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Adjuvants for Vaccines to Drugs of Abuse and Addiction

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Abstract

Immunotherapeutic vaccines to drugs of abuse, including nicotine, cocaine, heroin, oxycodone, methamphetamine, and others are being developed. The theoretical basis of such vaccines is to induce antibodies that sequester the drug in the blood in the form of antibody-bound drug that cannot cross the blood brain barrier, thereby preventing psychoactive effects. Because the drugs are haptens a successful vaccine relies on development of appropriate hapten-protein carrier conjugates. However, because induction of high and prolonged levels of antibodies is required for an effective vaccine, and because injection of T-independent haptenic drugs of abuse does not induce memory recall responses, the role of adjuvants during immunization plays a critical role. As reviewed herein, preclinical studies often use strong adjuvants such as complete and incomplete Freund's adjuvant and others that cannot be, or in the case of many newer adjuvants, have never been, employed in humans. Balanced against this, the only adjuvant that has been included in candidate vaccines in human clinical trials to nicotine and cocaine has been aluminum hydroxide gel. While aluminum salts have been widely utilized worldwide in numerous licensed vaccines, the experience with human responses to aluminum salt-adjuvanted vaccines to haptenic drugs of abuse has suggested that the immune responses are too weak to allow development of a successful vaccine. What is needed is an adjuvant or combination of adjuvants that are safe, potent, widely available, easily manufactured, and cost-effective. Based on our review of the field we recommend the following adjuvant combinations either for research or for product development for human use: aluminum salt with adsorbed monophosphoryl lipid A (MPLA); liposomes containing MPLA [L(MPLA)]; L(MPLA) adsorbed to aluminum salt; oil-in-water emulsion; or oil-in-water emulsion containing MPLA.

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1. Introduction

A worldwide epidemic of use and abuse of addictive drugs is responsible for massive, socially disruptive, and still increasing medical, social, economic, and political problems, and is associated with widespread suffering, including high risks of morbidity, disability, and death [1]. Among young injection drug users in San Francisco between 1997 and 2007 overall mortality rates were 10 times higher than those in the general population [2]. Use of addictive drugs is also associated with many diseases that vary in type and prevalence in different populations. For example, prevalence of HIV-1 infection among injection drug users in Argentina from 1987 through 1999 ranged from 27% to 80% [3]. According to the United Nations Office On Drugs And Crime [4]: “of the estimated 16 million injecting drug users worldwide, UNODC estimates that almost one in five is HIV-positive. Approximately the same proportion are infected with hepatitis B, whereas some 8 million — about half of all injecting drug users — are infected with hepatitis C.”

Current pharmacologic methods for treatment of individuals suffering from substance abuse often have problems of high cost, limited availability, compliance difficulties, diversion of opiate agonists such as methadone, and the inefficiency that patients often have high relapse rates [5]. Because of these many problems innovative alternative therapeutic approaches are being explored, and vaccines may represent a unique and potentially attractive supplemental approach that could be useful for treatment of chemical addiction. Some of the drugs of abuse that are current targets for experimental therapeutic vaccines include: opiates, such as heroin and morphine; stimulants such as cocaine and methamphetamine; prescription pain-killers such as hydrocodone and oxycodone; and general use chemicals such as nicotine, a widely used addictive drug which has complex stimulatory and social effects [6,7,8,9].

What is the mechanism by which addictive drugs work, and how would a vaccine work? In the course of typical drug use, crude or partially refined chemicals are introduced into the blood of the user, and the active chemical must then cross the blood-brain barrier in order to exert psychoactive effects by binding to an appropriate receptor in the brain. Repeated injections of psychoactive drugs have varying degrees of physical and psychological reinforcing effects that often lead to addiction. The theoretical strategy for a creating a therapeutic vaccine to an offending drug lies in the induction of high levels of specific antibodies that capture the drug in the blood to prevent the drug from crossing the blood-brain barrier, thus blocking the psychoactive effect. A further important aspect of the dynamics of drug abuse is that the reinforcing actions of intravenously administered drugs are usually directly related to the speed of infusion. Thus, even if antibodies to the drug could only retard the rate of transport of the drug from the blood to the brain a beneficial effect would still be achieved [5]. Therefore, an effective vaccine could work either by complete sequestration of the drug in the blood, or by serving as a “pharmacokinetic

antagonist” to slow the speed of entry of the drug into the brain, either of which approaches would result in a diminished psychoactive effect [5].

