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Effective induction of protective systemic immunity with nasally administered vaccines adjuvanted with IL-1

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ABSTRACT

IL-1α and IL-1β were evaluated for their ability to provide adjuvant activity for the induction of serum antibody responses when nasally administered with protein antigens in mice and rabbits. In mice, intranasal (i.n.) immunization with pneumococcal surface protein A (PspA) or tetanus toxoid (TT) combined with IL-1β induced protective immunity that was equivalent to that induced by parenteral immunization. Nasal immunization of awake (i.e., not anesthetized) rabbits with IL-1-adjuvanted vaccines induced highly variable serum antibody responses and was not as effective as parenteral immunization for the induction of antigen-specific serum IgG. However, i.n. immunization of deeply anesthetized rabbits with rPA + IL-1α consistently induced rPA-specific serum IgG ELISA titers that were not significantly different than those induced by intramuscular (IM) immunization with rPA + alum although lethal toxin-neutralizing titers induced by nasal immunization were lower than those induced by IM immunization. Gamma scintigraphy demonstrated that the enhanced immunogenicity of nasal immunization in anesthetized rabbits correlated with an increased nasal retention of i.n. delivered non-permeable radio-labeled colloidal particles. Our results demonstrate that, in mice, IL-1 is an effective adjuvant for nasally administered vaccines for the induction of protective systemic immunity and that in non-rodent species, effective induction of systemic immunity with nasally administered vaccines may require formulations that ensure adequate retention of the vaccine within the nasal cavity.

1. Introduction

There is a growing interest in needle-free, non-invasive methods of vaccination, especially in developing countries, since the re-use of consumable non-sterile supplies is often responsible for transmission of infectious agents [1–6]. One modality for immunization that is needle-free is nasal (mucosal) immunization. The development of safe and effective nasal immunization regimens would provide a relatively non-invasive approach for vaccination that may be useful for mass vaccination campaigns [1,4,7–9] as well as for use in people who desire an alternative to injected vaccines due to fear of needles [10–14]. The FDA approval and efficacy of the nasally administered influenza vaccine Flumist (an unadjuvanted, attenuated replicating virus) [15–17], demonstrates that nasal immunization has the potential to be safe and effective for use in humans. The ability to induce both systemic and mucosal immunity (i.e., S-IgA) is a benefit of mucosal immunization [18,19]. However, even if mucosal immunity is not required to protect the host against the pathogen of interest, nasal immunization should be considered as a non-invasive method of immunization that provides an alternative to delivery of vaccines via a needle injection.

When non-replicating antigens (proteins, peptides, polysaccharides) are used for nasal immunization, adjuvants must be used

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to maximize the induction of antigen-specific immune responses since nasal immunization in the absence of adjuvant may not induce the desired immune response and may induce antigen-specific tolerance [20–24]. The balance between the induction of tolerance and adjuvant-induced immunity is thought to be associated with the ability of adjuvants to prevent the induction of regulatory T cells (T regs [25]) secondary to adjuvant-dependent enhanced antigen uptake by dendritic cells (DC), altered DC migration and enhanced DC co-stimulatory activity [26–29]. Although cholera toxin (CTx) and related toxins have been used as mucosal vaccine adjuvants, the list of observed adverse effects (induction of IgE, lethal anaphylaxis, pulmonary inflammation, diarrhea, accumulation in olfactory tissues and Bell's Palsy [30–36]) associated with their use as mucosal vaccine adjuvants will likely prevent their use in humans. To identify non-toxin nasal vaccine adjuvants, we evaluated recombinant cytokines for their ability to provide adjuvant activity comparable to that provided by CTx. Our previous studies in mice demonstrated that interleukin-1-alpha (IL-1α) and beta (IL-1β) provided adjuvant activity when nasally administered with protein antigens and induced both antigen-specific serum IgG and mucosal IgA comparable to that induced by CTx [37,38].

Effective nasal immunization in mice models may not translate into efficient immunization in humans, or other animal species with an upper respiratory tract anatomy similar to humans. Unlike rodents which have organized nasal-associated lymphoid tissue (NALT) in the floor of the nasal cavity [39–43], the nasal inductive (NALT-like) tissues in larger animals such as rabbits, non-human primates and humans, likely include tonsils, adenoids and Waldeyer's ring [44,45] which anatomically are less accessible to nasally delivered antigen/adjuvant compared to NALT in rodents. Indeed, we have previously demonstrated in mice that the nasal route of immunization was able to induce serum IgG comparable to that induced by an injected vaccine [46]. However, our past experience with nasal immunization of non-human primates demonstrated that although nasal immunization (antigen + adjuvant) was able to induce statistically increased serum IgG responses, the antibody responses induced by adjuvanted nasal immunization were approximately 10-fold lower than those induced by adjuvanted intramuscular immunization [47]. Accordingly, for non-rodent species, more research is needed to optimize nasal immunization regimens in non-rodent species to achieve and maximize the induction of antigen-specific immunity, in both the systemic and mucosal compartments.

