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Oral vaccination of animals with antigens encapsulated in alginate microspheres

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Abstract

Most infectious diseases begin at a mucosal surface. Prevention of infection must therefore consider ways to enhance local immunity to prevent the attachment and invasion of microbes. Despite this understanding, most vaccines depend on parenterally administered vaccines that induce a circulating immune response that often does not cross to mucosal sites. Administration of vaccines to mucosal sites induces local immunity. To be effective requires that antigen be administered often. This is not always practical depending on the site where protection is needed, nor comfortable to the patient. Not all mucosal sites have inductive lymphoid tissue present as well. Oral administration is easy to do, is well accepted by humans and animals and targets the largest inductive lymphoid tissue in the body in the intestine. Oral administration of antigen requires protection of antigen from the enzymes and pH of the stomach. Polymeric delivery systems are under investigation to deliver vaccines to the intestine while protecting them from adverse conditions that could adversely affect the antigens. They also can enhance delivery of antigen specifically to the inductive lymphoid tissue. Sodium alginate is a readily available, inexpensive polymer that can be used to encapsulate a wide variety of antigens under mild conditions. Orally administered alginate microspheres containing antigen have successfully induced immunity in mice to enteric (rotavirus) pathogens and in the respiratory tract in cattle with a model antigen (ovalbumin). This delivery system offers a safe, effective means of orally vaccinating large numbers of animals (and perhaps humans) to a variety of infectious agents.

1. Introduction

Most infectious diseases begin at a mucosal surface. Local (mucosal) immunity is very important in protecting a host from infection. Mucosal immunity neutralizes viruses and bacteria and keeps them from attaching and crossing the mucosal surface. The most effective way to induce mucosal immunity is to administer a vaccine directly to the site where a pathogen invades the host. However, this is not always practical — for example, intranasal administration of vaccines to prevent respiratory diseases in livestock where there are hundreds of animals that must be individually caught and treated is both time consuming and labor intensive. An alternative method of delivery of vaccines to induce local immunity is needed. Fortunately, the mucosal immune sites are interconnected by a common mucosal immune system (CMIS) whereby stimulation of an inductive immune site results in migration of antigen specific lymphocytes to other affective mucosal sites in the body [1–3]. The largest accumulation of lymphoid tissue in the body is in the gastrointestinal tract (GIT) [4]. The oral administration of vaccines results in an immune response not only in the GIT, but also in distant mucosal sites. Although this method of administration has many benefits, successful oral vaccination requires that vaccine antigens be protected from the low pH and enzymes of the stomach enroute to the inductive sites in the intestine. One method of protection of antigens is by encapsulation in microparticles [5–8].

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Microparticles (MS) or microspheres (MS) are useful for oral administration because they protect the vaccine from the adverse conditions of the GIT, and they are of a size (< 10 μm) to be taken up by M cells where they can then reach the inductive immune site [5].

Many polymers have been used to produce MS. We have chosen to use sodium alginate as the material to encapsulate antigens. Sodium alginate is a complex carbohydrate composed of guluronic and mannuronic acid that forms gels in the presence of divalent cations [9]. Alginate is readily available, relatively inexpensive, and polymerizes under very mild conditions thereby avoiding degradation or alteration of antigens during encapsulation. We propose that alginate microspheres can be used to encapsulate a variety of antigens that can be mucosally (orally) administered to a wide variety of animals for any infectious disease. In this report we describe the oral administration of antigen to mice, rabbits, and cattle.

2. Materials and methods

2.1. Animals

2.1.1. Cattle

Female 4–6-month old Holstein calves weighing 150–200 kg were used in this study. Calves were housed indoors at the Purdue University Dairy Center and fed a diet of corn–alfalfa crumbles, mixed hay and haylage and water free choice.

2.1.2. Rabbits

Pasteurella multocida free New Zealand white male rabbits weighing 2–2.4 kg (Hazelton Research Products, Inc., Kalamazoo, MI), housed individually in stainless steel cages and fed commercial rabbit chow and water free choice.

2.1.3. Mice

Six–eight-week old BALB/c female (Harlan, Indianapolis, IN) fed commercial mouse chow and water free choice were used in this study.