2. Immunogenicity of drugs and the need for adjuvants

The challenge to production of antibodies to addictive drugs is that the drugs are haptens, i.e., substances that are not immunogenic by themselves. The term hapten, originally introduced as a concept by Landsteiner in the 1920s and 1930s, was defined functionally as a small chemical entity that cannot induce antibodies by itself but which can induce specific binding antibodies upon conjugation to a protein [10]. A more succinct modern definition is that a hapten is a small functional group corresponding to a single antigenic determinant [11,12].

The demonstration by Landsteiner that numerous types of small molecular weight natural and synthetic chemicals and drugs could serve as haptens [10] led to a long history of creation of polyclonal antisera containing specific antibodies as reagents for analytic and diagnostic immunoassays for drugs, including assays for drugs of abuse [13,14,15,16,17,18,19,20]. Antisera that were used for immunoassays were routinely obtained by immunizing animals with a protein-hapten conjugate that was emulsified with complete Freund's adjuvant usually followed by immunization with the conjugated protein-hapten emulsified with either complete or incomplete Freund's adjuvant. Freund's adjuvants are both water-in-oil (w/o) emulsions in which the stabilizing emulsifier (usually mannide monooleate) causes tiny droplets of water to be stabilized and distributed throughout the larger bulk oil phase [21,22]. This general immunizing procedure has now resulted in development of a large number of clinical immunoassays that utilize either polyclonal or monoclonal antibodies for detection of many other types of haptenic drugs [23,24].

With the validation from the initial studies that conjugates of proteins with small synthetic chemicals and drugs could serve as antigens to induce specific antibodies, efforts began in the 1970s to explore the possibility that animals immunized with protein-morphine conjugates emulsified with Freund's adjuvant could serve as models for *in vivo* blocking of the psychoactive effects of morphine [25]. As described below, the tradition of immunization of animals with Freund's adjuvant to obtain therapeutic antisera to drug haptens has persisted to this day. However, it is well known that complete Freund's adjuvant is unacceptable for human use. Although incomplete Freund's adjuvant, and similar w/o adjuvants, have been used extensively for a variety of prophylactic and therapeutic human vaccines [26,27], they are not currently considered to be at the forefront of modern vaccine adjuvants because of potential toxicities [28,29].

2.1 The need for adjuvants for immunization against drugs of abuse

“Behind every great vaccine is a great adjuvant [but] behind a great adjuvant may be an outdated carrier protein” [8]. This cautionary remark by Janda and Treweek is a reminder that chemical and formulation strategies for vaccines to haptenic drugs require a combination of creative synthetic organic chemistry for obtaining surrogate haptens, use of suitable carrier proteins, conjugation of the surrogate hapten to the carrier, and formulation of the conjugate with a safe and powerful adjuvant to induce high levels and long duration

of high quality antibodies to the offending drug. In the quest for development of candidate vaccines to morphine, heroin, cocaine, methamphetamine, oxycodone, nicotine, and similar addictive drugs, a wide variety of adjuvants and carrier proteins has been employed in various studies to immunize different species of experimental animals as well as humans [8,30]. Table 1 provides a broad perspective of the different adjuvants that have been employed both for obtaining antibodies for clinical immunoassays and for research on candidate vaccine formulations in animals and humans.

What is an adjuvant? In this context, a vaccine adjuvant has been defined by the European Medicines Agency (EMA) in a regulatory guideline as “a component that potentiates the immune responses to an antigen and/or modulates it towards the desired immune responses. An active ingredient of a combined vaccine that has an adjuvant effect on other active ingredients of the vaccine is excluded from the scope of this Guideline. Also excluded are carriers for haptens, antigens (e.g., CRM197, meningococcal OMP, tetanus toxoid and diphtheria toxoid that are used to conjugate polysaccharides) and excipients such as HAS” [67]. In the guideline EMA also lists 25 specific examples of adjuvants that are arranged in six categories.

