Immunization with DNA, adenovirus or both in biodegradable alginate microspheres: effect of route of inoculation on immune response.

Suresh K. Mittal, Neeraj Aggarwal, G. Sailaja, Alberto van Olphen, Harm HogenEsch, Adam North, John Hays, Stanley Moffatt

Department of Veterinary Pathobiology, School of Veterinary Medicine, Purdue University, 1290 Lynn Hall, West Lafayette, IN 47907, USA
Laboratory Animal Program, Purdue University, West Lafayette, IN 47907, USA

Abstract

To determine the potential for biodegradable alginate microspheres to be used as a delivery vehicle for DNA based vaccines, we constructed a plasmid, pMNe-gal-SV40, containing the bacterial β-galactosidase (LacZ) gene under the control of the murine cytomegalovirus (MCMV) immediate-early promoter and the simian virus 40 (SV40) polyadenylation signal. The effect of the route of administration and co-administration of adenovirus on systemic and mucosal immune responses were investigated. Mice were inoculated orally, intranasally (i.n.), intramuscularly (i.m.), subcutaneously (s.c.) or intraperitoneally (i.p.) on days 0, 14 and 28 with microspheres containing plasmid DNA, bovine adenovirus type 3 (BAd3) or plasmid DNA + BAd3. Systemic routes of immunization (i.m., s.c. and i.p.) resulted in higher LacZ- or BAd3-specific IgG ELISA titers compared to those obtained by mucosal routes of inoculation (oral and i.n.). Mucosal immunization led to slightly higher titers of LacZ- or BAd3-specific IgA at mucosal sites compared to those obtained by the various systemic routes. All the routes of immunization induced LacZ-specific lymphoproliferation. Co-administration of BAd3 enhanced the LacZ-specific IgG response irrespective of the route of administration. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Administration of nucleic acid (NA) coding for pro-teins has gained considerable interest in the areas of vaccine development and gene therapy [1–4]. NA vaccines seem to combine basic advantages of attenuated and inactivated viral vaccines without their obvious limitations. Induction of protective immune responses with NA appears to be similar to that of either live attenuated viral or recombinant vaccines, and the safety is similar to that of subunit or inactivated viral vaccines without the adverse side effects of adjuvants or animal-derived proteins. Early experiments with NA vaccines started with the injection of naked plasmid DNA encoding immunogenic proteins that led to transgene expression resulting in a protective immune response [1,5]. It was soon realized that the cellular uptake of the naked DNA, when injected into a muscle, is not very efficient and a large portion of the DNA is degraded before it reaches the nucleus for transcription [6,7]. Subsequently, a number of delivery systems, such as bombardment with gold microparticles coated with NA [8–10], incorporation of NA into liposomes and other poly-cationic lipids [11–13], biological erodible polymers [14] and others [15–17], have been evaluated for both their transfection efficiencies and NA persistence both in cultured cells and animals.

Sodium alginate is a naturally occurring polysaccharide which can be easily cross-linked into a solid matrix with the addition of divalent cations (cross-linking in a water-in-oil emulsion results in the formation of microspheres). Alginate microparticles are safe to use in animals and they have been used to encapsulate purified protein, bacterial outer membrane proteins and viruses [18–22]. Oral immunization of animals with microspheres containing antigenic pro-teins elicited both humoral (mucosal and systemic) and cell-mediated immune responses [18–22]. Recently, we demonstrated that alginate microspheres could be used for the encapsulation, delivery and expression of plus- mid DNA [23].

It has been demonstrated that transgene expression is increased when cells in culture are simultaneously exposed to NA and adenovirus [24]. The adenovirus-mediated enhancement of transgene expression is mainly due to the ability of adenovirus to disrupt endosomes before they fuse with lysosomes, thereby inhibiting DNA degradation by lysosomal enzymes [25,26]. Inoculation of mice with microspheres containing both plasmid DNA and bovine adenovirus type 3 (BAd3) resulted in a significant increase in transgene expression compared to those inoculated with micro-spheres containing only the plasmid DNA [23]. We were interested to know whether the level of transgene expression by microspheres containing plasmid DNA would be enough for the development of an immune response against the expressed protein. This paper describes the effect of route of inoculation on the type and level of immune response elicited with plasmid DNA encapsulated into alginate microspheres. Adenovirus-mediated augmentation of immune response elicited against the transgene product is also discussed.
2. Materials and methods

2.1. Cell culture and virus

MDCK and 3T3 cell lines, obtained from American Type Culture Collection (ATCC), were grown as monolayer cultures using Eagle's minimum essential medium (MEM) [Life Technologies, Rockville, MD] supplemented with 10% fetalClone III (HyClone Lab-ortorogies, Logan, UT) and 50 µg/ml gentamicin. BAd3, purchased from ATCC, was grown in MDCK cells for the virus stock and for obtaining a purified virus preparation for microencapsulation and for enzyme-linked immunosorbent assay (ELISA). Virus purification was done by cesium chloride density-gradient centrifugation, as described elsewhere [27]. The titer of the purified virus preparation was determined by plaque assay on MDCK cells.