2.2. Preparation of alginate microspheres

Two methods of preparation of alginate microspheres were used in these studies. For the cattle and rabbit studies, alginate MS were prepared with modifications of a previously described aerosolization method [10]. Antigen, ovalbumin (Fraction V, Sigma, St. Louis, MO, USA) for cattle, or potassium thiocyanate (KSCN) extracts of *P. multocida* for the rabbits, were prepared as previously described [11] was added to a 1.5% (w/v) solution of sodium alginate (LV, Keltone, Kelco, Inc., Chicago, IL, USA) to a final concentration of 1.2% w/v of alginate.

The size of alginate MS prepared by the aerosolization technique ranged from 1–50 μm in diameter, with most less than 10 μm as measured by a particle analyzer (Microtrak Series 9200, Leeds and Northrup, Northwales, PA, USA). The alginate MS were stabilized by a coating of 0.05% (w/v) solution of poly-l-lysine (mean m.w. 100,000, Sigma). The final concentration of OVA was 50 mg of OVA per ml wet weight of MS, or 1 mg/ml for KSCN extracts of *P. multocida*. The loading efficiency (approximately 33%) of OVA in microspheres was determined indirectly using fluorescein labelled and immunogold labelled OVA to make alginate MS in a similar manner. The microspheres were lysed by suspending them in 0.1 M EDTA with stirring for 2 h, pelleting any particulate material by centrifugation and the supernatant assayed for labelled OVA by fluorescent microscopy, or immunogold by light spectrophotometry.

For preparation of bovine serum albumin (Sigma Chemical Company) MS for administration to mice, alginate microspheres were prepared using an emulsification method as described elsewhere [13]. To determine loading efficiency, MS containing BSA were mixed in a ratio of 1:1 v/v with 10× PBS overnight at room temperature. The alginate MS lysate was centrifuged to remove particulate debris and supernatant assayed for total protein using the bicinchoninic acid method (BCA-Pierce). Particle size was determined by assaying a 10–20 μl sample of a 1:10 dilution of MS in a particle size analyzer (Accusizer 770, Nicomp). Mean volume diameter was recorded for each batch of MS prepared for inoculation of mice.

2.3. Administration of vaccines in alginate microparticles

2.3.1. Cattle

For administration to cattle, microspheres containing OVA were mixed with alginate to produce macrospheres 4–5 mm in diameter. This size and density of hydrogels was previously shown to bypass the rumen and drop into the reticulum [12]. Each 5 mg dose of OVA encapsulated in microspheres within alginate macrospheres was placed in two 1/4 ounce gelatin boluses prior to administration to calves by balling gun.

The immunization schedule and description of groups of calves are shown in Table 1. Each group contained six calves. Calves were challenged at different times to determine the onset and length of the immune response induced by oral vaccination. A 5 mg dose of OVA encapsulated in microspheres was administered to calves for 5 consecutive days with either a subcutaneous (SC) or oral booster of OVA, or for one

Table 1
Experimental groups of calves inoculated orally with ovalbumin encapsulated in alginate microparticles

Group number	Inoculation schedule	Time of challenge (weeks)	Day of final BAL
1	SC + oral 5× OVA MS	7	54
2	oral 5 × + oral 5× OVA MS	7	54
3	oral 3× OVA MS	3	29
4	oral 3× OVA MS	2	22
5	oral 3× OVA MS	1	15
6	oral MS only/ no OVA	3	29
7	oral OVA no MS	3	29

SC means subcutaneous, 5× five daily inoculations, 3× three daily inoculations, 1× one single inoculation, OVA ovalbumin, MS microspheres and BAL bronchoalveolar lavage.

regimen of 3 consecutive days. One, two or three weeks after the last inoculation of vaccine, each calf was challenged by a transtracheal administration of 5 mg of OVA in 50 ml of 0.9% NaCl inserted into the right caudal lung lobe. Five days later a bronchoalveolar lavage (BAL) was performed on each calf. Lymphocyte enriched leukocytes from the BAL were used in an ELISPOT assay to enumerate anti-OVA isotype specific antibody secreting cells as previously described [14].

