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Review

Immunomodulatory biomaterials

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ABSTRACT

Vaccination is one of the most successful medical interventions for the prevention of disease in the twentieth century. However, with the development of new and less reactogenic vaccine antigens, which take advantage of molecular recombinant technologies, comes the need for more effective adjuvants that will facilitate the induction of adaptive immune responses. In this context, immunomodulatory biomaterials, particularly the ones based on biodegradable polymers, show great promise. This article discusses the various classes of immunomodulatory biomaterials and advocates a cross-disciplinary approach that brings together molecular concepts from various fields to rationally design vaccine adjuvants with immunomodulatory properties.

Contents

1. Introduction

Vaccines are one of the most successful interventions for infectious diseases. However, major challenges remain in vaccine design, including improving their efficacy significantly and developing new vaccines for emerging diseases. Current vaccines typically include an antigen or live attenuated microorganism, an adjuvant to enhance the immune response, and a delivery system to target delivery to the right location (Pashine et al., 2005). An adjuvant is an agent that stimulates the immune system, increasing the response to a vaccine, while not having any specific antigenic effect. Adjuvants perform one or more of three main functions. (i) They provide a "depot" for the antigen for slow release; (ii) they facilitate targeting of the antigen to immune cells and enhance phagocytosis, and (iii) they modulate and enhance the type of immune response induced by the antigen alone (Cox et al., 2006; Trujillo-Vargas et al., 2005; Lutsiak et al., 2006; Petrovsky, 2006). Adjuvants may also provide the danger signal the immune system needs in order to respond to the antigen as it would to an active infection (Janeway et al., 2001). Thus, adjuvants play a significant role on every aspect of the immune response.

However, currently very few adjuvants and delivery systems are licensed for human use, with alum being the most common one. Adjuvants and delivery systems play a much more significant role in newer vaccines consisting of isolated antigens as opposed to live microorganisms. However, most current adjuvants only stimulate one immune pathway, as described below. Therefore the development of immunomodulatory biomaterials as adjuvants and delivery systems can have a significant impact on vaccines. This review discusses the current approaches to designing immunomodulatory adjuvants and presents some future research trends in this area. We begin with a brief discussion of the immune response.

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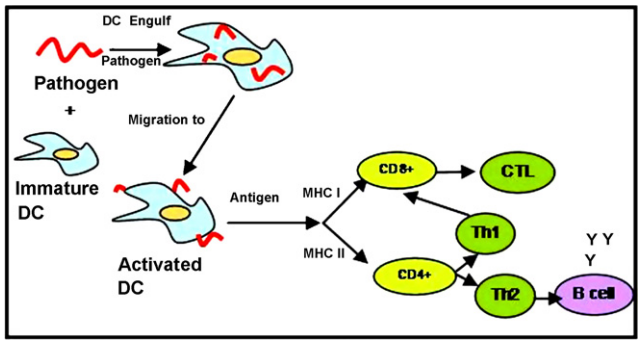

Fig. 1. Activation of immune response.

2. Immune response mechanisms

A physiological immune response begins with the antigen presenting cell (APC). This is the crucial step of the activation of the immune system. The best APCs responsible for activation of helper T cells, killer T cells and B cells are dendritic cells (DCs). Immature DCs are found under the skin and mucous membranes where they sample surrounding for possible pathogens through pattern recognition receptors. After detecting pathogen, these cells engulf it via phagocytosis and pinocytosis and migrate to lymph nodes where they become mature. Once inside the DC, pathogens are degraded into small fragments that are further expressed at their surface where they can be presented to T cells and B cells (Fig. 1). After the T cells and B cells become activated, they generate a cascade of events that lead to attack the disease.

There are two antigen presenting pathways within DCs that lead to the major histocompatibility complex (MHC) molecules. These molecules bind peptide fragments from pathogens and display them on the DC surface for recognition by T cells (O'Neill, 2005). One of these pathways is endogenous, which involves presentation of MHC I molecules to $CD8^+$ T cells. The $CD8^+$ T cells activated by DCs presenting antigens can kill infected cells directly by activating cytotoxic T lymphocytes (CTLs). On the other hand, the exogenous pathway involves presentation of MHC II molecules to $CD4^+$ T cells. A subset of the activated $CD4^+$ T cells, known as helper T cells, Th1 and Th2, are responsible for cell-mediated immunity (CMI) and humoral immunity, respectively. T cells of the immune system which generate CMI or a Th1 response require the antigen to be processed and presented on the surface of an antigen presenting cell to stimulate T cells. B cells are responsible for the humoral or Th2 response, and they recognize antigens through B cell receptors and can secrete antibodies in response. Most current vaccine designs generate an acquired immune response but do not stimulate the Th1 pathway. An immune response of the Th2-type is characterized by the production of cytokines such as IL-4, IL-5, IL-10 and IL-13 producing elevated IgG1 and IgE antibody isotypes. Th1-type responses are characterized by the production of the cytokines IFN-γ and TNF-β, antibodies of the IgG2 isotype and are usually associated with CMI including activated macrophages and delayed-type hypersensitivity (McNeela and Mills, 2001). Immune responses of the Th1 type are directed more towards intracellular pathogens and are necessary for clearance of many viruses, some bacteria (e.g., *Mycobacterium tuberculosis*) and anti-tumor effects, whereas a Th2 response is generally sufficient for the neutralization of toxins, viruses, and bacterial adhesion (Brewer and Pollock, 2004; Finkelman and Urban, 1992). Further, $CD4^+$ cells can be classified as Th17 or Treg (Annunziato et al., 2007; Villadangos and Schnorrer, 2007).

For efficacious vaccines, it is essential to induce the appropriate immune response is essential for vaccine efficacy (Finkelman and Urban, 1992; Woodland, 2004). For example, in the BALB/c model of leishmaniasis, a Th2-biased immune response does not afford protection and the mice are unable to clear the infection (Sedlik et al., 1997; Chatelain et al., 1999; Brady et al., 1998). In another example, the currently used Bacille Calmette Guerin (BCG) vaccine for tuberculosis is ineffective in preventing disease because it is unable to redirect a pre-existing immune response (due to previous infection) to a protective, Th1 dominant immune response (Rook et al., 2005; Heath and Carbone, 2003). Similarly, current vaccines against feline infectious peritonitis viruses are ineffective because they are biased towards enhancing humoral immunity, which has been shown to exacerbate the disease, whereas a CMI or regulatory response would be protective (Hebben et al., 2004).