2.2. Plasmid

A 3.8 kb XbaI-BglII fragment containing the bacterial β-galactosidase (LacZ) gene under the control of the murine cytomegalovirus (MCMV) immediate-early promoter and the simian virus 40 (SV40) polyadenylation signal was excised from pCa36 [28] (kindly pro-vided by Dr. F.L. Graham, Departments of Biology and Pathology, McMaster University, Hamilton, Ontario, Canada) and inserted into the XbaI-BamHI site of pUC18 to yield pMNe-gal-SV40. Plasmid DNA was purified by isopycnic centrifugation in cesium chloride-ethidium bromide gradients [29].

2.3. Transfection and β-galactosidase assay

The transfection protocol was described previously [23]. Briefly, 3T3 cells in 6-well plates (Nalge Nunc International, Hina-dale, IL) were transfected with pMNe-gal-SV40 (1 or 5 µg) mixed with 10 µg of Lipofectin (Life Technologies, Rockville, MD). Following 30 min incubation at 37°C, cells were infected with BAd3 at a multiplicity of infection (m.o.i.) of 500 pla-que forming unit (p.f.u.) per cell. The cells were harvested by scraping at 48 and 72 h post-transfection and the cell pellet was assayed for LacZ activity. LacZ activity was measured as previously described [23].

2.4. Microencapsulation of plasmid DNA and BAd3

To encapsulate either DNA or BAd3, a 3.5 ml sus-pension in phosphate-buffered saline (PBS) pH 7.2, was slowly mixed with 14 ml of a 1.5% solution of sodium alginate (Keltone HVCR, sterile filtered) pre-
pared in PBS. To this was added 1.58 ml of Span-85 (Sigma, St. Louis, MO), followed by 70 ml of a sur-
face-tant-free canola oil (Canadian Harvest, Cambridge, MN). The oil/water mix was emulsified for 1 min at 5500 rpm with a Servodyne mixer equipped with a 1.5 in. diameter propeller head. While continuing mixing a 17.5 ml solution of 0.5% CaCl2 and 0.05% ZnCl2 in PBS was added dropwise (~1 min adding time), and the mixing was continued for an additional 2 min. The microspheres were pelleted at 2750 x g and all of the oil residues were carefully removed. The microspheres were washed once in three volumes of water and pel-
leted. The pellet was resuspended in one volume of water, and two volumes of 0.2% poly-L-lysine were added. The microspheres were pelleted, brought up to an appropriate volume with sterile saline and sonicated before use. PBS was used to make mock-encapsulated control microspheres and the microspheres containing both DNA and BAd3 required a slight increase in the scale of the aforementioned protocol due to a 4.5 ml starting volume. One milliliter suspension of micro-spheres was estimated to contain a maximum of either 1.8 x 10⁹ p.f.u. of BAd3, 380 µg of plasmid DNA or 1.8 x 10⁹ p.f.u. of BAd3 and 380 µg of plasmid DNA. The majority of microspheres were of 5-10 µm in di-
ameter as measured by Microtrak Particle Analyzer.

2.5. Animal inoculation

Eighty 6-8-week-old female BALB/c mice were ran-domly divided into 20 groups (4 animals/group) and inoculated by either the oral, intra- nal (i.n.), intra-muscular (i.m.), subcutaneous (s.c.) or intraperitoneal (i.p.) route on days 0, 14 and 28 with alginate micro-spheres containing either PBS, BAd3, pMNe-gal-SV40 or pMNe-gal-SV40 + BAd3. For oral, i.m., s.c. or i.p. inoculations, 250 µl of the microsphere suspension was used, whereas for i.n. inoculation, only 100 µl of the microsphere suspension was used. Serum samples were collected on days 0, 28 and 40 to monitor the development of LacZ- and BAd3-specific IgG and IgA antibodies by ELISA. Animals were euthanized on day 40 by an overdose (90 mg/kg) of sodium pentobarbital (pH 9-10.5) and spleens were collected for lymphocyte proliferation assay. Animals were euthanized on day 40 by an overdose (90 mg/kg) of sodium pentobarbital (pH 9-10.5) and spleens were collected for lymphocyte proliferation assay. On day 40, 1 ml of PBS was infused into the lungs through trachea and then recover-er to collect lung lavages to evaluate the development of Lacz- and BAd3-specific mucosal immune responses by ELISA. The fecal samples were collected from intestines on day 40, homogenized in cold PBS (1 g/ml) and the supernatants were used to evaluate the development of Lacz- and BAd3-specific mucosal immune responses by ELISA.

