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Immunological responses to nasal delivery of free and encapsulated tetanus toxoid: studies on the effect of vehicle volume

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Abstract

In light of growing interest in the intranasal route as a non-invasive mode of immunisation, we have investigated the relationship between the volume of liquid instilled into the nasal passages and the development of subsequent immunological responses. Groups of six mice were intranasally immunised with soluble or microsphere encapsulated tetanus toxoid on days 1, 14 and 28 of the experiment. Microsphere suspensions and tetanus toxoid solutions were nasally instilled in two different volumes of buffer (10 or 50 μl). Nasal instillation of microspheres in 10 μl of buffer generated statistically depressed ($P < 0.001$) tertiary serum anti-toxoid IgG responses in comparison to animals immunised with 10 or 50 μl of soluble vaccine, or 50 μl of microsphere suspension. Relative to other treatments, nasal inoculation of encapsulated toxoid suspended in 50 μl generated statistically ($P < 0.05$) superior levels of specific IgG and IgA antibodies in day 49 lung wash samples. When radiolabelled microspheres were nasally instilled into mouse nares in 50-μl volumes of buffer, a significant portion of the dose (48%) entered the lungs ($P < 0.001$), whereas more particles remained in the nasal passages when a smaller (10 μl) volume of suspension was given ($P < 0.001$). These biodistribution and immunological data indicate that to generate optimal bronchopulmonary and systemic responses in concert following nasal administration, microparticulated vaccines should be administered with a delivery device that targets the formulation to distal regions of the nasal passages and the lower respiratory tract.

Keywords: Vaccines; Mucosal immunization; Intranasal; Microparticles; Nasal associated lymphoid tissues; M-cells

1. Introduction

There is an increasing body of evidence to suggest that the intranasal (i.n.) route is an extremely effective non-invasive means of vaccination (Almeida et al., 1993; Almeida and Alpar, 1996; Lemoine et al., 1998). Nasally applied anti-

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gens have been shown to engender both local and systemic immunological responses, and confer protection against a variety of infections (Eyles et al., 1998). The results of several studies suggest that particulated antigens, for example those encapsulated within biodegradable microspheres, stimulate superior immunity to soluble immunogens when administered by the i.n. route. Despite an expanding body of information concerning the uptake and distribution of microparticulate material following peroral administration (Florence, 1997), comparatively little is known about the fate of nasally delivered particulates. Early studies in our laboratories demonstrated that nasally applied latex microspheres could rapidly enter the blood circulation, and hence access systemic immunoresponsive tissues in the spleen, indicating that microparticulates are translocated through the nasal epithelium (Alpar et al., 1994).

In the present study we have quantified specific antibody responses in the serum and lungs of mice after i.n. administration of tetanus toxoid (TT) encapsulated in biodegradable polyester microspheres, or in solution. Concomitantly, gamma ray emitting non-biodegradable microspheres were used to determine the particle distribution after nasal delivery. As clinical i.n. immunisation procedures will likely involve either the administration of vaccine in the form of 'nose drops', or delivery of antigen to the nasopharyngeal duct/bronchopulmonary tree by way of an aerosolised spray, we have attempted to simulate these procedures by nasally administering microspheres in either 10- or 50-μl volumes of buffer.

2. Materials and methods

Tetanus toxoid (Serum Institute of India, Hadapsar, India) was encapsulated in microspheres composed of poly-(L-lactide) of molecular weight 2 kDa (Alpha Chemicals, Berkshire, UK) using a double emulsion solvent evaporation process as previously described (Conway et al., 1997). The amount of TT encapsulated per unit weight of microspheres was determined using the bicinchoninic acid assay method (Smith et al., 1985) following alkaline hydrolysis and neutralisation. Mean particle size was determined using a Malvern Mastersizer (Malvern Instruments, Malvern, UK). The surface morphology of the particles was studied using a Cambridge Instruments (Cambridge, UK) Stereoscan 90B scanning electron microscope.

Groups of six female BALB/c mice (25 g, 6-week-old) were used for in vivo studies. Experimentation strictly adhered to the 1986 Scientific Procedures Act. All mice were lightly anaesthetised with an inhaled gaseous mixture of 3% (v/v) halothane (RMB Animal Health, UK) in oxygen (300 $cm^3\ min^{-1}$) and nitrous oxide (100 $cm^3\ min^{-1}$) for i.n. dosing procedures. Animals received nasal immunisations with 2.26 limits of flocculation units (LFU) TT, either encapsulated in microspheres or in its 'free' state in solution, on days 1, 14 and 28 of the experiment. Microsphere vaccinees were given 0.250 mg of particles (= 2.26 LFU TT) suspended in either 10-μl (group: MS 10 μl) or 50-μl (group: MS 50 μl) volumes of phosphate buffered saline (PBS) (pH 7.4). Similarly, animals nasally immunised with soluble TT were inoculated with either 10-μl (group: FREE 10 μl) or 50-μl (group: FREE 50 μl) volumes of PBS diluted vaccine. A fifth, un-treated group of six mice acted as a negative control. Experimental animals were bled by superficial venupuncture on days 13, 27 and 41 of the experiment. On day 49, cardiac puncture blood samples were removed from all animals under terminal inhalational anaesthesia prior to humane killing by cervical dislocation. Broncho-alveolar washings (BAL) were collected as described previously (Eyles et al., 1998). Titration of TT specific antibody in serum and BAL samples from immunised animals was achieved using an established ELISA (Almeida et al., 1993).