As mentioned before, DCs are critical components of innate immunity that affect the acquired immune response (Banchereau et al., 2000) and their activation is a powerful tool to manipulate the immune system (Banchereau and Steinman, 1998). DCs are present in an immature state where they cannot stimulate T cells, but the presence of an antigen causes the DCs to mature and mobilize. Therefore vaccines that can stimulate DC maturation are promising in order to obtain a more balanced immune response and to increase the efficacy of vaccines. Harnessing both immune system pathways and facilitating immunomodulation to obtain an unbiased immune response (i.e., Th0, which is a balance between Th1 and Th2) is key to effective vaccine treatments against infectious agents (Akira et al., 2001).

3. Limitations of current vaccines

Current vaccine designs do not target the DC system. DCs can readily stimulate T cells and can operate at mucosal surfaces, where early protection is needed in many infections, while existing vaccines are weak stimulators of T cells. T-cell activation is only guaranteed by repeated encounters with persistent low levels of antigens (Zinkernagel, 2006). Therefore therapeutic strategies based on modulating the immune response may significantly expand treatment options and circumvent the problem of rapid emergence of resistance. The major advantage of infectious disease therapies based on immunomodulation is that it harnesses a system that has evolved and is continuously evolving to protect against microorganism-related diseases. While both the innate immune system and antimicrobial agents show rapid onset of action, modulators of innate immunity are not likely to develop resistance because they do not disable a specific microbial target and their mechanisms of action involve multiple effector cells.

Vaccinology, including adjuvant design, has focused mainly on parenteral routes of administration. However, most pathogens enter through mucosal routes such as oral, nasal or genital and parenteral vaccines alone do not typically produce mucosal immune responses. The difference between the mucosal mode of entry of most pathogens versus the parenteral route of administration of most vaccines leads to stimulation of production of different isotype antibodies. Current vaccines elicit mainly IgG isotype antibodies, in contrast to natural infections that elicit a wide range of antibody isotypes. Therefore the protection afforded by the vaccines is not as long-lasting.

Immunization of one mucosal surface also sensitizes other remote mucosal surfaces because of a common mucosal immune system. However, mucosal delivery of vaccines poses several challenges. Most protein antigens are poor immunogens when administered mucosally and may induce immunological tolerance instead (Elson et al., 2005). There have been several intranasal vac-

cines that have been developed to counter infectious diseases such as diphtheria and pneumonic plague (Alpar et al., 2001; Bielinksa et al., 2008; Bielinska et al., 2008; Jones et al., 2006). These vaccines are more effective when adjuvanted with either microbially derived components or synthetic polymers and are usually inhaled in particulate form. Some of these intranasal vaccines induce cell-mediated immunity, leading to protection (Bielinska et al., 2008; Jones et al., 2006). On the other hand, oral administration provides access to the largest immunological organ of the body, the intestine. Digestive degradation following oral administration causes large and repeated doses of killed microbe or peptide antigens to be required.

Therefore potentially new solutions to this problem are imperative, and a U.S. National Research Council (NRC) panel recently addressed grand challenges in this area. The panel's main recommendations (Immunomodulation, 2006) to improve vaccine design include: (1) Research to identify good delivery mechanisms and (2) identification of potential molecular targets for targeting DCs and modulating innate immunity without undesired side effects. The NRC report identified the determination of how to elicit protective non-IgG responses and simulate the mucosal response, and devising strategies to target DCs and optimize antigen delivery to DCs as the most likely approaches to improve vaccinations and combat infectious diseases (Immunomodulation, 2006).

4. Immunomodulators

The list of potential molecular targets for modulators of innate immunity is quite extensive (Germain, 2004). In cancer patients, for example, the immune system is non-specifically stimulated with immunomodulators in addition to treatment (Hanks et al., 2005). Several immunomodulatory agents are currently being investigated as adjuvants. Examples include natural compounds (calf thymic hormones, glucans), synthetic compounds (oligodeoxynucleotides containing CpG motifs, maramyl peptides, lipopolysaccharide (LPS) derivates, isopronosine, pidotimod, linomide) and endogenous compounds such as cytokines. These immunomodulatory adjuvants are shown in Table 1 and discussed in more detail in this section. Several adjuvants are being investigated to enhance the immune response and to serve as immunomodulators. The advantages and drawbacks of these adjuvants are discussed below.

Sub-toxic doses of *E. coli* heat-labile enterotoxin (LT) as an adjuvant can lead to effective mucosal immunity (Brewer and Pollock, 2004; Freytag and Clements, 2005; Majde et al., 1995). LT has shown adjuvant efficacy for induction of mucosal as well as parenteral immunity in mice and in rhesus monkeys (Baqar et al., 1995). Cholera enterotoxin (CT) is another such related potent mucosal adjuvant for oral immunization (Elson et al., 2005). LT is highly homologous to CT, but CT stimulates predominantly Th2 responses while LT stimulates predominantly Th1 responses. However, CT adjuvants delivered by the nasal route have been found to be taken up by the olfactory nerve and the central nervous system, leading to potential unwanted effects (van Ginkel et al., 2000) and CT can induce diarrhea in humans. Not much is known about the cell-mediated immunity or delayed hypersensitivity response to CT. The ability of CT to act as a mucosal adjuvant has been confirmed by many investigators with a variety of antigens, and giving CT by a route different from the antigen is not effective (Majde et al., 1995).

Many antigen preparations, especially recombinant derived antigens, contain bacterial LPS and other compounds with adjuvant activity (Singh and O'Hagan, 2002). LPS is known to stimulate a variety of cells to produce cytokines and chemokines that control dendritic cell movement and maturation (Banchereau and Steinman, 1998). But despite its potency, LPS is toxic. Chemically modified forms of its active component such as monophosphoryl lipid A (MPLA), have been shown to possess many of the adjuvant effects of LPS itself with less toxicity (Gavin et al., 2006; Johansson et al., 2004; Mata-Haro et al., 2007). But its effects at mucosal surfaces on the immune system are complex and largely unknown (Singh and O'Hagan, 2002).

Pertussigen, a complex mixture including LPS and variable amounts of pertussis toxin has been used experimentally as an adjuvant. But potential toxicity and severe reactions in humans limits its usefulness as a mucosal adjuvant. Muramyl dipeptide, derived from the cell wall of mycobacteria has been used parenterally for experimental vaccines, but data on use as a mucosal adjuvant are very limited. Lipopeptides from bacterial lipoproteins, which are potent adjuvants for parenteral immunization, are also being explored as mucosal adjuvants (Elson et al., 2005; Singh and O'Hagan, 2002).

