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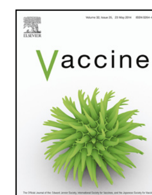

Mucosal immunization of calves with recombinant bovine adenovirus-3 coexpressing truncated form of bovine herpesvirus-1 gD and bovine IL-6

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ABSTRACT

Previous studies have suggested an important role of the cytokine adjuvant IL-6 in the induction of mucosal immune responses in animals, including mice. Here, we report the *in vivo* ability of bovine adenovirus (BAdV)-3 expressing bovine (Bo) IL-6, to influence the systemic and mucosal immune responses against bovine herpesvirus (BHV)-1 gDt in calves. To co-express both antigen and cytokine, we first constructed a recombinant BAdV-3 expressing chimeric gDt.BoIL-6 protein (BAV326). Secondly, we constructed another recombinant BAdV-3 simultaneously expressing gDt and BoIL-6 using IRES containing a bicistronic cassette gDt-IRES.IL-6, (BAV327). Recombinant proteins expressed by BAV326 and BAV327 retained antigenicity (gDt) and biological activity (BoIL-6). Intranasal immunization of calves with recombinant BAV326, BAV327 or BAV308 (gDt alone) resulted in demonstrable levels of gDt-specific IgG responses in sera and IgA response in nasal secretions, in all animals. In addition, all calves developed complement-independent neutralizing antibody responses against BHV-1. However, no significant difference could be observed in the induction of systemic or mucosal immune response in animals immunized with recombinant BAV326 or BAV327 co-expressing BoIL-6. Moreover, there was no difference in the protection against BHV-1 challenge particularly in the amount of virus excretion in the nasal cavity in calves immunized with BAV326, BAV327 or BAV308. These data suggest that the BoIL-6 had no modulating effect on the induction of gDt specific mucosal and systemic immune responses in calves.

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1. Introduction

Cytokines are signaling molecules which regulate both the type and the magnitude of immune response. The profile of cytokines produced by subpopulations of T cells influence the induction of humoral or cellular immune responses. For example, T helper subset 1 (Th1) cells secrete interleukin-2 (IL-2) and interferon gamma (IFN-γ), resulting in the induction of a cell-mediated response, while Th2 cells, which secrete IL-4, IL-5, IL-6 and IL-10 invoke high-titer antibody responses [1,2]. This indicates that particular cytokines may be used to optimize vaccine antigen response against infectious diseases [3]. As such researchers have been investigating the use of cytokines as a method of enhancing and modulating antigen specific immune responses to immunogens [4–6]. In several studies using different mouse challenge models, administration of recombinant cytokines along with vaccine antigens [7,8] has helped to bias the immune response as required, modulating disease progression [9,10]. However, despite the availability of different bovine cytokine genes, very few studies have been reported investigating the immunomodulation potential of known bovine cytokines in cattle [11–13].

Interleukin-6 produced by a variety of cells [14] is a multifunctional cytokine [15,16]. It has been identified as a major inducer of B-cell differentiation and T-cell activation in acute phase response [14]. Su et al. has shown that IL-6 and TNF-α can enhance cell mediated immunity induced by VP1 DNA vaccine for foot and mouth

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disease (FMD) [13]. Studies have shown that murine IL-6 plays a crucial role in the development of the mucosal antibody responses [17,18]. Reports suggest that the secretion of IL-6 by dendritic cells from Peyer's patches contributes to enhanced IgA production in the intestinal mucosa [19]. However, in general the use of recombinant cytokines has faced difficulties associated with toxicity and in targeting cytokines to sites of immune-reactivity. One way to deliver both antigen and cytokine is the use of live viral vectors [20,21], which may allow manipulating the immune system to favor the development of appropriate protective immune responses.

We have been evaluating bovine adenovirus (BAdV)-3 as a gene delivery system in the development of live viral vaccines for cattle [22–24]. BAdV-3 was chosen because of its low pathogenicity, species specificity and the ability of the virus to grow to high titers. Moreover, as BAdV-3 is a natural pathogen of cattle, it has an inherent advantage over other available systems for gene delivery in cattle. Although intranasal immunization of calves with the recombinant BAV308 induced clinical protection against the disease, the magnitude of the gD specific IgA response was not sufficient to reduce BHV-1 shedding in the first four days post challenge [23]. Since mice [9] and human [17] IL-6 have been demonstrated to induce secretory IgA antibody responses in the respective species, we chose to determine the effect of bovine (Bo) IL-6 in the induction of gD specific immune responses. In this report, we describe the construction of recombinant BAdV-3s co-expressing BHV-1 gDt and BoIL-6 either as a chimeric protein or as an individual protein, and their usefulness in the induction of an immune response.