2.3.2. Rabbits

Each experimental group consisted of five rabbits. Five ml of the desired dose of antigen (5 mg of protein of the *P. multocida* toxin) encapsulated in microparticles was mixed in 20 ml of tap water and placed in a water bottle which the rabbits drank within 2 h. Fresh water was then supplied ad libidum after the water containing the vaccine was totally consumed. Inoculation groups are listed below. All rabbits were inoculated at day 0, 7 and 14 followed by intranasal challenge with virulent *P. multocida* at day 16. All rabbits were euthanized and final samples (serum, nasal lavages and bacteriology specimens) collected on day 21. Table 2 provides an outline of the immunization and sample collection for rabbits in this study.

2.3.3. Mice

The desired dose of MS was administered in 250 μl of water by oral gavage to mice using an 18 gauge ball-tip stomach gavage needle. Intranasal administration of 20 μl of MS was performed by instilling the material dropwise into each naris using a micropipette tip. Groups were inoculated and samples collected as indicated in Table 3.

2.4. Sample collection

2.4.1. Cattle

Blood and bronchoalveolar lavage (BAL) fluids were collected at day 0 (prevaccination), 20, 42 and 54 for groups vaccinated orally five times with and without SC priming, day 29 for calves challenged at 3 weeks, day 22 for calves challenged at 2 weeks and day 15 for calves challenged 1 week following the oral administration of OVA). Blood was collected by jugular venipuncture. BAL was performed by injecting and immediately withdrawing 50 ml aliquots of 0.9% sodium chloride through a Foley-like catheter (Bivona, Goshen, IN, USA) wedged in the right caudal most bronchus as previously described [12].

2.4.2. Rabbits

Serum and nasal lavage fluids were collected on day 0, 10, 16, and 21 (day of euthanasia). Serum was collected by venipuncture of an ear vein. Nasal lavage fluid was collected and processed as previously described [15]. Bacterial cultures of the nasopharnyx, and each tympanic bulla, lung and liver were collected at day 21 and processed as previously described [15].

Table 2
Groups of rabbits used in study inoculated with KSCN extracts of *P. multocida*

Group number	Route of administration	KSCN encapsulated in MS	Days vaccinated	Serum and Nasal lavages
1	not immunized	no	none	0, 10, 16, 21
2	oral	no (blank MS— no antigen)	0, 7, 14	0, 10, 16, 21
3	oral	no	0, 7, 14	0, 10, 16, 21
4	oral	no, with cholera toxin	0, 7, 14	0, 10, 16, 21
5	IN	no	0, 7, 14	0, 10, 16, 21
6	oral	yes	0, 7, 14	0, 10, 16, 21
7	oral	yes, with cholera toxin	0, 7, 14	0, 10, 16, 21

Table 3
Groups of mice with description of inoculation and sample collection protocol

Group number	Group name	mg/dose	Encapsulated	Dose volume	Route of administration	Days of inoculation	Days serum collected
1	2.5 mg MS	2.5	yes	0.2	oral	0, 7, 14	7, 14, 21, 28, 35
2	1.0 mg MS	1.0	yes	0.2	oral	0, 7, 14	7, 14, 21, 28, 35
3	0.5 mg MS	0.5	yes	0.2	oral	0, 7, 14	7, 14, 21, 28, 35
4	0.1 mg MS	0.1	yes	0.2	oral	0, 7, 14	7, 14, 21, 28, 35
5	SC MS	0.1	yes	0.2	SC	0, 14	7, 14, 21, 28, 35
6	unencapsulated	2.5	no	0.2	oral	0, 7, 14	7, 14, 21, 28, 35
7	cholera toxin	5.0	no	0.2	oral	0, 7, 14	7, 14, 21, 28, 35
8	BSA + Imugen®	0.1	no	0.2	SC	0, 14	7, 14, 21, 28, 35
9	IN MS	1.0	yes	0.02	IN	0, 7, 14	7, 14, 21, 28, 35
10	blank MS	0	yes	0.2	oral	0, 7, 14	7, 14, 21, 28, 35

Bacterial colony forming units (CFU) were determined for each swab and per gram of tissue (liver and lung) wet weight.

