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Role of Adjuvants in Modeling the Immune Response

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Abstract

Purpose of review—Recent clinical trial results have indicated that it may be possible for vaccines to induce protection against HIV. To build on this result, strategies should be designed to enhance duration, breadth, and magnitude of antibody production. Strategic formulation of agonists of the innate immune system and carriers that selectively present the target antigen yield a class of pharmaceuticals, “adjuvants”, which greatly influence immunity resulting from vaccination. As researchers begin to focus not only on creating an immune response to an antigen, but also on the quality of that response, the role of adjuvants is becoming increasingly significant. This review is intended to give an overview of recent findings on how adjuvants model the immune response to antigens with a focus on the field of vaccines for HIV.

Recent findings—It is clear that innate and adaptive immunity are linked by communication channels that allow innate signals to influence the quality of adaptive responses as well as adaptive signals that temper innate responses. Adjuvants take advantage of this bridge to shape the immune response to antigens. In this review we will discuss the different classes of adjuvants currently available; recent findings on the relationship between adjuvants and the type of immune profile generated; and the breadth of neutralizing antibodies as influenced by adjuvants.

Summary—Because adjuvants influence the breadth of antibodies generated and the type of cells that proliferate in response to a vaccine this review is relevant for scientists clinicians involved in creating a new HIV vaccine.

Keywords

Toll-Like Receptor; Immunotherapy; Adjuvant; Innate Immunity

Introduction

Adjuvants are compounds that may enhance the magnitude, breadth, and longevity of specific immune responses to antigens, as well as direct the quality of the immune response, but have minimal toxicity or lasting immune effects on their own (1, 2). Addition of adjuvants to HIV vaccines could reduce the amount of antigen and/or number of immunizations required to achieve protection, as well as improve the efficacy of vaccines by enhancing neutralizing antibodies or the duration of the protective response (3-9). Despite the efforts over the past three or more decades to develop recombinant protein-based vaccines, the only product successes have been with viral proteins expressed as VLPs (virus-like particles) delivered with alum. Vaccines based on defined antigens, including

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recombinant proteins, while offering significant advantages over traditional vaccines in terms of safety and cost of production, generally have limited immunogenicity and require the addition of adjuvants in order to induce a protective and long-lasting immune response (7). If targeted HIV vaccines will require T cell immunity or a broad range of neutralizing antibody response, adjuvants will be an important solution (10).

Adjuvants as they are currently used

The most commonly used adjuvant formulations include aluminum salts (alum). Although some recombinant protein-based vaccines, including those for hepatitis B (HBV) and human papilloma virus (HPV) elicit protective antibody responses using only alum as adjuvant, the next generation of recombinant vaccines -- including those for HIV -- will require not only strong and lasting antibody responses, but also potent T cell responses, poorly induced by alum. The cellular and molecular mechanisms by which innate immune responses are induced have led to the development of a new generation of adjuvants that simulate pathogen-associated molecular patterns (PAMPs) and signal by means of pathogen-recognition receptors (PRRs), which include the Toll-like receptors (TLR). Recent data indicate that the addition of an agonist of TLR4, monophosphoryl lipid A (MPL®), a natural glycolipid derived from *Salmonella* cell membranes, increased the breadth of the immune response to alum adsorbed VLP of an HPV vaccine (Cervarix®, GSK) (11).

To be effective *in vivo*, proper formulation of the TLR ligand is essential. Several aspects of formulation (e.g. phase state, particle size, and charge...) may influence vaccine potency. Criteria involved in selecting the formulation for a given vaccine include the nature of the antigenic components, type of immune response desired, preferred route of delivery, avoidance of significant adverse effects, and stability of the vaccine. The optimally formulated adjuvant will be safe, stable prior to administration, readily biodegraded and eliminated, able to promote an antigen specific immune response, and inexpensive to produce. Furthermore, the ideally formulated adjuvant will be well defined chemically and physically to facilitate quality control that will ensure reproducible manufacture and potency.

Formulation design can aim to complement the inherent immunogenicity of vaccine antigens. For example, small, soluble, monomeric proteins (such as HIVgp120) tend to be poorly immunogenic compared to multimeric proteins that form virus-like particles (VLPs) like the Hepatitis B surface antigen (HBsAg)(12). To enhance the immunogenicity of monomeric proteins, a formulation that renders it multimeric (e.g., by incorporation into virosomes) might be most appropriate. For multimeric proteins, virosome formulation might not be appropriate; instead adsorption of the protein to mineral salts might enhance immunogenicity and stability.

Adjuvants can be classified according to their component sources, physiochemical properties, or mechanisms of action. Two major classes of adjuvants commonly found in modern vaccines include:

- **immunostimulants** that such as TLR ligands, cytokines, saponins, and bacterial exotoxins that directly act on the immune system to increase responses to antigens, and:
- **vehicles** that present vaccine antigens to the immune system in an optimal manner, including controlled release and depot delivery systems to increase the specific immune response to the antigen. The vehicle can also serve to deliver immunostimulants. Examples include: mineral salts, emulsions, liposomes,

virosomes, biodegradable polymer microspheres, and immune-stimulating complexes (i.e. ISCOM, ISCOMATRIX™).