The above EMA guideline covers only adjuvants in vaccines against infectious diseases, including those containing hapten-like oligosaccharides linked to carrier proteins as antigens. However, from a theoretical standpoint small synthetic chemicals probably pose greater complexity than oligosaccharides as haptens for vaccines because, unlike carbohydrates which are naturally present as complex polysaccharides on the surfaces of bacterial particles during infection [68,69], injected chemicals are soluble in plasma and are not attached to infectious particles during repeated injections. Because of this, repeated injections of a therapeutic vaccine will be required in order to maintain high levels of binding antibodies to the drug during the course of therapy for withdrawal from drug abuse. The need for potent adjuvants for induction of antibodies is emphasized by the observation that as many as one-third to two-thirds of patients vaccinated with candidate vaccines to drugs of abuse fail to achieve a sufficient antibody response [32,36,37,38]. This could have been due to the development of low affinity IgM antibodies during the course of drug use which results in suppression of induction of high affinity IgG by the anti-drug vaccine, or it could have reflected an idiosyncratic inability of a subset of individuals to produce high levels of antibodies or, more likely, it could have been due to low potency of the aluminum salt adjuvant that was used.

2.2 Types of adjuvants and adjuvant strategies

The purpose of this review is to examine the various types of adjuvants that have been used, and to present the rationales for utilization and optimization of modern adjuvants, or combinations of adjuvants, for candidate vaccines. The field of preclinical adjuvant research is quite large overall, and hundreds of adjuvants and strategies have been proposed either for various types of animal models or for human vaccines [8,70,71,72]. In the selection process for adjuvants it is useful to understand which adjuvants are present in vaccines that are currently approved or that are pending approval. Regulatory agencies have made it clear that adjuvants cannot be approved independently of a vaccine that contains the adjuvant, and the

vaccine formulation as a whole is the element considered for approval. In our experience the adjuvants that are present in licensed proprietary vaccines generally can be reproduced in generic forms with considerable freedom to operate regarding intellectual property. Discussion of this issue for monophosphoryl lipid A (MPLA) and liposomes containing MPLA has been reviewed elsewhere [73,74]. Methodologies for creation of oil-in-water emulsions by using mixtures of phospholipids or intact liposomes as emulsifiers have also been described [75,76,77]. Theoretical and practical details of the chemistry of emulsions can be obtained by consulting the classic monograph by Paul Becher [78]. We recommend that if a commercial formulation is not available for use, then preclinical and clinical studies can be conducted with generic adjuvants that either emulate or reflect the characteristics of one or more of the six adjuvants that are formulated in licensed vaccines [79]. These adjuvants consist of:

- Aluminum salts are present in at least 146 vaccines licensed to prevent single or multiple diseases in different countries worldwide (including more than 33 licensed in the U.S.) [67,80]. They are used for antibody production rather than cell-mediated immunity, but they are relatively weak stimulators when compared with many modern adjuvants.
- Virosomes (Crucell) are specialized liposomes containing natural and synthetic phospholipids together with the surface hemagglutinin and neuraminidase proteins purified from the influenza virus [81]. The influenza proteins provide the ability to fuse with cells of the immune system. Virosomes are present in influenza virus and hepatitis A vaccines in Europe. Although cell-mediated immunity may play a role, it is likely that antibodies are the main effector mechanism stimulated by the adjuvant.
- Oil-in-water emulsions. Three stable emulsions, MF59 (Novartis), AS03 (Merck), and AF03 (Sanofi-Pasteur) are present as adjuvants in vaccines to seasonal influenza in Europe (MF59) or pandemic influenza in Spain (AF03) or the United States (AS03). Each emulsion contains squalene oil together with an emulsifier consisting of either a Tween and α -tocopherol (AS03), or a Span and polyoxyethylene and cetostearyl ether (AF03), or both a Tween and a Span (MF59) [82]. The emulsifier stabilizes small droplets of oil to form an emulsion in a large volume of water [75]. The main adjuvant mechanism is stimulation of antibodies.
- Monophosphoryl lipid A adsorbed to an aluminum salt (GlaxoSmithKline), also known as AS04. The MPLA consists of MPL®, a natural monophosphoryl lipid A congener extracted and purified from *Salmonella minnesota* R595 lipopolysaccharide. MPLA, is a strong stimulator of both antibodies and cell-mediated immunity [74].
- Liposomes containing MPLA and QS 21 saponin (GlaxoSmithKline) (license approval pending) is also known as AS01. AS01E is used in RTS,S, a pediatric malaria vaccine. QS21, which is extracted from the bark of *Quillaja saponaria* tree in Chile [83], is a generic material noted mainly for stimulation of cell-mediated immunity, but it can also stimulate antibody production.