2.4.3. Mice

Blood was collected weekly through 5 weeks post-vaccination. Serum was collected and stored at −20°C until assayed.

2.5. Lymphocyte enriched leukocyte preparation for ELISPOT assays for cattle

Heparinized blood samples were diluted 1:1 with PBS, pH 7.4 and centrifuged over a density gradient (Histopaque 1.083, Sigma). The interface bands containing peripheral blood mononuclear cells (PBMC) were collected and washed twice. The BAL fluid was centrifuged and the cell pellet washed in RPMI-1640 supplemented with 10% FetalClone I serum (Hyclone, UT, USA), 50 μl/ml mercaptoethanol, 25 mM HEPES, 200 U/ml penicillin, 200 μg/ml streptomycin, 0.5 ug/ml amphotericin B and 10 ug/ml gentamycin. The BAL leukocyte population was enriched for lymphocytes as previously described [14]

2.6. Immunological assays

2.6.1. ELISPOT

Ovalbumin specific ASCs were identified in BAL cells immediately following isolation. PBMC were assayed following cultivation in vitro of 2×10^5 cells/ml in RPMI supplemented as described above plus 0.1 μg/ml OVA for 7 days at 37°C and 5% CO_2. The assay was performed as previously described [14]. ELISPOT microtiter plates were examined using an inverted microscope to enumerate the number of spots per well. The mean number of spots per six wells was converted to ASCs per 10^6 cells.

2.6.2. ELISA

Serum diluted 1:20 in PBS–tween and undiluted nasal lavages of rabbits and mouse serum diluted 1:20 for mice were assayed by ELISA as previously described [15–17] using polystyrene microtiter plates (Costar).

2.7. Statistical evaluation of data

2.7.1. Cattle

The Kruskal–Wallis nonparametric test was used to compare differences in numbers of ASCs and between the groups of calves. Once a significant group difference was determined, pairwise comparisons between groups was based on the Wilcoxon test using a two-sided rank test to compare the response of each orally vaccinated group to each other. Statistical significance was determined by $p \leq 0.05$.

2.7.2. Rabbits

Means of ELISA absorbance values of serum and nasal washes antibody to KSCN extracts and colony forming units of *P. multocida* were compared using the Wilcoxon rank sum test. Statistical significance was determined by $p \leq 0.05$.

2.7.3. Mice

Means of ELISA absorbance values for serum were compared using the Student's *t*-test with statistical significance determined by $p \leq 0.05$.

3. Results and discussion

3.1. Cattle

None of the calves had detectable anti-ovalbumin IgA ASCs in the BAL or blood at day 0. The Kruskal–Wallis test showed a significant group difference ($p = 0.008$) postvaccination. The mean number

Table 4
Number of anti-ovalbumin IgA antibody secreting cells in bronchoalveolar lavage fluids in groups significantly different from each other following oral administration of ovalbumin encapsulated in alginate microspheres. Values indicate p-value for two-sided pairwise Wilcoxon rank-tests. See Table 1 for description of groups

Cattle group	Mean ± S.D. ASCs	SC + oral	Oral 5 × + oral 5×	3 weeks	2 weeks	1 week	No MS	MS only
SC + oral	17 ± 17	×						0.04
Oral 5 × + oral 5×	20 ± 17		×					0.05
3 weeks	17 ± 19			×				0.022
2 weeks	46 ± 87				×			0.028
1 week	29 ± 23					×	0.006	0.003
No MS	4.3 ± 3.7						×	0.075

ASCs means antibody secreting cells and MS microspheres. Empty blocks indicate no significant difference between these groups ($p > 0.10$).