Adjuvants in approved human vaccines include alum, oil-in-water emulsions (Novartis' MF59™ and GSK's AS03™) and the TLR ligand MPL. Alum is in several licensed human vaccines, including diphtheria-pertussis-tetanus (DPT), diphtheria-tetanus (DT), DT combined with Hepatitis B (HBV), Haemophilus influenza B or inactivated polio virus (IPV), hepatitis A (HAV), *Streptococcus pneumoniae*, meningococcal, and human papilloma virus (HPV). The mechanisms of action of alum are thought to be: (1) depot formation facilitating continuous antigen release; (2) particulate structure formation promoting antigen phagocytosis by APC such as DC, macrophages, and B cells; (3) increased MHC class II expression and antigen presentation. Recent reports establish that alum induces secretion of chemokines by monocytes and macrophages. Inflammatory monocytes are recruited by alum to the site of injection, migrate draining lymph nodes, and further differentiate into inflammatory DC. Alum often increases Th2 antibodies but not significant T cell responses, which are required to control most intracellular pathogens. Advantages of alum include its overall safety record, augmentation of antibody responses (faster, higher antibody titers, longer-lasting antibody responses), antigen stabilization and relatively simple formulation for large-scale production. Alum can induce granulomas at the injection site, a concern for vaccines requiring frequent boosts.

The adjuvant AS04 is a combination of alum and MPL. AS04 has been shown to enhance vaccine potency in hepatitis vaccines (sero-conversion with fewer immunizations as compared with alum alone, and increased responses in dialysis patients), as well as in the HPV vaccine Cervarix® (increase in breadth of antibody responses to HPV serotypes not contained in the vaccine (13)). Considering the partial success of alum-gp120 in the Thai trial, formulating gp120 or other HIV antigens with a combination of alum and a TLR4 ligand may lead to improved vaccine efficacy.

After alum, oil in water (O/W) formulations are the most widely used adjuvants. MF59™ consists of an oil (squalene)-in-water nano-emulsion composed of droplets which are smaller than 250 nanometers. It is licensed in Europe for use in influenza vaccines. MF59™ has also been tested with herpes simplex- (HSV), hepatitis B- (HBV), and human immunodeficiency- (HIV) virus vaccine candidates including a recent study that demonstrated protection of macaques against SHIV challenge in a prime-boost setting (14). Here, the alphavirus replicon particle prime was boosted with *env* in MF59™ and afforded complete protection against mucosal challenge. This study builds upon previous experiences with the O/W adjuvant in preclinical and human studies where the use yielded neutralizing and cross-reactive antibodies both in protein/adjuvant (15, 16) as well as adenoviral prime/protein boost settings (17). Overall, MF59™ has an acceptable safety profile and with several antigens significantly increases antibody titers with a reportedly more balanced Th1/Th2 response than that obtained with alum alone. MF59™ is believed to act by creating a depot and by direct stimulation of cytokine and chemokine production by monocytes, macrophages, and granulocytes. Increased immunogenicity has been achieved with MF59-adjuvanted influenza vaccines in the elderly, and with MF59-adjuvanted vaccines against CMV and HIV in infants.

AS03, created by GlaxoSmithKline and contained in an influenza vaccine licensed in Europe, is also a squalene-based emulsion. Squalene is a naturally occurring chemical involved in the body's production of cholesterol and vitamin D. Vaccines containing AS03 have been evaluated in thousands of individuals, and an AS03-H5N1 influenza vaccine has been reported to be safe in both adults and children. AS03 is being developed for both seasonal and pandemic influenza vaccines, including pre-pandemic vaccines to prime

individuals against H5 viruses to induce at least partial immunity against related influenza variants. The vaccine has been reported to be immunogenic, dose-sparing compared to those without adjuvant, and able to induce immune responses against influenza viruses in the H5 family other than the one incorporated in the vaccine.

GSK's ASxx series of adjuvants have been in HIV studies in a variety of models including DNA prime / protein boosts of *env*, *nef*, and *tat* in mice (18), protein / AS02A immunizations using the oligomeric gp140(R2) and the surface region gp120(R2) in rabbits (19); trimeric and monomeric *env* in combination with AS01B, AS02A, and AS03 in guinea pigs (20); and HIV-1 *env*, *nef*, *tat*, and SIV *nef* in both prime/boost settings (21) as well as a vaccine formulation containing multiple components in non-human primates (22). In these studies increased antibody titers, the induction of neutralization and partial protection were reported in all the models studied with a bias towards the use of the O/W formulation containing MPL®, AS02A.

