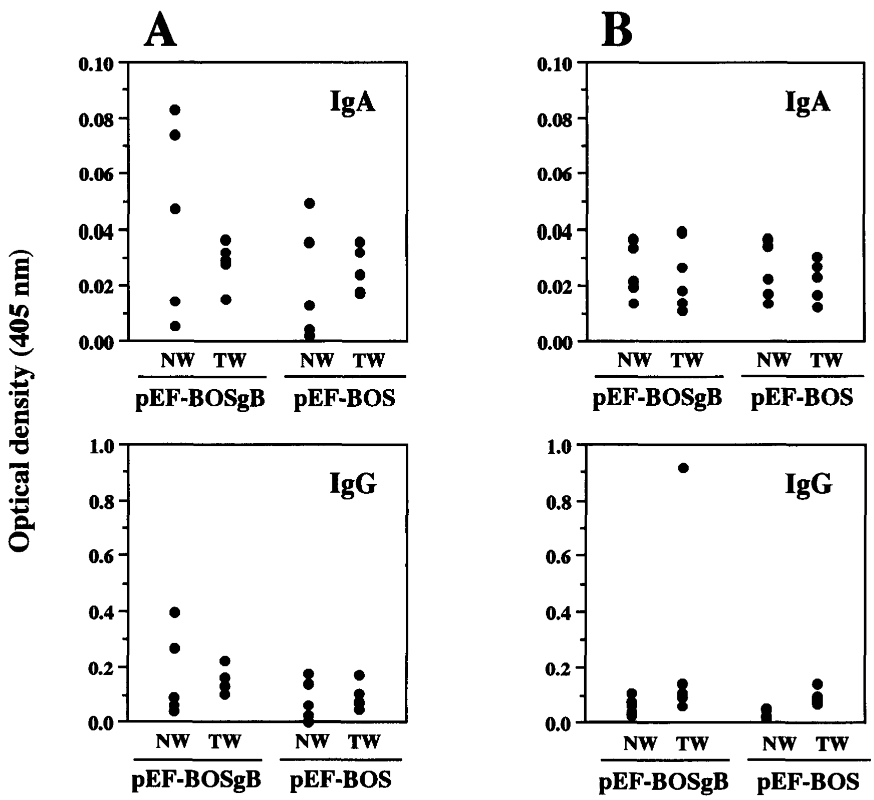
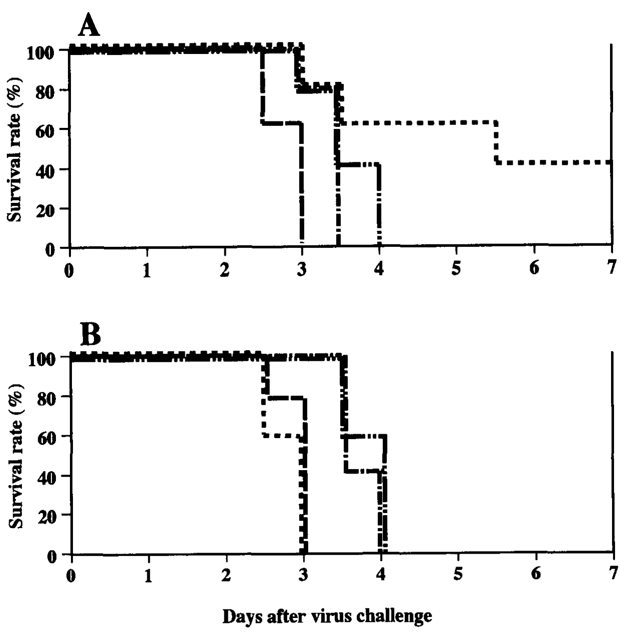


Fig. 4. IgA and IgG antibodies in the secretion of the respiratory tracts of mice vaccinated intranasally (A) or intramuscularly (B) with pEF-BOSgB or pEF-BOS. Virus-specific antibodies were measured by ELISA. Undiluted nasal wash (NW) and trachea-lung wash (TW) tested. Each point represents a single mouse. Samples which gave 3-fold higher absorbance than the average value of those from 5 mice vaccinated with control plasmid, pEF-BOS were considered to be positive.



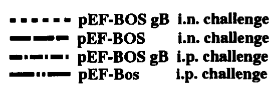


Fig. 5. Survival rates of mice vaccinated intranasally (A) or intramuscularly (B) with pEF-BOSgB or pEF-BOS. Mice were challenged intranasally (i.n.) or intraperitoneally (i.p.) with 10 LD50 of live virus.

Discussion

The present results indicate that intranasal administration of plasmid expressing gB can induce both systemic and mucosal antibody responses and confer protection from PrV infection on mice. In contrast, intramuscularly vaccinated mice produced much higher serum antibody response than intranasally vaccinated mice, and notwithstanding any of these mice did not resisted the virus challenge. In accord with our previous study[22], the present results substantiates that serum IgG antibody response is not fully contribute to the protection of mice from initial infection of PrV on the respiratory mucosal tissues. Serum and secretory antibody responses elicited by intranasal administration of plasmid in this study were indeed similar levels to those induced by vaccination with low dose (0.05mg) of gB together with CTB, which gave partial protection in our previous study[25].

It has been shown that parenteral immunization of animlas with PrV vaccine, including plasmid DNA, induced serum antibody response, sensitized lymphocytes, and hence suppressed the severity of disease[2,4,5,7,10,15,27]. In this study, however, intramuscular vaccination did not protect mice from lethal infection of PrV, although it induced systemic antibody response. Hence, in consistence with our previous study[26], complete protection is likely to be achieved by induction of secretory antibodies which preclude initial replication of the virus on the mucosal epithelial cells.

In this study, comparatively high levels of IgG antibodies were detected by ELISA in the sera of mice intramuscularly vaccinated with control plasmid, pEF-BOS, but these sera did not neutralize PrV infectivity even in the presence of complement. In addition, these sera did not immunoprecipitated any viral proteins, while sera from mice vaccinated with pEF-BOSgB immunoprecipitated gB (data not shown). Such a nonspecific immune reaction by injection with vector DNA was observed in several studies by others[3,6,28].

Fynan *et al.*[3] initially demonstrated that intranasal administration of plasmid encoding influenza virus hemagglutinin suppressed the severity of influenza and gave partial protection from lethal infection. The ability of intranasal administration of plasmid encoding gB of herpes simplex virus 1 to generate systemic and mucosal immune responses was reported[13]. In accord with these earlier studies, the present results suggest the potential of mucosal immunization of animals with plasmid DNA to generate local secretory antibodies which act as a barrier against viral infections. The development of DNA delivery systems such as a liposome and a microcapusule and investigation of a suitable promoter which efficiently works in the cells consisting in the mucosal tissues should be addressed for the practical use of an effective mucosal DNA vaccine.

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