2.6. ELISA

The serum samples were used to detect LacZ- and BAd3-specific IgG and IgA antibodies by ELISA follow-
ing a previously described protocol [30]. The intes-tinal supernatants and lung lavages were used to detect Lacz- and BAd3-specific IgA antibody by ELISA. Briefly, 96-well microtiter plates (Becton Dickin-
on, Franklin Lakes, NJ) were coated either with purified Lacz (Boehringer Mannheim, Indianapolis, IN) or purified BAd3 and incubated with different di-lutions of each sample to quantitate Lacz- or BAd3-specific antibody titers, respectively. A horseradish per-oxidase (HRP)-conjugated goat anti-mouse IgG (BioRad, Hercules, CA), or HRP-conjugated goat anti-mouse IgA (Southern Biotechnology Assoc., Birmingham, AL) were used as the secondary antibody. The reciprocal of the serum dilution showing an OD reading of at least the mean OD + 2SD of negative control sera was taken as the ELISA antibody titer.

2.7. Lymphocyte proliferation assay

Spleens were removed from euthanized mice and homogenized individually in sterilized glass tissue grinders to obtain single-cell suspensions. Viable spleen cells were counted by Trypan blue dye exclusion. The spleen cells were resuspended in RPMI 1640 supplemented with 10% FetalClone III, 100 units penicill-
in/ml and 100 µg streptomycin/ml and the concentration of spleen cells was adjusted to 2 x 10⁶
cells/ml. Two hundred microliter of the cell suspension (4 x 10^6 cells) was added in each well of 96-well flat-bottom plates (Corning Costar, Cambridge, MA). Ten microliter of RPMI 1640 containing various amounts of purified LacZ (1, 4 or 8 µg/well) was added in each set of wells (3 wells/antigen concentration) to induce an antigen-specific proliferative response. Concanavalin-A (ConA) (2 µg/well) or RPMI 1640 were used as a positive or negative control, respectively. Spleen cells either in the presence or absence of antigen were incubated at 37°C for 4 days and then labeled with 0.5 µCi/pH-thymidine (6.7 Ci/mmol, ICN Pharmaceuticals, Costa Mesa, CA) per well for 18 h. Cells were harvested and the incorporation of 3H-thymidine was determined with a Packard scintillation counter. A mean value of the counts per minute (cpm) of triplicate wells was calculated and used to determine the stimulation index (mean cpm of wells with antigen/mean cpm of wells without antigen).

2.8. Statistical analysis

The significance of differences between groups in ELISA antibody titers or stimulation indexes was determined by Student's t-test. A series of t-tests (between DNA and DNA + BAd3 inoculated groups; between BAd3 and BAd3 + DNA inoculated groups) were performed to determine whether the observed differences in the outcome demonstrated true differences in the populations or were the result of random sampling error. A value of P < 0.05 was considered significant.

3. Results

3.1. Expression of β-galactosidase in cells transfected with plasmid DNA

Since LacZ expression with the MCMV promoter was approximately three to six times higher in 3T3 cells as measured by a transient expression assay compared to those levels obtained either with the cytomegalo-galovirus (CMV) immediate-early promoter or the Rous sarcoma virus (RSV) early promoter (data not shown), we used the MCMV promoter for further study. To determine the effect of BAd3 infection on transient LacZ expression in cells transfected with pMMe-gal-SV40 were subsequently infected with BAd3. Transgene expression was increased 2.4–7.4-fold by adenovirus infection compared to mock-infected controls (Fig. 1). These results demonstrate that adenovirus augments transgene expression in this system. BAd3 does not replicate in 3T3 cells or in mice (data not shown).