MPL® is the first and only TLR ligand in licensed human vaccines. Derived from the lipopolysaccharide (LPS) of *Salmonella minnesota*, MPL® is a potent stimulator of T cell and antibody responses. LPS consists of two basic structures: a hydrophilic polysaccharide portion and a hydrophobic lipid moiety (lipid A). While lipid A is toxic, structural modifications such as the removal of specific phosphate groups or varied number and length of acyl chains, may greatly reduce toxicity. MPL® is a safe and effective adjuvant that has been administered to millions of individuals in various adjuvants in development as well as in products containing the combinations such as AS04 (in Fendrix® for HBV and Cervarix® for HPV). Cervarix® is licensed in the U.S., making MPL® the first FDA licensed TLR agonist adjuvant molecule. The molecule is a component of AS01, the liposomal adjuvant in GSK's promising malaria vaccine RTS,S which has been in many trials and is expected to be injected into 16,000 children as part of a multicenter Phase 3 clinical trial (23). MPL® is also licensed in Europe to treat allergies (Pollinex Quattro®). Synthetic TLR4 agonists include the aminoalkyl glucosamidine-4-phosphates (AGPs), one of which, RC-529 is in a licensed HBV vaccine (Dynavax™) and the lipid A mimic "glucopyranosyl lipid A" (GLA). The success of MPL® has validated the use of TLR4 agonists in vaccine adjuvants. Expression of functional TLR4 in humans is limited to macrophages and dendritic cells, making it an ideal target for a safe and effective way to target adaptive immune responses. GLA represents a completely synthetic TLR4 agonist shown to be safe and effective in a number of animal models as well as in humans. GLA can be formulated as an emulsion, in liposomes, and with alum, making it a versatile adjuvant molecule.

Neutralization and breadth of the antibody response

A neutralizing response is generated when a key target on the pathogen is bound by antibodies that then do not allow proper functioning of that target or that recruit secondary neutralizing factors to the target (24). For this to occur, the immune system must be presented with the target in the desired conformation and then create antibodies at an affinity that outcompetes the native function at a level that eliminates reservoirs of the pathogen. An example of this in HIV vaccines is gp120 as a target for a neutralizing antibody response (25, 26). Here, the key function of the target is binding and entrance to cells via the CD4 / gp120 interaction (27). Antibodies that bind to a specific conformation of the complex are able to abolish viral entry and are found in subjects that can resist infection over the long term (28).

A broad response allows antibodies that recognize a specific conformation to also bind proteins in a similar conformation that may have divergent amino acids sequences (29). This

type of response would be desirable in HIV where mutations that conserve the function of key targets would allow the virus to escape immunity induced by a specific vaccine.

Adjuvants can be used to create broad and neutralizing immunity to HIV and other pathogens by invoking innate signaling mechanisms (3, 6, 30-33). For humoral responses, B cells must expand and undergo affinity maturation while weak binders must be recruited and matured in order to recognize a diverse set of antigens. Innate signaling via the Toll-like receptors has also been shown to induce this expansion and differentiation even in the absence of BCR cross-linking (34) while also influencing induction of long term memory (35). Early signals during vaccination select naïve B cell from the large recognition repertoire of $>10^6$ molecules per individual (36-38) and recruit them to germinal centers. Somatic hypermutation then matures and diversifies the recruited cells (37, 39). This process is mediated by the adjuvant(s) used to initiate and enhance the immune response. Whether this response is mediated directly by innate signaling on the B cell or by other cellular help is unclear although signaling through the TLR on the B cell itself may not help diversification (34). In fact, strong signals during initial stages of activation abort B cell maturation prematurely (40). TLR4 may be a key regulator in creating high affinity B cells as it has been demonstrated that these agonists alter B cell trafficking into the GC (41) and result in a broadly neutralizing immune response.

The multiple forms of individual antigens represented in various conformations, clades and mutated viral antigens is a huge problem in designing a vaccine for HIV – a reason why it is thought the virus has escaped attempts at making an effective vaccine. Delivery of antigen in combination with TLR agonists can broaden the immune response to a point where this is not an effective survival mechanism. It is easy to envision the use of an agonist-based adjuvant to generate antibodies against varied regions of crucial protein antigens in the virus to protect subjects against AIDS.

Designing an HIV vaccine with adjuvants

Innate immunity forms an essential and important role in the defense against HIV infection. Current understanding of the role of innate immunity in restricting HIV transmission is making important gains. Because innate responses may allow successful control of HIV the concept of using vaccines that could prime adaptive responses to yield innate effectors in exposed individuals is attractive. Such responses, including those mediated by complement dependent pathways such as ADCC and CDC have demonstrated promise in animal models and through correlation in human studies (42, 43). It is highly likely that a successful vaccine for HIV will include potent adjuvant and based on the broad immune response needed that that adjuvant will include agonist molecules that signal through innate pathways.

CONCLUSION

There is renewed promise for successful HIV vaccination, in part because of indications that prime-boost strategies that include protein with adjuvant may induce at least some level of protection. Immunological advances have provided confidence that adjuvants that induce higher quality responses will be available for improved vaccination strategies for a number of infectious targets, including HIV.

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Abbreviations in this paper

HIV	Human Immunodeficiency Virus
AIDS	Acquired Immunodeficiency Syndrome
TLR	Toll-Like Receptor
GLA	Glucopyranosyl Lipid A, a proprietary, synthetic TLR4 agonist