The use of anesthesia with nasal immunization has been reported to augment antigen-specific immune responses in mice, including anti-anthrax protective antigen (PA) antibody responses as well as anthrax lethal toxin-neutralizing activity [48,49]. The anesthetic agents that augmented the vaccine-induced immune responses were agents such as propofol and pentobarbital and were delivered by needle injection. The ability of the anesthetics to increase the efficacy of nasal immunization may be attributed to an increased delivery of vaccine to the lower respiratory tract [50] and/or delayed mucociliary clearance due to decreased epithelial ciliary beat frequency [51]. Although the use of injectable anesthetics with nasal immunization regimens applied to humans is not realistic since it would negate our goal of developing needle-free methods of vaccines, the use of anesthetics with laboratory animals is needed to allow safe animal handling and effective vaccine delivery. Therefore, anesthetized laboratory models will continue to be used in nasal immunization studies and it is uncertain whether the use of anesthesia influences the efficacy of nasal immunization in larger research animals (rabbits, non-human primates). A clearer understanding of technical issues that influence the efficiency of nasal immunization in larger non-rodent species will provide valuable insight to guide the fruitful development of effective nasally administered vaccines for use in humans.

In the present study we evaluated the ability of IL-1, when used as a nasal vaccine adjuvant in mice, to induce protection against a systemic lethal challenge with *Streptococcus pneumoniae* and tetanus toxin. In addition, we assessed the adjuvant activity of IL-1 by the nasal immunization route in a rabbit model and monitored core body temperature as an index of pyrogenicity. For this rabbit model, systemic anesthesia was also investigated to evaluate its influence upon efficacy of nasal immunization adjuvanted with IL-1.

2. Materials and methods

2.1. Animals

Female BALB/c mice, 16–18 g, were purchased from Charles River. Female New Zealand White (NZW) rabbits, 2.5–3 kg, were purchased from Robinson Services (Mocksville, NC). Animals were housed in cages and provided food and water ad libitum. Mice were housed in groups of 5 mice per cage (all mice within a cage received the same vaccine) and rabbits were housed individually. Procedures for use and care of animals were approved by Duke University's Institutional Animal Care and Use Committee.

2.2. Reagents

Recombinant protective antigen (rPA), recombinant lethal factor (rLF), cholera toxin (CT) and tetanus toxin were purchased from List Biologicals (Campbell, CA). rPA and rLF were also obtained from BEI Resources. PspA protein was graciously obtained from Dr. Gary Nabors, Aventis, Lot # 589A-11, Rx1M1 PspA. Imject Alum was obtained from PIERCE (Cat # 77160; aluminum hydroxide). Tetanus toxoid was a kind gift from Pasteur Merrieux Connaught. ISA-51 [52–54] was obtained from Seppic (Fairfield, NJ) and was used to make water-in-oil emulsion vaccines. We have previously reported that both murine IL-1α and IL-1β exhibit nasal adjuvant activity [37]. Recombinant human (rhu) IL-1α was purchased from R&D Systems (Minneapolis, MN) and rhuIL-1β was provided by CISTRON Biotechnology (Pine Brook, NJ). Others have demonstrated that human IL-1α and IL-1β exhibit biological activity across species barriers [55–58] and that recombinant human IL-1α exhibits mucosal adjuvant activity in rabbits [59]. Therefore, to allow these preclinical studies to utilize the form of IL-1α or IL-1β that may be used in human studies, rhuIL-1α or rhuIL-1β was used in our murine and rabbit studies. Sterile and non-pyrogenic technetium-99m (Tc^{99m}) was acquired from Duke University radio-pharmacy and added to sodium thiosulfate according to the manufacturer's (Pharmalucence, Bedford, MA) instructions to generate Tc^{99m}-labeled sulfur colloid particles, as previously described [60]. The final labeled sulfur colloid preparation is isotonic and at neutral pH.

The adjuvant activity mediated by IL-1α/IL-1β is not due to contaminating bacterial components (i.e., endotoxin) since IL-1-containing vaccine formulations did not exhibit adjuvant activity in IL-1 receptor deficient mice while control vaccine formulations utilizing cholera toxin as the adjuvant exhibited adjuvant activity in IL-1 receptor deficient mice comparable to that observed in wild-type mice (data not shown). Vaccine formulations were prepared in endotoxin-free Dulbecco's phosphate buffered saline with calcium and magnesium (Mediatech Inc., Manassas, VA).

2.3. Mouse immunization, DTH and challenge

Nasal and subcutaneous immunization of mice with tetanus toxoid or PspA was performed as indicated in the legends for