- CpG oligodeoxynucleotide adsorbed to aluminum salt is a constituent in HEPLISAV™ Hepatitis B vaccine that is pending approval for licensing (Dynavax Technologies). This is mainly an adjuvant for stimulation of cell-mediated immunity, but it can also stimulate antibody production. [84,85,86].

3 Classes and types of adjuvants that have been used previously for preclinical and clinical evaluations of vaccines against drugs of abuse

3.1 Water-in-oil (w/o) and oil-in-water (o/w) emulsions

As mentioned above, Freund's adjuvant, a w/o emulsion (containing tiny stabilized droplets of water in the larger bulk oil phase), consisting of either complete (containing killed mycobacteria) or incomplete (lacking mycobacteria) adjuvant, was introduced for immunization with morphine conjugates in the 1970s, and these adjuvants are still widely used for immunization of animal models for evaluation of the quality of numerous candidate haptens to drugs of abuse (Table I). However, complete Freund's adjuvant when used in animals causes severe ulceration at the site of injection, and it is unacceptable as a human vaccine adjuvant.

It should be noted that Freund's adjuvant emulsion should always be tested for stability as judged by phase separation of the water and oil. When it is properly made the emulsion can be stable at 37°C for a week or more, but phase separation occurs gradually thereafter [87]. The concentration of the stabilizing agent is critical for emulsion stability and this in turn is critical for adjuvant activity. Amphipathic molecules such as phospholipids (or even certain proteins) that are added to the emulsion can sometimes change the degree of phase separation.

Two commercial w/o emulsions, Montanide® ISA 720 (containing squalene oil) and ISA 51 (containing mineral oil), have considerable stability when formulated with antigen, and have been used extensively in clinical studies, mainly in candidate cancer and malaria vaccines [26]. However, w/o emulsions in general sometimes have issues of reactogenicity in humans, including severe tenderness, pain and swelling, or erythema, or a non-erythematous nodule at the injection site, and toxicity could limit their use as a human adjuvant formulation [28,88].

Oil-in-water (o/w) emulsions, in which tiny stabilized droplets of oil are distributed in the larger bulk water phase, are relatively easier to manufacture and use than w/o emulsions. Antigen can be simply mixed with preformed emulsion, and the emulsions have increased stability and are used for approved human vaccines. As with Freund's, the stability of the emulsion is also dependent on the chemical composition and concentration of the amphipathic stabilizer. By themselves o/w emulsions reportedly have less inherent adjuvant potency for induction of antibodies than w/o emulsions [89]. However, they can serve as constituents of multi-adjuvant formulations that have increased potency, one of which is known as the Ribi Adjuvant System (RAS) [90]. This is also available in modified form as the Sigma Adjuvant System® [91]. This interesting and complex o/w emulsion, which is mainly an adjuvant for veterinary use, contains monophosphoryl lipid A from *Salmonella minnesota* R595 (MPLA); trehalose 6,6' diesters (a high molecular weight glycolipid known

as ‘cord factor’) from the cell walls of *Mycobacterium phlei*; and mycobacterial cell wall skeleton which includes a peptidoglycan that is a polymerized form of muramyl dipeptide [90]. Because of the mycobacterial components the RAS is intended to be a relatively purified o/w substitute for complete Freund's adjuvant. Indeed, RAS outperformed complete/incomplete Freund's adjuvant for producing anti-hapten antibodies after immunizing with a protein-hapten conjugate in a direct comparison [92]. Although the RAS is a potent adjuvant formulation, it is mainly useful for enhancement of cell-mediated immunity (in addition to antibody-mediated immunity), which is a characteristic that would probably not be beneficial for vaccines against drugs of abuse. There appears to be little current commercial interest in developing RAS or Sigma Adjuvant System® as an adjuvant for any human vaccine.