Two groups of mice were intranasally treated with 0.250 mg of scandium-46 labelled styrene-divinyl benzene 7-μm diameter microspheres (NEN-TRAC®; NEN Life Science Products, UK), using either 10- or 50-μl volumes of PBS to deliver the particles. Mice were killed 15 min after radiolabel administration and the amount of radioactivity in the upper and lower respiratory tract, and gastrointestinal tract, was quantified using a 1282 compugamma universal gamma counter (LKB WALLAC, Finland).

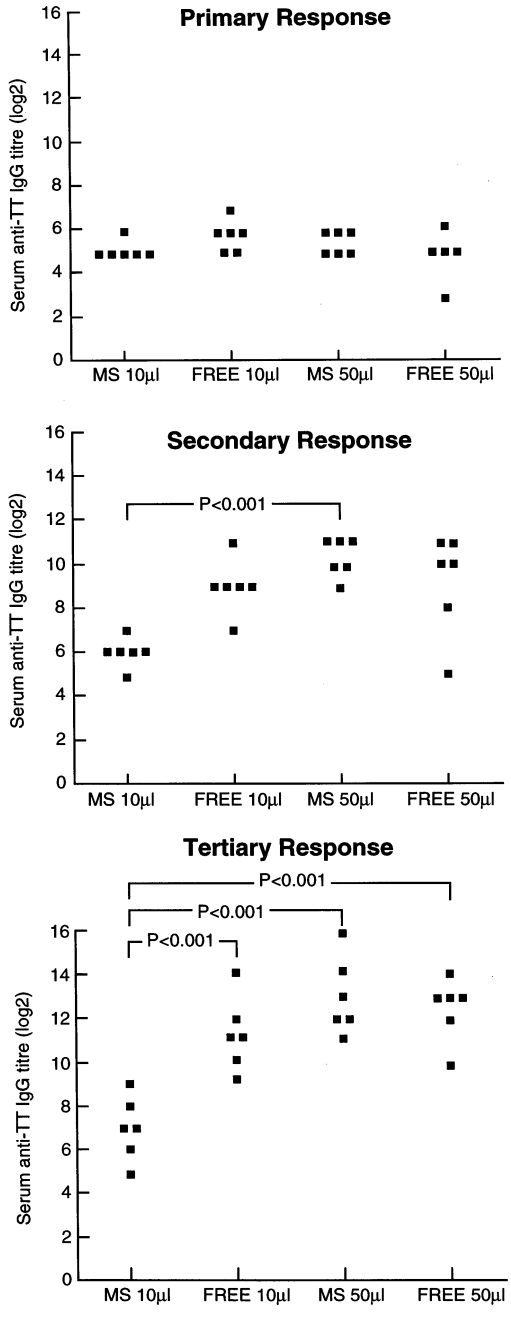

Fig. 1. Primary (upper panel), secondary (middle panel) and tertiary (lower panel) serum anti-TT IgG titres (presented on a log2 scale) following intranasal immunisation of mice ($n = 6$) with 2.26 LFU TT on days 1, 14 and 28 of the experiment. Mice received either microencapsulated (MS), or soluble TT (FREE) in 10- or 50-µl volumes of phosphate buffer. For comparison, data was treated statistically with a t-test.

3. Results

Efficiency of TT entrapment in the 2 kDa poly-(L-lactide) microspheres was 96.5%. Structural integrity of TT during microencapsulation was preserved, as evidenced by SDS–PAGE analysis of the spheres (not shown). Each mg of particles contained 9.033 ± 0.130 LFU TT. Scanning electron microscopy revealed particles with a narrow size distribution, and smooth surface topography. Laser diffraction measurements showed a volume mean particle diameter of 1.8 ± 0.7 µm. The observed tertiary specific serum responses (Fig. 1) and levels of anti-TT specific antibody recovered from BAL (Fig. 2) indicate that both the physical status of the antigen, and the volume of liquid instilled into the nasal passages, influences subsequent immunological reactions. IgG subclass analysis of terminal bleed serum revealed that for all TT vaccinees, anti-TT IgG_1 conclusively dominated over anti-TT IgG_{2a}, suggesting that it is the Th_2 subset of T-helper cells which is involved in the generation of humoral immunity to microsphere formulated or soluble TT following nasal instillation.

The biodistribution of radiolabelled microspheres following i.n. administration is shown in Table 1. Administration vehicle volumes of 50 µl served to efficiently deliver an average of $47.6 \pm 12.1\%$ of the administered microparticle dose to the lung. Conversely, comparatively more particles were retained within the nasal passages if 10-µl volumes of buffer were used to instil the microspheres ($P < 0.001$). Similar ($P = 0.83$) quantities of particles were detected in the gastrointestinal tract, irrespective of vehicle volume. This suggests that differences in observed immunological responses, following i.n. application of 10- and 50-µl volume suspensions of microencapsulated TT, are unlikely to be attributed to differences in particle uptake into gut associated lymphoid tissues (GALT).