Bacterial DNA such as CpG oligodeoxynucleotides or synthetic oligonucleotides containing immunostimulatory "CpG" motifs is effective at triggering the innate immune system (Diwan et al., 2002; Singh and O'Hagan, 1999). CpGs are effective in experimental

Table 1
Immunomodulatory adjuvants, their basic characteristics, and their immune deviation preferences

Adjuvant	Examples	Basic characteristics	Dominant antibody isotype	Reference
Microbially derived	MPLA	Detoxified TLR-4 ligand	IgG1 and IgG2a/c	Gavin et al. (2006), Johansson et al. (2004), Mata-Haro et al. (2007)
	LT/CT	Modified bacterial toxins for mucosal adherence heat-liable enterotoxin and cholera toxin	LT: IgG1, IgG2a, and IgA CT: IgG1	Brewer and Pollock (2004), Freytag and Clements (2005), Majde et al. (1995)
	CpG	Non-methylated bacterial DNA, a TLR-9 ligand	IgG2a	Diwan et al. (2002), Singh and O'Hagan (1999)
Cytokines	IL-1	Pro-inflammatory cytokine	IgG2a, IgA	Cox et al. (2006), McNeela and Mills (2001)
	IL-2	Lymphoproliferative cytokine	IgG2a	McNeela and Mills (2001)
	IL-12	Pro-inflammatory cytokine	IgG2a, IgA	Lynch et al. (2003)
	IL-6	Anti-inflammatory cytokine	IgG1, IgA	Griffin et al. (2002)
Natural polymers	Polysaccharides	Coating or emulsified with solid antigen	IgG1 or IgG2a depending on route	Brewer and Pollock (2004)
Synthetic polymers	Polyesters	Antigen and immunostimulators emulsified into biodegradable particles ranging from 50 μm to 20 nm	Variable depending on immunostimulants and antigen incorporated	Lutsiak et al. (2006), Brewer and Pollock (2004), Raghuvanshi et al. (2002)
	Polyanhydrides	Antigen emulsified into biodegradable particles ranging from 50 μm to 20 nm	Variable depending on polymer chemistry	Hanes et al. (1998), Hanes et al. (1997), Kipper et al. (2006)

infection models against several pathogens including *L. monocytogenes* (Chong et al., 2005). Cytokines themselves have been explored as adjuvants to provide potentially less toxic approaches. Not much work has been done in this area, but possible candidates include several interleukins such as IL-1, IL-2, IL-6, and IL-12 (Cox et al., 2006; McNeela and Mills, 2001; Griffin et al., 2002; Lynch et al., 2003).

Some synthetic adjuvants are based on the concept of packaging the antigen into micro/nanoparticles or micelles, where the particle size is a crucial determinant of efficient uptake. Cationic stealth liposomes significantly increased the uptake of these oligonucleotides by immune cells, suggesting that use of these liposomes can improve the delivery and activity of CpG in vivo. Quil A, a detergent derived from plants has been employed as an adjuvant, but suffers from toxicity issues (Elson et al., 2005). Multiple emulsions involving block copolymers and lipids have been used for parenteral immunization, but only recently for mucosal immunization. These include poloxamers that act more as delivery systems rather than as adjuvants. Combined DNA vaccines to four dissimilar bio-warfare pathogens (anthrax, Ebola, Marburg and Venezuelan equine encephalitis (VEE)) was demonstrated using this approach, enabling rapid development of multi-agent vaccines (Reimenschneider et al., 2003). These DNA vaccines can also be combined with other immunostimulatory agents such as CT and LT (Dean et al., 2005).

There are potential drawbacks of the immunomodulation approach. Toll-like receptor agonists can trigger autoimmune disorders in genetically predisposed mice (Lang et al., 2005). Excessive stimulation of the innate immune system can lead to pro-inflammatory mediators that promote host damage. Enhancing T-cell regulatory activity in vivo has been shown to limit infection-associated inflammation and this can be used as a strategy to minimize inflammation (Belkaid and Rouse, 2005). Possible toxic side effects due to potent activation of innate immunity can be minimized by use of suitable delivery systems to minimize diffusion from the injection site (Pashine et al., 2005).

5. The promise of polymeric biomaterials

The adjuvants discussed above, while promising, suffer from several drawbacks. Pathogens have evolved mechanisms against host immune systems. Moreover, toxicity is a huge concern with several of these adjuvants. Synthetic polymers with specific characteristics can be used as adjuvants and immunomodulators as an alternative to the microbially derived adjuvants currently being investigated. This is a relatively new interdisciplinary area involving a marriage of immunology and materials chemistry. The use of hydrophobic and amphiphilic biodegradable polymers as both adjuvants and as delivery agents has not been explored much in this context for immune modulation, but holds great promise, for the reasons described below.

The innate immune system has a dual role, and is associated with defense against pathogens, and also some normal physiological processes such as tissue remodeling after damage and during development. A model proposed by Matzinger suggests that APCs are tuned to endogenous danger/alarm signals from distressed tissues (Seong and Matzinger, 2005). Therefore the danger model suggests that immune responses occur against any entities associated with host damage, and not just by other microorganisms. There is evidence to suggest that the toll-like receptors on DCs have evolved to recognize and react to hydrophobic portions ("hypos') of molecules should they suddenly become exposed, as can happen after injury and during repair and remodeling (Seong and Matzinger, 2005). These exposed hyppos can aggregate intracellularly or extracellularly, leading to potential cytotoxicity as in the case of prions, amyloid-β proteins etc. Thus, hyppos are potentially immunostimulatory. It is also well known that inflammation increases with polymer hydrophobicity, hydrophobic bacteria are more readily phagocytosed by macrophages, and LPS and other microbial immunostimulatory products have large hydrophobic portions that are exposed when the microorganisms are damaged.

Therefore a new strategy involving use of hydrophobic synthetic biodegradable polymeric biomaterials as immunomodulators can eliminate the use of microbially derived adjuvants that suffer from toxicity issues and other drawbacks. Moreover, these polymers can simultaneously serve as delivery devices for the antigens in the form of microspheres or nanospheres (Kipper et al., 2002) for enabling alternate routes of delivery, and provide sustained release to facilitate single-dose vaccines and eliminate the need for booster shots. These materials obviate the need for surgical removal, and most are manufactured from synthetic base compounds, eliminating many potential reactive antigenic or allergenic epitopes associated with animal or plant derived materials. Polyesters and polyanhydrides are the two most widely studied biodegradable materials for controlled release of antigens. In addition, other polymers have been evaluated and shown to successfully deliver antigen to laboratory animals.