3.2. Cellular immune response in mice inoculated with microspheres containing plasmid DNA and BAd3

In order to assess the potential for alginate micro-spheres to be used for DNA immunization, mice were inoculated with microspheres containing either PBS, DNA or DNA + BAd3 on days 0, 14 and 28 by either the oral, i.n., s.c. or i.p. route. Serum samples were collected on days 0, 28 and 40 to monitor LacZ- and BAd3-specific IgG and IgA titers by ELISA. Immunization with microspheres containing both plasmid DNA and BAd3 yielded significant (P < 0.05) increases in LacZ-specific serum IgG titers irrespective of the route of administration compared to those obtained with microspheres containing only plasmid DNA (Fig. 2). Similarly, a combination of BAd3 and plasmid DNA also yielded significant (P < 0.05) higher BAd3-specific serum IgG titers at least on day 28 compared to those obtained with BAd3 alone (Fig. 3). The highest LacZ-specific as well as BAd3-specific serum IgG titers were observed in i.p. immunized group followed by s.c., i.m., i.n. and orally inoculated animals (Figs. 2 and 3).

Mice inoculated i.n. yielded the maximum LacZ-specific serum IgA titers followed by i.p., orally, s.c. and i.m. immunized animals (Fig. 4), whereas, the highest BAd3-specific serum IgA titers were obtained in animals immunized i.p. followed by i.n., s.c. and i.m. inoculated groups (Fig. 5). Encapsulation of plasmid DNA along with BAd3 did not result in significant (P > 0.05) enhancement in neither LacZ-specific serum IgA titers nor BAd3-specific serum IgA titers compared to titers obtained either with plasmid DNA or BAd3, respectively (Figs. 4 and 5). The highest LacZ-specific serum IgG as well as IgA titers (Figs. 2–5).

3.3. Mucosal immune response in mice inoculated with microspheres containing plasmid DNA and BAd3

To determine the effect of the route of inoculation on mucosal immune response, lung lavages and fecal samples were collected 12 days after the third inoculation and LacZ- and BAd3-specific IgA titers were determined by ELISA. Animals immunized with alginic micro-particles containing both plasmid DNA and BAd3 yielded approximately a 10–40 and 17–67% increase in LacZ-specific IgA titers in the lung lavages and fecal samples respectively, compared to those obtained with plasmid alone (Fig. 6). These increases in levels of LacZ-specific IgA titers were not significant (P > 0.05).

The highest BAd3-specific IgA titers in lung lavages were observed in i.p. immunized group followed by i.n., s.c., i.m. and orally inoculated animals, whereas in fecal samples the maximum BAd3-specific IgA titers were observed i.p. and i.m. in immunized mice followed by i.n., s.c. (Fig. 7). BAd3-specific mucosal IgA titers in animals immunized with a combination of BAd3 and plasmid DNA were not significantly (P > 0.05) different compared to those obtained with BAd3 alone.

3.4. Cellular immune response in mice inoculated with microspheres containing plasmid DNA and BAd3

To determine the effect of the route of adminis-tration on the cellular immune response, mice were immunized three times by various routes with micro-spheres containing either PBS, DNA or DNA + BAd3 and spleens were collected 12 days after the third inoculation. Spleen cells were analyzed for LacZ-specific proliferation by lymphocyte proliferation assay. Lymphocyte proliferation, as indicated by a stimulation index, were higher in animals immunized orally, i.m. or i.p. than those immunized either i.e. or s.c. (Fig. 8). There were significant (P < 0.05) increases in stimulation indexes of animals immunized orally or i.p. with micro-particles containing both plasmid DNA and BAd3 compared to those obtained with plasmid DNA alone. Due to technical difficulties we were not able to determine BAd3-specific lymphoproliferation.
4. Discussion

In addition to the type of transgene, dosage and number of inoculations, there are four other factors that have a major impact on the level and type of immune response produced by a NA vaccine. These factors are (i) the use of suitable heterologous regulatory sequences for transgene expression [23,31,32], (ii) the choice of an appropriate delivery system [5,8,11,14–16], (iii) the route of inoculation [33–35] and (iv) the use of immunomodulatory molecules [36–40]. A number of NA delivery systems have been developed, each of which has certain advantages and disadvantages. The NA delivery system based on biodegradable alginate microspheres, described in this paper, provides versatility by being able to immunize the host through a number of different routes. Our results demonstrate that alginate microspheres can also be used successfully for the delivery of adenovirus with or without plasmid DNA. The route of immunization will eventually be dependent on the type and level of immune response required for the immunogen of interest. All the routes of inoculation we tested led to detectable levels of both humoral and cellular immune responses against the transgene product (LacZ); however, the type and levels of immune response varied with the route of inoculation. Mucosal routes of inoculation (oral and i.n.) resulted in higher levels of LacZ- or BAD3-specific IgA titers at mucosal sites as compared to systemic administration (i.m., s.c. and i.p.). In contrast, systemic routes of inoculations led to a stronger systemic immune response against either LacZ or BAD3 compared to those obtained with mucosal routes. The alginate microsphere-based delivery system appears to be similar to other delivery systems in that mucosal delivery led to a stronger mucosal response and systemic delivery resulted in a stronger systemic response.