AS03 (Glaxo), an o/w adjuvant, has been tested as a possible adjuvant for a nicotine vaccine [51]. The major reasons that were stated for using this adjuvant were that antibodies were strongly induced in animals and that o/w adjuvants, such as MF59 (Novartis) or AS03, are used in licensed human influenza vaccines. AS03, which also contains alpha-tocopherol as a constituent, is used in the US stockpile as a vaccine to pandemic influenza. Although it is regarded as a safe adjuvant, it is noted that AS03 has been reported to be associated (albeit rarely) with narcolepsy as a side effect in humans [93].

3.2 Aluminum salt adjuvants

For many years aluminum salts have been the most commonly used adjuvants for licensed vaccines worldwide [67]. In 2002 it was estimated that 33 vaccines licensed in the US contained aluminum salt adjuvant [80], and others have since been added. Among these, 12 contained aluminum hydroxide, 15 contained aluminum phosphate, 1 contained both aluminum hydroxide and aluminum phosphate, and 5 contained aluminum potassium sulfate which is also known technically as alum. It should be pointed out that it is highly confusing that vaccinologists routinely, and improperly, refer informally to all aluminum salt adjuvants as “alum”, and this practice should decline with greater technical understanding. Aluminum salts, which are extremely complex minerals, are mined as aluminum-containing clay-like materials worldwide. Because of their widespread use in antiperspirants, cosmetics, antacids, foods, and many other applications, aluminum salts are very inexpensive, and this is a useful attribute for reducing the cost of a vaccine formulation.

Aluminum hydroxide gel is technically mainly crystalline aluminum oxyhydroxide, $\text{AlO}(\text{OH})$. As noted by Hem and HogenEsch [94], “Aluminum phosphate adjuvant is amorphous to x-rays but its infrared spectrum identifies it as aluminum hydroxide in which phosphate has substituted for some hydroxyls. It is correctly termed amorphous aluminum hydroxyphosphate, $\text{Al}(\text{OH})_x(\text{PO}_4)_y$. Unlike aluminum hydroxide adjuvant, it is not a stoichiometric compound. Rather, the degree of phosphate substitution for hydroxyl depends on the reactants and method of preparation.” Further, when alum [i.e., $\text{AlK}(\text{SO}_4)_2$] adjuvant is used to precipitate a protein antigen, the adjuvant consists of “amorphous aluminum hydroxysulfate, as some sulfate anions substitute for hydroxyls. If a phosphate anion is present at the time of precipitation, phosphate will substitute some hydroxyls and the adjuvant will be amorphous aluminum hydroxyphosphate sulfate.” [94].

It should be noted further that Imject® Alum, which somewhat surprisingly has been widely employed in animal studies (Table I), is a combination of amorphous aluminum hydroxycarbonate and crystalline magnesium hydroxide and it has the magnesium and aluminum salt composition that is used as an antacid [95]. Although it may have adjuvant activity in animals, this particular salt composition has never been present as an adjuvant in any licensed vaccine and it is recommended that it not be used in research when the intent is to study the effect of either aluminum hydroxide adjuvant or aluminum phosphate adjuvant [95,96].

Why does the mineral salt composition matter from a practical standpoint? There are many answers to that, but it mainly relates to the belief that a protein antigen usually must physically adsorb to the aluminum salt in order to obtain adjuvant activity [97]. This latter reference can also be consulted for preclinical dose guidelines for aluminum salts. Adsorption can occur through electrostatic attraction, hydrophobic interactions, hydrogen bonding, phosphate (or sulfate)-hydroxyl ligand exchange, and by Van der Waals forces. In addition, the adsorption can be dramatically different depending on the isoelectric point of the protein, and can be influenced by the type of buffer that is used. For example, phosphate-buffered saline can convert aluminum hydroxide to aluminum phosphate by ligand exchange and this can lead to different adsorption characteristics [94]. In some cases this can cause desorption of the protein, necessitating the use of an alternate buffer such as Tris-buffered saline [98]. Addition of phosphorylated compounds, such as MPLA or liposomes containing MPLA, readily adsorb to aluminum hydroxide, and it is possible that they also might change the pH for adsorption of certain proteins. In any case, the degree of adsorption of the protein should always be determined.

Aluminum salts have complicated mineralogical properties and they can be difficult materials to use as vaccine adjuvants, but because of their low cost, and long track record of safety, and because they have been administered to humans in billions of doses, they are still regarded by some as credible adjuvants for vaccines to drugs of abuse (Table 1). The major problem is that when compared to many modern adjuvants aluminum salts generally have the lowest potency for induction of high levels of antibodies to many purified protein antigens [38,72,79]. In view of this, when used as adjuvants for vaccines to drugs of abuse aluminum salts represent a relatively weak strategy for induction of specific antibodies to the target haptens.