Copolymers of lactic acid and glycolic acid (i.e., poly(lactide-co-glycolide) or PLGA) have been widely utilized in biomedical applications such as soluble sutures and have been recently shown to induce protective immunity (Hanks et al., 2005; Chong et al., 2005; Hamdy et al., 2007; Alonso et al., 1994; O'Brien and Guidry, 1996). However, as the polyester degrades, an acidic microenvironment is created by the lactic or glycolic acid. A prolonged exposure to acidic environments may be detrimental to the stability and immunogenicity of recombinant proteins used in vaccines, e.g., tetanus toxoid (TT) and diphtheria toxoid (Jiang and Schwendeman, 2001; Xing et al., 1996). Vaccine formulations based on PLGA have been successful in inducing immune responses to many antigens (McNeela and Mills, 2001; Alpar et al., 1997; Conway et al., 2001; Gupta et al., 1998; Kim et al., 2002; Raghuvanshi et al., 2002). Furthermore, encapsulation of antigens in PLGA microspheres was shown to enhance antigen presentation via MHC I leading to increased activation of antigen specific cytotoxic T cells (Conway et al., 2001; Audran et al., 2003; Evans et al., 2004). However, some of these investigations included MPLA, a known Th1 immune response activator, in the microsphere while others used multiple injection regimens in vivo. Some studies have suggested an immune response shift after immunization with PLGA microspheres. Moore et al. showed that HIV gp120 protein loaded PLGA microspheres shift the immune response from Th2 or mixed Th1/Th2 to Th1 (Moore et al., 1995). It has also been shown that when CpG DNA is formulated together with PLGA microspheres and HIV-1 enc gp120 recombinant protein, an enhanced immune response is observed (Fig. 2) compared to the response with PLA microspheres or CpG DNA alone (Singh and O'Hagan, 2002). In other studies, the Th2-biased Hepatitis B core antigen has been formulated with MPLA in PLGA nanoparticles to develop a stronger Th1 response (Lutsiak et al., 2006). More recently, a vaccine formulation against malaria with PLGA microspheres and Montanide ISA 720 has shown to exhibit a Th1 response (Mata et al., 2007). There is no consensus opinion, however, as to whether PLGA-based vaccines are immunomodulatory by themselves (i.e., without the addition of an immunomodulator like MPLA or CpG DNA) or more efficacious than current adjuvant systems. Additionally, no studies have been reported on PLGA-based vaccine formulations that attest to protective immunity in humans.

Polyanhydrides are surface erodible polymers that have been widely used as carriers for controlled delivery of drugs, proteins,

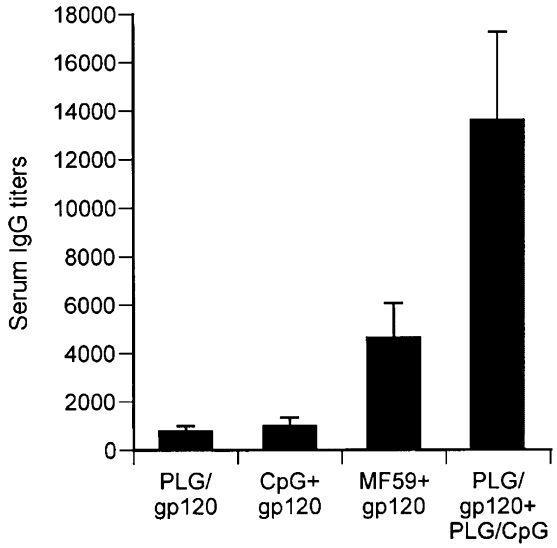

Fig. 2. Effect of CpG DNA and PLGA microsphere adjuvants on the antibody responses after two i.m. injections 4 weeks apart in mice. The adjuvants were provided together with HIV-1 env gp120 recombinant protein. The induced responses are compared to gp120 in MF59 adjuvant. The data is presented as geometric mean titer ± standard error. Reproduced with permission.

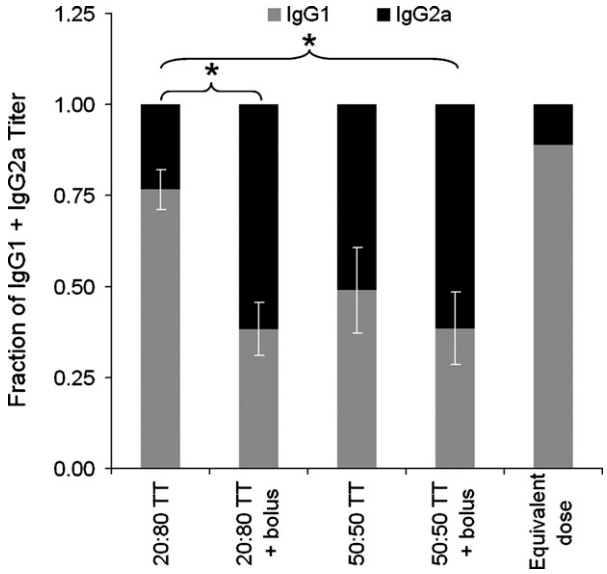

Fig. 3. Fraction of the IgG1 and IgG2a TT-specific antibody titers at week 12. The 20:80 group represents TT antigen encapsulated in 20:80 CPH:SA microspheres and the 50:50 group represents TT antigen encapsulated in 50:50 CPH:SA microspheres. The asterisk (*) represents statistical significance ($p < 0.05$). Reproduced with permission.

and antigens (Leong et al., 1986a,b; Narasimhan and Kipper, 2004; Tamada and Langer, 1992; Torres et al., 2006a). These degradable biomaterials break down into carboxylic acids, which are non-mutagenic and non-cytotoxic (Katti et al., 2002; Kumar et al., 2002). Their surface erosion mechanism leads to a controlled release profile with predictable degradation profiles, which can range from days to months, depending on the polymer chemistry (Kipper et al., 2002; Shen et al., 2002). As the hydrophobicity of the polymer decreases, these materials also exhibit characteristics of bulk erosion (Torres et al., 2007; Vogel and Mallapragada, 2005). Polyanhydride-based systems for antigen delivery have exhibited improved adjuvanticity, antigen stabilization, and enhanced immune responses (Leong et al., 1986a,b; Tamada and Langer, 1992; Schwendeman et al., 1997; Determan et al., 2004). A desirable feature of polyanhydrides as antigen carriers is the enhanced protein stability conferred by them (Hanes et al., 1998, 1997; Kipper et al., 2006). Polyanhydrides are capable of stabilizing polypeptides and sustaining their release without the inclusion of potentially reactive excipients or stabilizers (Torres et al., 2007; Ron et al., 1993; Tabata et al., 1993; Determan et al., 2006). Furthermore, the degradation products of polyanhydrides are less acidic than those of polyesters, which may further enhance the stability of encapsulated antigens and reduce tissue reactions to the polymer (Torres et al., 2007; Determan et al., 2006).