Since adenovirus can efficiently enter both replicating and quiescent cells, it may be used as a mediator for macromolecular transport into cells. Exposure of cultured cells to human adenovirus and unencapsulated plasmid DNA leads to co-inertialization of the plasmid DNA and the virus by receptor-mediated endocytosis [24]. Earlier we demonstrated that adenovirus enhances transgene expression in vivo using alginate microspheres containing both DNA and BAD3 [23]. Our immunization experiments demonstrate that the presence of adenovirus led to at least consistently stronger IgG antibody response compared to those obtained with DNA alone. Inclusion of adenovirus along with plasmid DNA did not result in significant rise in LacZ-specific IgA titers compared to those obtained with DNA alone. There may be a number of explanations for adenovirus-mediated augmentation of IgG antibody response against the transgene product: (i) Adenovirus has been shown to cause the lysis of endosomes carrying DNA before they fuse with lysosomes, thereby preventing DNA degradation by lyso-somal enzymes [41,42]. (ii) Adenoviral E1A transactivates a number of cellular and viral promoters [41,42]. (iii) The presence of adenoviral proteins may inhibit transgene protein degradation. (iv) Cells infected with adenovirus may be more easily recognized by cells involved in immune recognition. It has been demonstrated that DNA and vaccinia virus vectors work synergistically to enhance the immune response against the transgene product [43,44]. Surprisingly, the presence of plasmid DNA resulted in stronger BAD3-specific IgG response as compared to those obtained with BAD3 alone. DNA containing CpG motifs stimulates the growth, activation and maturation of dendritic cells [45], and enhances antigen-specific humoral and cell-mediated immune responses [46]. Whether the plasmid DNA used in our study acts as a source of CpG motifs needs further investigation.

Transfection of antigen presenting cells, such as dendritic cells, is important in eliciting an immune response against the expressed antigen [47,48]. Since alginate microspheres are most likely taken up by macrophages and dendritic cells, it will be interesting to evaluate the role of these cells in modulation of immune response. The alginate microsphere-based delivery system may also have implications in gene therapy especially for targeting macrophages and dendritic cells.

In general, LacZ- or BAD3-specific humoral immune response in mice inoculated orally was low as compared to the other routes of administration. It appears that in orally inoculated animals, a large proportion of microspheres are not taken up by the M cells at Peyer’s patches [19]. The efficiency of microsphere uptake could be improved by designing microspheres having the capability of binding particularly to M cells. It should be noted that for i.n. inoculation, the amount of microspheres was only 40% of the amount used for other routes of inoculation. The utility of the i.n. route of inoculation would be immensely improved if we could devise a method to aerosolize alginate microspheres. Use of immune stimulatory molecules, such as cytokine genes [35,37,49], cholera toxin [38], CpG motifs [36,45,46] and others will be useful in modulating immune responses by alginate microspheres containing DNA, virus/protein or both.