4. Conclusions

We speculate that some aspects of the data reported here can be explained, at least in part, by

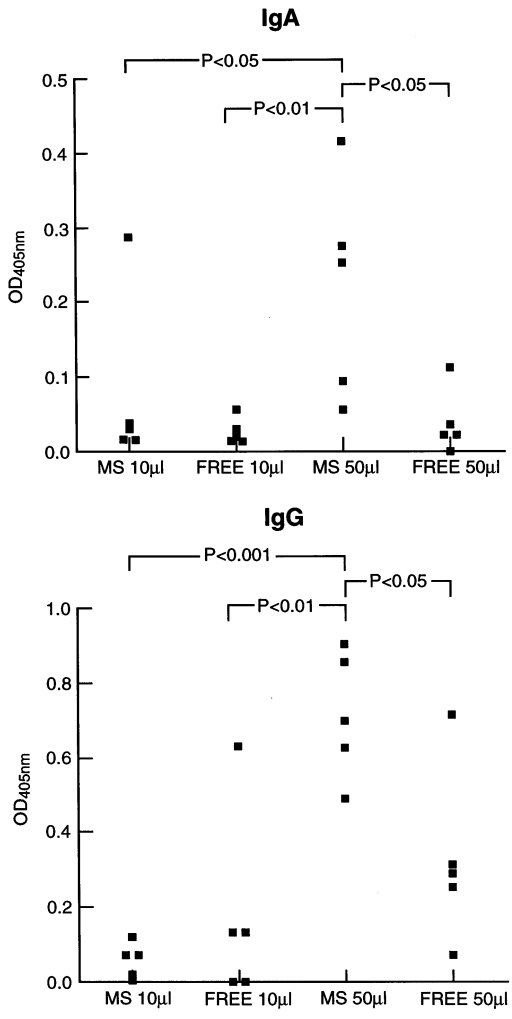

Fig. 2. Anti-TT IgA (upper panel) and IgG (lower panel) levels in day 49 broncho-alveolar washings ($n = 5$) following intranasal immunisation with 2.26 LFU TT. Mice received either microencapsulated (MS), or soluble TT (FREE) in 10- or 50-µl volumes of phosphate buffer. For comparison, data was treated statistically with a t-test.

the nature of postulated antigen sampling mechanisms in the upper and lower respiratory tract: M-cells, thought to be the principal uptake site of particulate antigen, are found in the distal regions of the nose, the nasopharyngeal and palatine tonsils, and bronchial associated lymphoid tissues (BALT) in the lung (Siminia et al., 1989; Spit et al., 1989; Neutra et al., 1996; Giannasca et al., 1997; Heritage et al., 1997; Perry and White, 1998; Ridley Lathers et al., 1998). Hence nasal administration of microsphere-encapsulated TT suspended in comparatively small (10 µl) volumes of PBS may not have delivered the vaccine carriers to the most appropriate regions of the tract to facilitate particle translocation into systemic and mucosal immunoresponsive tissues. In the case of 'free' vaccine, it is thought that soluble antigen is sampled by accessory cells in the pseudostratified respiratory epithelium, found on the ventral surfaces of the nasal cavity and lining the entrance and the length of the nasopharyngeal duct. Hence it might be anticipated that i.n. application of TT solutions, even with a relatively small volume, would facilitate exposure of immunogen to elements conducive to the induction of immunological responses towards this type of antigen.

Taken in context, these data corroborate the thesis that microencapsulated antigens stimulate superior local and systemic responses following i.n. delivery. This work expands on previous findings, in that it highlights the importance of targeting different types of antigen to the appropriate region of the respiratory tract. For optimal results, microparticulated vaccines should be administered with a device that facilitates delivery to the distal nasopharangeal duct and bronchopulmonary tree. We found that soluble TT could

Table 1
Biodistribution (represented as percent of administered radioactivity detected in each compartment) of scandium-46 labelled 7-µm diameter NEN-TRAC® microspheres 15 min after intranasal administration in either 10- or 50-µl volumes of PBS ($n = 6$)

Body compartment	Mean ($\pm$S.D.) percent administered radioactive dose in compartment		t-test comparison
	10-µl vehicle volume	50-µl vehicle volume	
Nasal passages	44.773 ± 11.292	13.339 ± 4.519	$P < 0.001$
Lungs	0.017 ± 0.001	47.612 ± 12.097	$P < 0.001$
Gastrointestinal tract	31.207 ± 8.56306	32.431 ± 10.326	$P = 0.829$

stimulate systemic responses, but not secretory responses in the lung, even if only a comparatively small volume was introduced into the nose. Thus in the case of vaccine formulations that consist of antigen(s) in solution, it is feasible that systemic responses may be generated by simple 'nose-drop' application. Under these circumstances, for most antigens, co-administration of mucosal adjuvants or absorption enhancers will probably be needed in order to elevate humoral responses to protective levels.

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