2. Materials and methods

2.1. Cells and viruses

Madin Darby bovine kidney (MDBK) and primary fetal bovine retinal (PFBR) cells were grown in Eagle's minimum essential medium (MEM) supplemented with 5% fetal bovine serum (FBS). The wild-type (WBR-1 strain) and recombinant BAV3s were propagated in MDBK cells as described [22]. The BHV-1 (108 strain) was propagated and quantified as described earlier [23].

2.2. Antibodies

Production of monoclonal antibodies against BHV-1 gD has been described [25]. Monoclonal anti-bovine IgA antibody was provided by Dr. K. Nielson, Animal Disease Research Institute, Ontario, Canada. Goat anti-bovine IgG was purchased from Kirkegaard and Perry Laboratories, USA. Anti-BoIL-6 MAb (47-8) and anti-BoIL-6 serum (96-84) was produced by immunizing mice and rabbits, respectively, with β-Gal-BoIL-6 fusion protein (Godson, unpublished data).

2.3. Construction of recombinant plasmids

Plasmids were constructed (Supplementary file S1) by standard procedures.

2.4. Construction of recombinant BAV-3s

Recombinant BAV308 and BAV302 (BAV.E3d) have been described [22]. Recombinant BAV325 expresses bovine IL-6 inserted in E3 region of BAdV-3 (Godson, unpublished data). Other recombinant BAdV-3 were constructed as described (Supplementary file S1).

2.5. Radiolabeling and immunoprecipitation

Confluent cell monolayers were infected with 10 pfu of virus. At different times post infection, the cells were incubated in methionine-cysteine-free Dulbucco's MEM for 60 min before labeling with [^{35}S] methionine-cysteine (100 uCi/well). Similarly, the cells were incubated in glucose free DMEM for 60 min before labeling with [^{3}H] glucosamine (100 uCi/well). After 8–12 h of labeling, cells and medium were harvested. Proteins were immunoprecipitated from the medium or the cells and analyzed by SDS-PAGE [22].

2.6. Determination of biological activity of BoIL-6

MDBK cells were infected with individual recombinant BAdV-3 at an MOI of 5. At 24–48 h post infection, the supernatants were collected and clarified by centrifuging at 2000 rpm for 15 min. Dilutions of clarified supernatants were incubated with ovine retropharyngeal lymph node (RPLN) cells for 6 days and the supernatants were assayed for IgA production by sandwich ELISA [26].

To block the induction of ovine IgA responses, three dilutions of supernatant from BAV327 infected MDBK cells were incubated with 5×10^5 RPLN cells in the presence of rabbit anti-BoIL-6 serum (96-84) or normal rabbit serum (1/100) for 6 days. Finally, the supernatants were collected and analyzed for the presence of IgA by sandwich ELISA [26].

2.7. Animal inoculations

The calf experiments were carried out in accordance with the regulations of the Canadian Council for Animal Care. The calves were housed in high-security isolation rooms throughout the experiment. Twelve, BHV-1 negative calves (3–4 months old) were randomly allocated into four groups with three calves in each group. At day 0, the calves were exposed intranasally for 5 min to an aerosol of 10^9 pfu of BAV302, BAV308, BAV326 or BAV327 using a Devilbiss Nebulizer, model 65 (devilbiss Barry, Ontario Canada). Four week post primary immunization, the calves were given a second immunization. Two weeks post second immunization, the calves were challenged with an aerosol of 10^7 pfu/ml of BHV-1. Calves were bled at the times of first immunization, booster immunization, BHV-1 challenge and 10 days after challenge for antibody responses.

2.8. Virus isolation

The virus isolation assays were performed as described earlier [23].

2.9. ELISA

BHV-1 gD and BAdV-3 specific antibodies were determined in sera and nasal secretions by ELISA as described earlier [23].

2.10. Virus neutralization test

The virus neutralization test was performed as described earlier [23].

2.11. Clinical evaluation

Calves were clinically evaluated by a veterinarian for 10 days following challenge. The clinician was blind to vaccine groups. Rectal temperatures, nasal scores and clinical scores were measured daily. Nasal and clinical scores were given between 0 (normal) and

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