of anti-OVA IgA ASCs in the BAL of calves is shown in Table 4. Calves vaccinated with five oral doses of OVA in alginate MS following oral or SC priming had a significant increase in anti-OVA IgA ASCs compared to sham vaccinated calves. Calves inoculated with OVA for 3 consecutive days and challenged 1, 2 or 3 weeks later had an increased number of anti-OVA IgA ASCs with no significance difference between groups. However, each of these groups had a significantly greater number of anti-OVA IgA ASCs than the MS only or unencapsulated oral OVA groups. The number of anti-OVA IgA ASCs in BAL was similar to that seen in calves primed by a SC injection or oral inoculation of OVA followed by an oral booster. None of the calves that received OVA in microspheres orally only had detectable anti-OVA IgG_1 or any other isotype ASCs in the BAL. This is in contrast to the SC primed calves which had a large number of anti-OVA IgG_1 ASCs (100 ± 124) in the BAL. There was no significant increase in any antibody isotype in serum or BAL of calves vaccinated orally only with OVA with or without microspheres. There was no significant increase in the titer of any isotype of anti-OVA ASCs in peripheral blood mononuclear cells, nor was there a statistically significant increase of antibodies of any isotype in the serum for any group vaccinated orally only. The serum and BAL levels of anti-OVA IgG and IgA were consistent with the number of ASCs in PBMC and BAL in calves primed SC and boosted orally. The number of anti-OVA IgA ASCs was greater in each group that was inoculated orally with OVA encapsulated in alginate microspheres than in calves that received MS only with no OVA. The mean number of anti-OVA IgA ASCs in calves vaccinated with OVA encapsulated in MS was much greater than that in the unencapsulated OVA group. This suggests that the encapsulated OVA was protected from the digestive enzymes, low pH of the abomasum (fourth stomach of ruminants) and rumenal microflora activity. There was considerable variation in the immune responses in calves due most likely to the fact that four stomachs were traversed before the antigen could stimulate the intestinal Peyer's patches.

3.2. Rabbits

There was a significant increase in ELISA absorbance value for IgA specific for the KSCN extracts of *P. multocida* in nasal washes of rabbits beginning at day 10 and increasing until day 21 (Table 5). Groups inoculated with KSCN extracts encapsulated in alginate MS had greater increases in nasal wash antibody to KSCN extracts than rabbits inoculated with unencapsulated antigen. Oral administration of antigen encapsulated in MS was as effective as IN administration of antigen in inducing specific IgA and in reducing bacteria in the upper and lower airways (Table 6). The addition of cholera toxin to the KSCN extracts in

Table 5
Change in optical density of IgA present in nasal lavage of rabbits inoculated with KSCN extracts of *P. multocida*

Group	Day 0	Day 10	Day 16	Day 21
Nonimmunized	0.025	0.070	0.050	0.050
Microspheres by oral	0.065	0.050	0.030	0.050
KSCN by oral	0.060	0.090	0.125	0.140
KSCN + cholera toxin by oral	0.050	0.105	0.135	0.145
KSCN by Intranasal	0.058	0.130	0.500	0.530
KSCN by oral in microspheres	0.055	0.140	0.560	0.600
KSW by oral in microspheres with cholera toxin	0.070	0.135	0.610	0.725

KSW: potassium thiocyanate extracts of outer membrane proteins.

Table 6
Isolation of bacteria from tissues following intranasal challenge in immunized rabbits

Group	Positive cultures*	Number of bacteria in lung	Number of bacteria in nasopharynx
Nonimmunized and nonchallenged	0/15	0	0
Microspheres	7/15	84 ± 6	321 ± 11
KSCN oral	4/15	73 ± 2	302 ± 9
KSCN + CT oral	4/15	69 ± 7	287 ± 13
KSCN intranasal	1/15	38 ± 7	198 ± 14
KSCN oral in microspheres	3/15	40 ± 3	205 ± 15
KSCN oral in micro spheres with CT	1/15	40 ± 4	188 ± 10

*Five rabbits per group. Liver and right and left tympanic bullae were cultured from each rabbit. KSCN is potassium thiocyanate extracts of outer membrane proteins and CT cholera toxin.

the alginate MS resulted in a further decrease in bacteria in the lung and nasopharnyx, reduced the number of positive cultures, and had the highest ELISA absorbance value of anti-KSCN extract IgA of any group. However, this difference was not significantly greater than the value for the other encapsulated KSCN extracts vaccinated groups.