Polyanhydrides based on hydrophobic moieties such as sebacic acid (SA) and 1,6-bis(*p*-carboxyphenoxy)hexane (CPH) have been shown to modulate the immune response, which holds great promise for immunomodulatory vaccines (Kipper et al., 2006). In vivo release studies in mice of tetanus toxoid from poly(CPH-co-SA) microspheres with different CPH:SA ratios has shown that the microspheres provided adjuvant-like activity and a prolonged exposure to TT was sufficient to induce an enhanced immune response following a single administration (Kipper et al., 2006). More significantly, in this single-dose polymer-based vaccine, altering the copolymer composition and hydrophobicity allowed the dominant Th2 immune response induced by TT to be selectively diminished (Fig. 3), resulting in a balanced (Th0) immune response. The preferential enhancement of the Th1 immune response resulting in more balanced immune response (i.e., immune deviation) is a unique and valuable feature of this delivery vehicle that makes it a promising adjuvant candidate for vaccines. Potent adjuvants such as polyanhydrides with the ability to shift Th1-Th2 potential have the ability to improve current vaccine strategies that are often inappropriate.

Related work has shown that amphiphilic biodegradable polyanhydrides can be used to establish optima for both surface hydrophobicity-controlled phagocytosis and amphiphilicity-controlled antigen stabilization by tailoring polymer chemistry and microenvironment to yield superior adjuvanticity, enhanced antigen stability, modulation of antigen uptake and presentation, and induction of a balanced immune response characterized by both B and T-cell responses. Studies have shown that polyanhydrides are capable of stabilizing polypeptides and sustaining their release (Torres et al., 2007; Determan et al., 2004; Determan et al., 2006). A new class of amphiphilic polyanhydrides based on oligomeric ethylene glycol-containing anhydrides (e.g., (1,8-bis(*p*-carboxyphenoxy)-3,6-dioxaoctane) (CPTEG)) (Vogel and Mallapragada, 2005; Torres et al., 2006b) have shown promise as novel vaccine carriers with immunomodulatory capabilities. Using ovalbumin (Ova) as a model antigen, stimulation of DCs using various adjuvants such as LPS and CPTEG:CPH polyanhydride microspheres shows that the highest proliferative response occurs with the CPTEG:CPH microspheres in combination with Ova (Torres et al., submitted for publication). Therefore these degradable biomaterials serve as effective immunomodulators without the toxicity issues associated with LPS. They can also be used as microspheres for single-dose vaccines and inhalation delivery mechanisms to help target mucosal tissues directly, which would mimic the route of entry for most pathogens.

6. Discussion and conclusions

In summary, good immunostimulatory vaccine adjuvants activate DCs to mature and migrate to the draining lymph node, coincident with induction of the cytokine profile appropriate to the desired immune response mechanism (i.e., IFN-γ, IL-2, and IL-12 for the Th1 response and IL-4, IL-5, and IL-6 for the Th2

response). Like adjuvants that target DCs, some immunostimulatory vaccine adjuvants also interact with TLR proteins. Regardless of the mechanism of adjuvanticity, vaccine adjuvants must activate the desired adaptive immune response without over stimulating innate immune function. From an economic and practical standpoint, immunomodulatory biomaterials need to be stable and cost-effective for them to have widespread use. This creates potential problems for cytokines and some biodegradable polymers. There is also the need to for the biomaterial to be delivered to mucosal surfaces in many instances and for the delivery to occur in a single dose. Finally, it is important that these delivery systems be manufactured with ease and under GMP conditions, which may provide challenges for micro- and nanoparticles, which are typically fabricated in solvent-intensive processes.

The balance of Th1 and Th2 immune responses has been shown to be important in the favorable outcome of many disease states. In this regard, vigorous and inappropriate Th1-biased immune responses have been implicated in the induction of autoimmune diseases (multiple sclerosis and Crohn's disease) while Th2-biased immune responses are associated with allergic reactions (Akira et al., 2001). In order to control the induction of appropriate immune responses and reduce the risk of autoimmunity or allergic responses, there is an urgent need to develop new, well-characterized adjuvants that allow for tailored immune activation and deviation. In spite of these implications of immune deviation, the mechanisms by which adjuvants influence whether Th1 or Th2 cells dominate an immune response are not well understood. It is also critical to re-think the importance of antibody titer as a correlate of vaccine efficacy, since this does not translate into the best protection (Magyar et al., 2008).

While microbially derived adjuvants have shown promise, toxicity issues can limit their usage, and the use of non-toxic biodegradable synthetic polymeric biomaterials as adjuvants can greatly improve immunomodulatory capabilities by tuning polymer chemistry and hydrophilicity. These polymers can simultaneously serve as delivery agents as well, with aerosol delivery involving polymer micro and nanospheres mimicking the natural routes of entry of many pathogens. These polymers can also lead to single-dose vaccines that can stimulate DCs by forcing them to respond to danger signals induced by the hydrophobic moieties in the polymers.

The recommendations of the NRC panel on Immunomodulation (Immunomodulation, 2006) to improve vaccine design included an enhanced molecular level understanding of the innate immune system, the need for effective delivery mechanisms, the identification of potential molecular targets to modulate innate immunity without undesirable side effects, and new strategies to target DCs and optimize antigen presentation. A key need that was identified was that in order to solve these problems, it is important to develop novel cross-disciplinary approaches that bring together researchers from multiple disciplines. These fields include biochemistry, immunology, materials science, cell biology, computational biology/materials science, pathology, oncology, microbiology, and combinatorial science. Our own view is concurrent with this strategy and the development of tomorrow's successful adjuvants and vaccine delivery devices will likely be traced to the seamless melding of ideas from these fields.