3.3 Liposomes containing monophosphoryl lipid A (MPLA)

Lipid A, the lipid moiety of Gram-negative lipopolysaccharide (LPS), which contains a diphosphorylated diglucosamine polar head group to which are attached multiple esterified and amidated fatty acids, is responsible for many of the biological activities, including all of the endotoxic activities of LPS, such as pyrogenicity, and most importantly it possesses extremely potent adjuvant activities [72,74,99]. Much of the toxic activity of lipid A is reduced, but not eliminated, by removing the C1-glucosamine phosphate group to form MPLA, and all of the residual toxicity of MPLA is eliminated by embedding the fatty acid groups in the lipid bilayers of liposomes [74,99]. MPLA adsorbed to aluminum hydroxide (Adjuvant System 04, or AS04; GSK) is present in licensed human vaccines [78].

Liposomes containing both MPLA and QS21 saponin are present in Adjuvant System 01 (AS01; GSK) that has experienced success in phase III trials of human malaria vaccines, and vaccines containing AS01 will likely eventually be licensed [72,74,79]. In our laboratory, liposomes containing MPLA, which we designate as L(MPLA), have been used successfully and safely as adjuvant constituents in 16 phase I or phase II human trials in which they exhibited strong adjuvant effects for candidate vaccines to malaria, HIV-1, meningococcal type B, and as therapeutic vaccines to prostate cancer and gastrointestinal cancer. Because of commercial interest in L(MPLA) and because of our own experiences in 16 human trials we introduced the use of L(MPLA) as an adjuvant in animal models for inducing high levels of specific antibodies for a therapeutic vaccine to heroin [64,65]. L(MPLA), or liposomes containing bis(palmitoyloxy)-(2RS)-propyl]-N-palmitoyl-(R)-cysteinyl-alanylglycine (Pam₃CAG), have also been investigated as adjuvants for a candidate nicotine vaccine [56] (Table 1).

4 Adjuvant mechanisms

For rational development of vaccine adjuvants there is a natural desire to understand how the adjuvant works. The benefits of using multiple adjuvants as combinations in formulations are derived from additive or synergistic effects of different adjuvant mechanisms [8,30]. A partial list of biological effects that have been ascribed to adjuvants includes: depot formation; conversion of soluble antigens to particulate forms; protection of certain antigens from being degraded by interstitial fluids after injection; display of conformational specificities of complex proteins; activation of complement; transportation of antigen to antigen presenting cells; promotion of endocytosis or phagocytosis; promotion of autophagy; display of danger-associated molecular patterns; display of pathogen-associated molecular patterns; induction of Toll-like receptor-dependent cellular effects leading to caspase-1-dependent inflammasomes; induction of Toll-like receptor-independent cellular effects, such as caspase-11-dependent inflammasomes, and oxidative bursts; and combinations of the above.

It should be noted that there is often a tendency to assign only a single mechanism to a single adjuvant, such as being a depot, or a toll-like receptor agonist, or a complement activator, each of which represents a presumed single adjuvant mechanism. However, most commonly used adjuvants have a complex profile or pattern of activity involving more than one mechanism, and the relative role of each mechanism in the overall adjuvant effect might not be clear. For example, aluminum salts were long thought to be only a depot for prolonged presentation or uptake of particles containing adsorbed antigen; however, although adsorption of antigen is a major mechanism, under some circumstances unadsorbed antigen can be adjuvanted by aluminum salt [96]. In addition it is now further believed that aluminum salts can work by release of uric acid from dendritic cells, activation of NLRP3-inflammasomes, binding to plasma membrane lipid rafts, and by participation in many other mechanisms that contribute to or detract from the adjuvant activity [96]. MPLA is often referred to solely as a TLR4 agonist, but LPS and MPLA can also activate TLR4-independent cellular responses by autophagy, endocytosis, phagocytosis, oxidative bursts, and generation of noncanonical TLR4-independent caspase-11-dependent inflammasomes [100,101,102]. Lipid A also sits as a depot for at least several weeks at the site of

subcutaneous injection [103]. Combinations of aluminum salt-adsorbed liposomes containing both MPLA and encapsulated antigen have exhibited strong potency and safety in humans for vaccines to malaria or HIV-1 [28,104]. Thus, addition of MPLA or L(MPLA) to aluminum salt theoretically could introduce many additional mechanisms that enhance those of the aluminum particles and the other constituents to provide more potent adjuvant effects.