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References


Fig. 1. Expression of LacZ in cells initially transfected with plasmid DNA carrying the LacZ gene and subsequently infected with BAd3. 3T3 cells were transfected with pMNe-gal-SV40 and subsequently infected with BAd3 at an m.o.i. of 500 p.f.u. per cell. At 48 and 72 h post-transfection the cells were harvested, cell extracts were prepared and used to assay for LacZ activity. Mock and virus-infected cell extracts were used as negative controls. Purified bacterial LacZ was used as a standard. Each bar represents the mean of two independent samples. 1 + 10, 1 μg DNA and 10 μg Lipofectin; 5 + 10, 5 μg DNA and 10 μg Lipofectin; 1 + 10 + V, 1 μg DNA, 10 μg Lipofectin and BAd3; 5 + 10 + V, 5 μg DNA, 10 μg Lipofectin and BAd3.
Fig. 2. LacZ-specific IgG response in sera of mice inoculated with microspheres containing DNA. Mice were immunized with microspheres containing either PBS, DNA or DNA + BAd3 on days 0, 14 and 28 by either the (A) oral, (B) intranasal, (C) intramuscular, (D) subcutaneous or (E) intraperitoneal route. The serum samples were collected on days 0, 28 and 40, and LacZ-specific IgG antibody titers were determined by ELISA. Each point represents the mean value for 3–4 animals ± SD. i.n., intranasal; i.m., intramuscular; s.c., subcutaneous; i.p., intraperitoneal; V, BAd3; * 16,000 ± 6400; ** 192,000 ± 7390.
Fig. 3. BAd3-specific IgG response in sera of mice inoculated with microspheres containing BAd3. Mice were immunized with microspheres containing either PBS, BAd3 or DNA + BAd3 on days 0, 14 and 28 by either the (A) oral, (B) intranasal, (C) intramuscular, (D) subcutaneous or (E) intraperitoneal route. The serum samples were collected on days 0, 28 and 40, and BAd3-specific IgG antibody titers were determined by ELISA. Each point represents the mean value for 3–4 animals ± SD. i.n., intranasal; i.m., intramuscular; s.c., subcutaneous; i.p., intraperitoneal; V, BAd3.
Fig. 4. LacZ-specific IgA response in sera of mice inoculated with microspheres containing DNA. Mice were immunized with microspheres containing either PBS, DNA or DNA + BA3 on days 0, 14 and 28 by either the (A) oral, (B) intranasal, (C) intramuscular, (D) subcutaneous or (E) intraperitoneal route. The serum samples were collected on days 0, 28 and 40, and LacZ-specific IgA antibody titers were determined by ELISA. Each point represents the mean value for 3–4 animals ± SD. i.n., intranasal; i.m., intramuscular; s.c., subcutaneous; i.p., intraperitoneal; V, BA3; * 800 ± 320; ** 960 ± 370.
Fig. 5. BAd3-specific IgA response in sera of mice inoculated with microspheres containing BAd3. Mice were immunized with microspheres containing either PBS, BAd3 or DNA + BAd3 on days 0, 14 and 28 by either the A) oral, B) intranasal, C) intramuscular, D) subcutaneous or E) intraperitoneal route. The serum samples were collected on days 0, 28 and 40, and BAd3-specific IgA antibody titers were determined by ELISA. Each point represents the mean value for 3–4 animals ± SD. i.n., intranasal; i.m., intramuscular; s.c., subcutaneous; i.p., intraperitoneal; V, BAd3; * 5333±1800.
Fig. 6. LacZ-specific IgA response in lung lavages and fecal samples of mice inoculated with microspheres containing DNA. Mice were immunized with microspheres containing either PBS, DNA or DNA + BAd3 on days 0, 14 and 28 by either the (A) oral, (B) intranasal, (C) intramuscular, (D) subcutaneous or (E) intraperitoneal route. Animals were euthanized on day 40, lung lavages and fecal samples were collected, and LacZ-specific IgA antibody titers were determined by ELISA. Each point represents the mean value for 3–4 animals ± SD. i.n., intranasal; i.m., intramuscular; s.c. subcutaneous; i.p., intraperitoneal; V, BAd3.
Fig. 7. BAd3-specific IgA response in lung lavages and fecal samples of mice inoculated with microspheres containing BAd3. Mice were immunized with microspheres containing either PBS, BAd3 or BAd3 + DNA on days 0, 14 and 28 by either the (A) oral, (B) intranasal, (C) intramuscular, (D) subcutaneous or (E) intraperitoneal route. Animals were euthanized on day 40, lung lavages and fecal samples were collected, and BAd3-specific IgA antibody titers were determined by ELISA. Each point represents the mean value for 3-4 animals ± SD. i.n., intranasal; i.m., intramuscular; i.p., intraperitoneal; s.c., subcutaneous; V, BAd3; * 260±120; ** 280±80; *** 266±45.
Fig. 8. Lymphocyte proliferation in response to LacZ in freshly isolated spleen cells from immunized mice. Mice were immunized with microspheres containing either PBS, DNA, BAd3 or DNA + BAd3 on days 0, 14 and 28 by either the oral, intranasal, intramuscular, subcutaneous or intraperitoneal route. Animals were euthanized on day 40, spleens were collected, homogenized and spleen cells were collected. Spleen cells were stimulated with 20 µg/ml LacZ (4 µg/well) for 72 h and then proliferation was measured by ³H-thymidine incorporation. Each point represents the mean stimulation indices for 3–4 animals ± SD. i.n., intranasal; i.m., intramuscular; s.c., subcutaneous; i.p., intraperitoneal.