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References

Akira, S., Takeda, K., Kaisho, T., 2001. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat. Immunol. 2, 675–680.
Alonso, M.J., Gupta, R.K., Min, C., Siber, G.R., Langer, R., 1994. Biodegradable microspheres as controlled-release tetanus toxoid delivery systems. Vaccine 12, 299–306.
Alpar, H.O., Ozsoy, Y., Bowen, J., Eyles, J.E., Conway, B.R., Williamson, E.D., 1997. Potential of particulate carriers for the mucosal delivery of DNA vaccines. Biochem. Soc. Trans. 25, 337S.
Alpar, H.O., Eyles, J.E., Williamson, E.D., Somavarapu, S., 2001. Intranasal vaccination against plague, tetanus, and diphtheria. Adv. Drug Deliv. Rev. 51, 173–201.
Annunziato, F., Cosmi, L., Santarlasci, V., Maggi, L., Liotta, F., Mazzinghi, B., et al., 2007. Phenotypic and functional features of human Th17 cells. J. Exp. Med. 204, 1849–1861.
Audran, R., Peter, K., Dannull, J., Men, Y., Scandella, E., Groettrup, M., et al., 2003. Encapsulation of peptides in biodegradable microspheres prolongs their MHC class-I presentation by dendritic cells and macrophages in vitro. Vaccine 21, 1250–1255.
Banchereau, J., Steinman, R.M., 1998. Dendritic cells and control of immunity. Nature 392, 245–252.
Banchereau, J., Briere, F., Caux, C., Davoust, J., Lebecque, S., Liu, Y.-J., et al., 2000. Immunobiology of dendritic cells. Ann. Rev. Immunol. 18, 767–811.
Baqar, B., Bourgeois, A.L., Schultheiss, P.J., Walker, R.I., Rollins, D.M., Haberberger, R.L., et al., 1995. Safety and immunogenicity of a prototype oral whole-cell killed campylobacter vaccine administered with a mucosal adjuvant in non-human primates. Vaccine 13, 22–28.
Belkaid, Y., Rouse, B.T., 2005. Natural regulatory T-cells in Infectious Disease. Nat. Immunol. 6, 353–360.
Bielinksa, A.U., Janczak, K.W., Landers, J.J., Markovitz, D.M., Montefiori, D.C., Baker, J.R., 2008. Nasal immunization with a recombinant HIV gp120 and nanoemulsion adjuvant produces Th1 polarized responses and neutralizing antibodies to primary HIV type 1 isolates. AIDS Res. Hum. Retroviruses 24, 271–281.
Bielinska, A.U., Chepurnov, A.A., Landers, J.J., Janczak, K.W., Chepurnova, T.S., Luker, G.D., et al., 2008. A novel killed-virus nasal vaccinia vaccine. Clin. Vaccine Immunol. 15, 348–358.
Brady, M.T., Mahon, B.P., Mills, K.H.G., 1998. Pertussis infection and vaccination induces Th1 cells. Immunol. Today 19, 534.
Brewer, J.M., Pollock, K.G.J., 2004. Adjuvant-induced Th2 and Th-1 dominated immune responses. In: Kaufmann, S.H.E. (Ed.), Novel Vaccination Strategies. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, pp. 147–163.
Chatelain, R., Mauze, S., Coffman, R.L., 1999. Experimental leishmania major infection in mice: role of IL-10. Parasite Immunol. 21, 211–218.
Chong, C.S., Cao, M., Wong, W.W., Fischer, K.P., Addison, W.R., Kwon, G.S., et al., 2005. Enhancement of T helper type 1 immune responses against hepatitis B virus core antigen by PLGA nanoparticle vaccine delivery. J. Control. Release 102, 85–99.
Conway, M.A., Madrigal-Estebas, L., McClean, S., Brayden, D.J., Mills, K.H., 2001. Protection against *Bordetella pertussis* infection following parenteral or oral immunization with antigens entrapped in biodegradable particles: effect of formulation and route of immunization on induction of Th1 and Th2 cells. Vaccine 19, 1940–1950.
Cox, E., Verdonck, F., Vanrompay, D., Goddeeris, B., 2006. Adjuvants modulating mucosal immune responses or directing systemic responses towards the mucosa. Vet. Res. 37, 511–539.
Dean, H.J., Haynes, J., Schmaljohn, C., 2005. The role of particle mediated DNA vaccines in biodefense preparedness. Adv. Drug Deliv. Rev. 57, 1315–1342.
Determan, A.S., Trewyn, B.G., Lin, V.S.-Y., Nilsen-Hamilton, M., Narasimhan, B., 2004. Encapsulation, stabilization, and release of BSA-FITC from polyanhydride microspheres. J. Control. Release 100, 97–109.
Determan, A.S., Wilson, J.H., Kipper, M.J., Wannemuehler, M.J., Narasimhan, B., 2006. Protein stability in the presence of polymer degradation products: consequences for controlled release formulations. Biomaterials 27, 3312–3320.
Diwan, M., Tafaghodi, M., Samuel, J., 2002. Enhancement of immune responses by co-delivery of a CpG oligodeoxynucleotide and tetanus toxoid in biodegradable nanospheres. J. Control. Release 85, 247–262.
Elson, C.O., Dertzbaugh, M.T., 2005. Mucosal adjuvants. In: Mestecky, J., Lamm, M.E., Strober, W., Bienestock, J., McChee, J.P., Mayer, L. (Eds.), Mucosal Immunology. Academic Press, New York, pp. 967–986.
Evans, J.T., Ward, J.R., Kern, J., Johnson, M.E., 2004. A single vaccination with protein-microspheres elicits a strong CD8 T-cell-mediated immune response against *Mycobacterium tuberculosis* antigen Mtb8.4. Vaccine 22, 1964–1972.
Finkelman, F.D., Urban, J.F.J., 1992. Cytokines: making the right choice. Parasitol. Today 8, 311–314.
Freytag, L.C., Clements, J.D., 2005. Mucosal adjuvants. Vaccine 23, 1804–1813.
Gavin, A.L., Hoebe, K., Duong, B., Ota, T., Martin, C., Beutler, B., et al., 2006. Adjuvant-enhanced antibody responses in the absence of toll-like receptor signaling. Science 314, 1936–1938.
Germain, R.N., 2004. An innately interesting decade of research in immunology. Nat. Med. 10, 1307–1320.

Griffin, K.F., Eyles, J.E., Spiers, I.D., Alpar, H.O., Williamson, E.D., 2002. Protection against plague following immunisation with microencapsulated V antigen is reduced by co-encapsulation with IFN-gamma or IL-4, but not IL-6. Vaccine 20, 3650–3657.