Overall, these experiments demonstrated that oral administration of antigens of an infectious agent can induce protective immunity in a distant mucosal site (the respiratory tract). The encapsulation of antigen in alginate microspheres was necessary to protect antigens and to assure uptake by inductive lymphoid tissue in the intestinal tract as shown by the poor immune response induced by oral administration of unencapsulated antigens. The results of this study suggest that there is promise of an effective oral vaccine using alginate microspheres to control respiratory diseases in rabbits. The results in cattle and mice further support the hope that this delivery system can be used in multiple host species to provide protection to infectious agents that begin at mucosal surfaces.

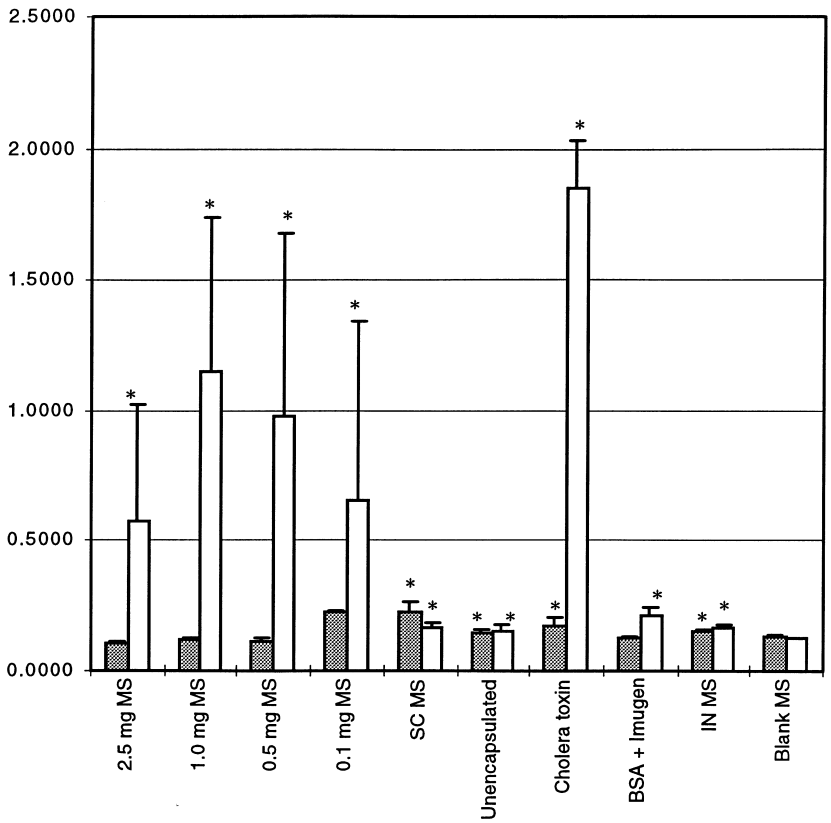

Fig. 1. Absorbance values of ELISA IgA to bovine serum albumin in serum of mice vaccinated by oral, intranasal, or subcutaneous inoculation of vaccine encapsulated in alginate microspheres. See Table 3 for explanation of groups. *indicates statistically significant increase in titer compared to blank MS group. Darkened bars — absorbance value at week one and open bars — absorbance at week 5 following inoculation.

3.3. Mice

There was a significant increase in serum anti-BSA IgA in mice vaccinated orally with and without a SC priming dose of antigen (Fig. 1). For this study only the serum IgA responses are reported. There was a dose dependent increase in the anti-BSA IgA response in mice vaccinated orally with a 0.1–1.0 mg dose of BSA with the greatest dose inducing the greatest antibody response. However, as the dose increased to 2.5 mg the response was similar to the 0.1 mg dose. The most likely explanation is that this batch of microparticles had a mean size volume of 12 μm whereas the microspheres in the other groups were all less than 10 μm. Since particles less than 10 μm are not as likely to be taken up by the Peyer's patches [5], the probability of inducing an immune response was reduced in this group of mice. The SC or IN inoculation of BSA in MS or using another adjuvant (Imugen®) resulted in the lowest serum IgA responses in mice. The response to IN administration was unexpected as unencapsulated antigen as well as encapsulated antigen have previously been shown to be effective in inducing both a local and systemic immune response to other antigens [18–20] The poorer response here may be due to slower uptake and processing of the antigen in alginate MS by nasal associated lymphoid tissue in the mouse or due to the poorer processing of the antigen BSA by these tissues. The multiple doses of antigen in MS also seemed to greatly increase the IgA immune response consistent with previous studies [21]. The boost at 28 days resulted in a much greater increase in IgA titer with the responses from 1–4 weeks being more similar for most of the groups. Further studies are indicated to see how high and how long the IgA titers could be maintained. Although not shown, all groups except the mice vaccinated with blank MS had an increased IgG response with an absorbance 0.95 to 1.65. As for IgA responses, the lowest response was in mice vaccinated orally with unencapsulated BSA.