Because of the complexities of groups of mechanisms involved with many adjuvants and adjuvant formulations, and because of many profound immunological and anatomical differences between animal models and humans, it is wise to note that extrapolation of comparative degrees of activities of different types of adjuvants directly from animal models to humans can be notoriously unreliable [28,72]. In view of this it is hoped that a new series of phase I clinical research trials that introduce modern adjuvants and adjuvant formulations that emulate adjuvants already present in licensed and approved vaccines, and that are known to be safe and highly effective in humans, will provide higher titers, higher affinities, and more prolonged levels of specific antibodies for immunotherapy based on vaccines to drugs of abuse and addiction.

5 Recommendations for Adjuvant Selection

For adjuvant selection, safety is a major consideration. Because there are no currently approved or licensed vaccines to drugs of abuse the guidance gained from experiences with vaccines to infectious diseases is useful. Most approved infectious disease vaccines have striven to induce high levels of antibodies that block infection upon exposure or re-exposure to the infectious organism. Thus, adjuvants that are primarily noted for stimulating cell-mediated immunity, such as QS21 or CpG, might be viewed as less attractive for development of anti-hapten vaccines because of their greater emphases on cellular rather than antibody-mediated immunity.

It is noteworthy that the human trials conducted for testing candidate vaccines to cocaine or nicotine employed aluminum hydroxide adjuvant. This seems logical because aluminum hydroxide is safe and inexpensive and is currently used in more than 12 licensed vaccines in the US. However, low titers, magnitudes, affinities, and durations of polyclonal antibodies induced by the vaccines against nicotine and cocaine often did not provide sufficient support to achieve adequate therapeutic effects in humans [8,36,37,38,105]. It is not clear yet whether this was an idiosyncratic result in a subset of volunteers or due to poor aluminum salt potency. Although aluminum hydroxide is noted for its ability to induce antibodies instead of cellular immunity, and even though it may sometimes serve as an effective adjuvant in animal models, it has only modest adjuvant activity in humans when compared with many modern adjuvants [28,72,82]. Although aluminum salts have a strong safety record, we do not recommend them for drugs of abuse vaccines because of relatively weak or inconsistent stimulation of antibodies in human infectious disease vaccines, and also because the phase III results in humans with aluminum-adjuvanted nicotine and cocaine vaccines were unimpressive.

In general, single adjuvants alone, such as aluminum hydroxide, or liposomes, or monophosphoryl lipid A (MPLA), are less stimulatory in humans for induction of antibodies than combinations of adjuvants. Aluminum hydroxide is a relatively weak adjuvant by itself, but when MPLA, or liposomes containing MPLA, are adsorbed to it these can be strong adjuvant formulations. One illustration of a successful adjuvant strategy that we recommend for inducing high titers of specific antibodies to heroin (or nicotine) haptens in animals is to use liposomes containing L(MPLA), a formulation that has been widely employed in human trials [28,74]. The L(MPLA) can be mixed together with an antigen consisting of a hapten conjugated to a carrier protein such as tetanus toxoid, as shown in Fig. 1 [56,64,65]. Oil-in-water emulsions by themselves can be strong stimulators of antibodies, but immunological effectiveness can be enhanced by addition of MPLA [72]. Adjuvant combinations, particularly all of those mentioned above, have the advantages that they are known to be safe in humans, and they can all be pre-manufactured and mixed directly with the antigen, and multiple additive or synergistic mechanisms are mobilized by each combination. In view of the above, it appears that MPLA adsorbed to aluminum salt, or L(MPLA), or L(MPLA) adsorbed to aluminum salt; or an o/w emulsion, or an o/w emulsion containing MPLA, is each an excellent adjuvant formulation that would be expected to have considerable safety and potency, and that could potentially could be successfully developed in candidate vaccines to drugs of abuse and addiction.