Gupta, R.K., Chang, A.C., Siber, G.R., 1998. Biodegradable polymer microspheres as vaccine adjuvants and delivery systems. Dev. Biol. Stand. 92, 63–78.

Hamdy, S., Elamanchili, P., Alshamsan, A., Molavi, O., Satou, T., Samuel, J., 2007. Enhanced antigen-specific primary CD4+ and CD8+ responses by codelivery of ovalbumin and toll-like receptor ligand monophosphoryl lipid A in poly(D,L-lactic-co-glycolic acid) nanoparticles. J. Biomed. Mater. Res. 81, 652–662.

Hanes, J., Cleland, J.L., Langer, R., 1997. New advances in microsphere-based single-dose vaccines. Adv. Drug Deliv. Rev. 28, 97–119.

Hanes, J., Chiba, M., Langer, R., 1998. Degradation of porous poly(anhydride-co-imide) microspheres and implications for controlled macromolecule delivery. Biomaterials 19, 163–172.

Hanks, B.A., Jiang, J., Singh, R.A., Song, W., Barry, M., Huls, M., et al., 2005. Re-Engineered CD40 receptor enables potent pharmacological activation of dendritic cell cancer vaccines in vivo. Nat. Med..

Heath, W.R., Carbone, F.R., 2003. Immunology: dangerous liaisons. Nature 425, 460–461.

Hebben, M., Duquesne, V., Cronier, J., Rossi, B., Aubert, A., 2004. Modified vaccinia virus Ankara as a vaccine against feline coronavirus: immunogenicity and efficacy. J. Feline Med. Surg. 6, 111–118.

Immunomodulation, 2006. Treating Infectious Diseases in a Microbial World: Report of Two Workshops on Novel Antimicrobial Therapeutics. National Research Council of the National Academies, Washington DC.

Janeway, C.A., Travers, P., Walport, M., Shlomchik, M.J. (Eds.), 2001. Immunobiology The Immune System in Health and Disease, 5th ed. Garland Publishing, New York, NY.

Jiang, W., Schwendeman, S.P., 2001. Stabilization of a model formalinized protein antigen encapsulated in poly(lactide-co-glycolide)-based microspheres. J. Pharmaceut. Sci. 90, 1558–1569.

Johansson, J., Ledin, A., Vernesson, M., Lovgren-Bengtsson, K., Hellman, L., 2004. Identification of adjuvants that enhance the therapeutic antibody response to host IgE. Vaccine 22, 2873–2880.

Jones, T., Adamovicz, J.J., Cyr, S.L., Bolt, C.R., Bellerose, N., Pitt, L.M., et al., 2006. Intranasal Protollin/F1-V vaccine elicits respiratory and serum antibody responses and protects mice against lethal aerosolized plague infection. Vaccine 24, 1625–1632.

Katti, D.S., Lakshmi, S., Langer, R., Laurencin, C.T., 2002. Toxicity, biodegradation and elimination of polyanhydrides. Adv. Drug Deliv. Rev. 54, 933–961.

Kim, W., Lee, W., Ryoo, J., Kim, S., Kim, J., Youn, J., et al., 2002. Suppression of collagen-induced arthritis by single administration of poly(lactic-co-glycolic acid) nanoparticles entrapping type II collagen: a novel treatment strategy for induction of oral tolerance. Arthritis Rheum. 46, 1109–1120.

Kipper, M.J., Shen, E., Determan, A., Narasimhan, B., 2002. Design of an injectable system based on bioerodible polyanhydride microspheres for sustained drug delivery. Biomaterials 23, 4405–4412.

Kipper, M.J., Wilson, J.H., Wannemuehler, M., Narasimhan, B., 2006. Single dose vaccine based on biodegradable polyanhydride microspheres can modulate immune response mechanism. J. Biomed. Mater. Res. 76, 798–810.

Kumar, N., Langer, R.S., Domb, A.J., 2002. Polyanhydrides: an overview. Adv. Drug Deliv. Rev. 54, 889–910.

Lang, K.S., Recher, M., Junt, T., Navarini, A.A., Harris, N.L., Freigang, S., et al., 2005. Toll-like receptor engagement converts T-cell autoreactivity into overt autoimmune disease. Nat. Med. 11, 138–145.

Leong, K.W., D'Amore, P., Marletta, M., Langer, R., 1986a. Bioerodible polyanhydrides as drug-carrier matrices, II. Biocompatibility and chemical reactivity. J. Biomed. Mater. Res. 20, 51–64.

Leong, K.W., Kost, J., Mathiowitz, E., Langer, R., 1986b. Polyanhydrides for controlled release of bioactive agents. Biomaterials 7, 364–371.

Lutsiak, M.E., Kwon, G.S., Samuel, J., 2006. Biodegradable nanoparticle delivery of a Th2-biased peptide for induction of Th1 immune responses. J. Pharm. Pharmacol. 58, 739–747.

Lynch, J.M., Briles, D.E., Metzger, D.W., 2003. Increased protection against pneumococcal disease by mucosal administration of conjugate vaccine plus interleukin-12. Infect. Immun. 71, 4780–4788.

Magyar, T., Donko, T., Kovacs, F., 2008. Atrophic rhinitis vaccine composition triggers different serological profiles that do not correlate with protection. Acta Vet. Hung. 56, 27–40.

Majde, J., Pavlovskis, O., Baqar, S., Katz, J., Walker, R., Clements, J.D., 1995. *E. coli* heat-labile enterotoxin, an oral adjuvant for protection against mucosal pathogens. In: Stewart-Tull, D.E.S. (Ed.), The Theory and Practical Application of Adjuvants. Wiley, pp. 337–351.

Mata, E., Carcaboso, A.M., Hernandez, R.M., Igartua, M., Corradin, G., Pedraz, J.L., 2007. Adjuvant activity of polymer microparticles and Montanide ISA 720 on immune responses to *Plasmodium falciparum* MSP2 long synthetic peptides in mice. Vaccine 25, 877–885.

Mata-Haro, V., Cekic, C., Martin, M., Chilton, P.M., Casella, C.R., Mitchell, T.C., 2007. The vaccine adjuvant monophosphoryl lipid A as a TRIF-biased agonist of TLR4. Science 316, 1628–1632.

McNeela, E.A., Mills, K.H., 2001. Manipulating the immune system: humoral versus cell-mediated immunity. Adv. Drug Deliv. Rev. 51, 43–54.