The results in mice also demonstrate that an alternative approach, i.e. emulsion technology, to making alginate microspheres is possible. Microspheres made in this manner were effective in stimulating IgA response in mice following oral administration. This method of production is more amenable to scale up for commercial production and results in more repeatable production of MS of a size that is necessary for oral administration. With the spray method, production of MS less than 10 μm was difficult to control and hard to consistently achieve. Microspheres made by emulsion also had better loading of antigen within microspheres with loading efficiencies of 40–80% of material compared to less than or equal to 33% for MS made with the spray method. This is the one of the first studies to show the efficacy of alginate MS made in this manner to induce an immune response in animals when administered orally.

These results show that oral administration of antigen in alginate microspheres can induce an antigen specific pulmonary immune response in cattle and rabbits and serum IgA response in mice. The pulmonary antibody response was present in rabbits that had reduced bacterial infection suggesting at least some protection afforded by this immune response. It will be interesting to determine how the presence of IgA alone as induced by oral vaccination alone protects cattle when challenged by an infectious agent. This has never been examined in cattle. Previous studies have demonstrated that oral administration of infectious agent antigens can induce partial protection in calves, but the isotypic nature of the protection could not be determined [12].

The immune response induced by oral administration of antigen was somewhat different in the three different hosts. The present study confirmed that IgA is the primary antibody isotype induced by the oral administration of antigen encapsulated alginate microspheres to cattle. This is somewhat different from the results seen in mice and rabbits which agrees with previous studies in which the oral administration of antigen encapsulated in microspheres in which production of both IgG and IgA is induced [6, 15, 22].

The present study demonstrated the successful induction of pulmonary immunity in cattle is consistent with the migration of lymphocytes from the gut to the lung following the oral administration of antigen encapsulated in alginate microspheres. The antigen specific IgA response seen in the BAL of calves suggests that homing of gut-associated lymphoid tissue induced lymphocytes to other mucosal sites is antigen independent consistent with previous results [17, 23]. The lack of antibodies detected in serum and BAL in orally primed calves may be due to the presence of T-helper cells at the mucosal sites being primarily responsible for local antibody detection when stimulated by antigen. The extremely low levels of antibody in serum would then result in low levels in BAL diffusing across alveoli.

Ours is among the first studies to show that a primary oral inoculation of vaccine-containing alginate microspheres is effective in stimulating antigen specific mucosal immune response at other mucosal (respiratory) sites in animals. This study has begun to elucidate the parameters involved in producing alginate microspheres for administration of antigens to animals. Because of the ease and low cost of production, they could also be used to deliver other biologically active materials as well as pharmaceuticals by parenteral or oral administration to a variety of animal species. It is our long term goal to use alginate microspheres to administer vaccines orally to food producing as well as

companion animals by incorporation in the feed or water. Successful stimulation of an antigen specific pulmonary immune response in cattle, rabbits and mice was achieved by oral administration of three different antigens encapsulated in alginate microspheres. The oral administration of vaccines to animals would reduce labor costs and increase the effective use of vaccines, permit vaccination in a more timely manner before animals are stressed and exposed to pathogens, provide an easy way to booster a large number of animals without the need for individual handling, eliminate injection site reactions and minimize adverse reactions to vaccines. Most importantly, effective immunity at the mucosal site where infection begins can be induced with oral vaccination. Future studies can now be performed to better evaluate the role of IgA in protection against respiratory pathogens. This will enhance our understanding of the role mucosal antibodies play in preventing mucosally acquired diseases in animals. The results of the studies reported here indicate the need to further evaluate the use of oral vaccines as a means of inducing protective immunity at distant mucosal sites in animals.

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