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Figure 1.
Schematic illustration of a conjugate of TT-heroin hapten (DiAmHap) adjuvanted by liposomes containing monophosphoryl lipid A [L(MPLA)]. See [65] for further details.

Table 1

Adjuvants, carriers, and species injected for selected candidate vaccines to drugs of abuse

Adjuvant	Drug of Abuse	Carrier	Species	Ref.
ALUMINUM SALTS				
Aluminum hydroxide (Alhydrogel)	Nicotine	VLPs from coat protein of phage Q β	Mouse, Human	31
	Nicotine	<i>P. aeruginosa</i> r-exoprotein A	Human	32
	Methamphetamine	Immunocyanin KLH	Rat	33
Aluminum hydroxide (Alu-Gel S)	Nicotine	CTB	Mouse	34
Aluminum hydroxide (brand not specified)	Cocaine	KLH	Human	35
	Nicotine	VLPs from coat protein of phage Q β	Human	36
	Cocaine	CTB	Human	37, 38
	Morphine	BSA	Human	39
	Nicotine	DT	Mouse, NHP	40
Aluminum potassium sulfate (alum)	Methamphetamine	KLH	Rat	41
	Morphine	KLH	Rat	42
Amorphous aluminum hydroxycarbonate and crystalline magnesium hydroxide (Imject® Alum)	Heroin	TT	Rat	43
	Heroin	KLH	Rat	44, 45
	Heroin	KLH	Mouse	46, 47
	Oxycodone and Hydrocodone	KLH	Rat, Mouse	48
	Morphine, Oxycodone	KLH	Rat	49
	Oxycodone or Hydrocodone	KLH	Mouse	50
OIL EMULSIONS				
Oil-in-water emulsion containing alpha tocopherol (AS03)	Nicotine	KLH, TT, CRM197	Mouse, Rat	51
Oil-in-water emulsion containing MPLA and mycobacterium cell wall components (Sigma Adjuvant System, also known as Ribi Adjuvant system)	Cocaine	KLH	Rat	52, 53
	Methamphetamine	KLH	Mouse	54
	Methamphetamine	KLH	Rat	55
	Nicotine	KLH	Mouse	56
	Nicotine	OVA	Mouse	57

Adjuvant	Drug of Abuse	Carrier	Species	Ref.
Water-in-oil emulsion (Complete/Incomplete Freund's adjuvant)	Mescaline, Methamphetamine	Methylated BSA	Rabbit	13
	Morphine	BSA	Rabbit	14, 15, 18
	Morphine	BSA	NHP	25
	LSD	PLL	Rabbit, guinea pig	16
	Morphine	PLL, succinylated hemocyanin	Rabbit	17
	Morphine	PLL	Rabbit	58
	Nicotine	HSA, KLH, PLL	Rabbit	19
	Cocaine	BSA	Mouse	59
	Nicotine	<i>P. aeruginosa</i> r-exoprotein A	Rat	60, 61
	Nicotine	<i>P. aeruginosa</i> r-exoprotein A, KLH	Rat	62
LIPOSOMES CONTAINING MONOPHOSPHORYL LIPID A OR OTHER ADJUVANT	Morphine and Oxycodone	KLH	Rat	49
	Morphine/Heroin	KLH	Rat	63
	Oxycodone, Hydrocodone	KLH	Mouse, Rat	48
	Heroin	TT, HIV-1 MPER peptide	Mouse	64
	Nicotine	KLH	Mouse	56
	Heroin	TT	Mouse	65
	Nicotine	KLH	Mouse	56
	Heroin	KLH	Mouse	46, 47
	Nicotine	DT	Mouse, NHP	40
	Nicotine	Peptide from MUC1	Rat	66
DNA				
PEPTIDE				
CpG ODN	Heroin	KLH	Mouse	46, 47
C5a agonist peptide	Nicotine	DT	Mouse, NHP	40

Abbreviations: BSA, bovine serum albumin; CRM197, cross-reacting material 197 from DT; CTB, B subunit of cholera toxin; DT, diphtheria toxoid; HSA, human serum albumin; KLH, keyhole limpet hemocyanin; MPER, membrane proximal external region of HIV-1 gp41; MPLA; monophosphoryl lipid A; NHP, non-human primate; OVA, ovalbumin; PLL, poly-L-lysine; TT, tetanus toxoid; VLP, virus-like particle