Moore, A., McGuirk, P., Adams, S., Jones, W.C., Paul McGee, J., O'Hagan, D.T., et al., 1995. Immunization with a soluble recombinant HIV protein entrapped in biodegradable microparticles induces HIV-specific CD8+ cytotoxic T lymphocytes and CD4+ Th1 cells. Vaccine 13, 1741–1749.

Narasimhan, B., Kipper, M.J., 2004. Surface-erodible biomaterials for drug delivery. Adv. Chem. Eng. 29, 169–218.

O'Brien, C.N., Guidry, A.J., 1996. Formulation of poly(DL-lactide-co-glycolide) microspheres and their ingestion by bovine leukocytes. J. Dairy Sci. 79, 1954–1959.

O'Neill, L.A.J., 2005. Immunity's early warning system. Sci. Am. 292, 38–45.

Pashine, A., Valiante, N.M., Ulmer, J.B., 2005. Targeting the innate immune response with improved vaccine adjuvants. Nat. Med. 11, S63–S68.

Petrovsky, N., 2006. Novel human polysaccharide adjuvants with dual Th1 and Th2 potentiating activity. Vaccine 24, S2-26-9.

Raghuvanshi, R.S., Katare, Y.K., Lalwani, K., Ali, M.M., Singh, O., Panda, A.K., 2002. Improved immune response from biodegradable polymer particles entrapping tetanus toxoid by use of different immunization protocol and adjuvants. Int. J. Pharm. 245, 109–121.

Reimenschneider, J., Garrison, A., Geisbert, J., Jahrling, P., Hevey, M., Negley, D., et al., 2003. Comparison of individual and combination DNA vaccines for B. Anthracis, Ebola Virus, Marburg Virus and Venezuelan Equine Encephalitis Virus. Vaccine 21, 4071–4080.

Ron, E., Turek, T., Mathiowitz, E., Chasin, M., Hageman, M., Langer, R., 1993. Controlled release of polypeptides from polyanhydrides. In: Proceedings of the National Academy of Sciences of the United States of America, vol. 90, pp. 4176–4180.

Rook, G.A.W., Dheda, K., Zumla, A., 2005. Do successful tuberculosis vaccines need to be immunoregulatory rather than merely Th1-boosting? Vaccine 23, 2115–2120.

Schwendeman, S.P., Costantino, H.R., Gupta, R.K., Langer, R., 1997. Peptide, protein, and vaccine delivery from implantable polymeric systems. In: Park, K. (Ed.), Controlled Drug Delivery: Challenges and Strategies. ACS, Washington, DC, pp. 229–267.

Sedlik, C., Dériaud, E., Leclerc, C., 1997. Lack of Th1 or Th2 polarization of $CD4^+$ T cell response induced by particulate antigen targeted to phagocytic cells. Int. Immunol. 9, 91–103.

Seong, S.-Y., Matzinger, P., 2005. Hydrophobicity: an ancient damage associated molecular pattern that initiates innate immune responses. Nat. Rev. Immunol. 4, 469–478.

Shen, E., Kipper, M.J., Dziadul, B., Lim, M.-K., Narasimhan, B., 2002. Mechanistic relationships between polymer microstructure and drug release kinetics in bioerodible polyanhydrides. J. Control. Release 82, 115–125, 2002/7/18.

Singh, M., O'Hagan, D., 1999. Advances in vaccine adjuvants. Nat. Biotechnol. 17, 1075–1081.

Singh, M., O'Hagan, D.T., 2002. Recent advances in vaccine adjuvants. Pharm. Res. 19, 715–728.

Tabata, Y., Gutta, S., Langer, R., 1993. Controlled delivery systems for proteins using polyanhydride microspheres. Pharmaceut. Res. 10, 487–496.

Tamada, J., Langer, R., 1992. The development of polyanhydrides for drug delivery applications. J. Biomater. Sci. Polym. Ed. 3, 315–353.

Torres, M.P., Determan, A., Mallapragada, S.K., Narasimhan, B., 2006a. In: Lee, S. (Ed.), Polyanhydrides Encyclopedia of Chemical Processing. Marcel Dekker, New York.

Torres, M.P., Vogel, B., Narasimhan, B., Mallapragada, S.K., 2006b. Synthesis and characterization of novel polyanhydrides with tailored erosion mechanisms. J. Biomed. Mater. Res. 76, 102–110.

Torres, M.P., Determan, A.S., Anderson, G.L., Mallapragada, S.K., Narasimhan, B., 2007. Amphiphilic polyanhydrides for protein stabilization and release. Biomaterials 28, 108–116, 2007/1.

Torres, M.P., Wilson-Welder, J.H., Lopac, S.K., Mallapragada, S.K., Wannemuehler, M.J., Narasimhan, B. In vitro activation of dendritic cells using polyanhydride microspheres. Biomaterials, submitted for publication.

Trujillo-Vargas, C.M., Mayer, K.D., Bickert, T., Palmetshofer, A., Grunewald, S., Ramirez-Pineda, J.R., et al., 2005. Vaccinations with T-helper type 1 directing adjuvants have different suppressive effects on the development of allergen-induced T-helper type 2 responses. Clin. Exp. Allergy 35, 1003–1013.

van Ginkel, F.W., Jackson, R.J., Yuki, Y., McGhee, J.W., 2000. Cutting edge: the mucosal adjuvant cholera toxin redirects vaccine proteins into olfactory tissues. J. Immunol. 165, 4778–4782.

Villadangos, J.A., Schnorrer, P., 2007. Intrinsic and cooperative antigen-presenting functions of dendritic-cell subsets in vivo. Nat. Rev. Immunol. 7, 543–555.

Vogel, B.M., Mallapragada, S.K., 2005. Synthesis of novel biodegradable polyanhydrides containing aromatic and glycol functionality for tailoring of hydrophilicity in coltrolled drug delivery devices. Biomaterials 26, 721–728.

Woodland, D.L., 2004. Jump-starting the immune system: prime-boosting comes of age. Trends Immunol. 25, 98–104.

Xing, D.K., Crane, D.T., Bolgiano, B., Corbel, M.J., Jones, C., Sesardic, D., 1996. Physicochemical and immunological studies on the stability of free and microsphere-encapsulated tetanus toxoid in vitro. Vaccine 14, 1205–1213.

Zinkernagel, R., 2006. On observing and analyzing disease versus signals. Nat. Immunol. 8, 8–10.