REVIEW

OPEN

Modulation of HIV-1 immunity by adjuvants

M. Anthony Moody

Purpose of review
To summarize the role of adjuvants in eliciting desirable antibody responses against HIV-1 with particular emphasis on both historical context and recent developments.

Recent findings
Increased understanding of the role of pattern recognition receptors such as Toll-like receptors in recruiting and directing the immune system has increased the variety of adjuvant formulations being tested in animal models and humans. Across all vaccine platforms, adjuvant formulations have been shown to enhance desirable immune responses such as higher antibody titers and increased functional activity. Although no vaccine formulation has yet succeeded in eliciting broad neutralizing antibodies against HIV-1, the ability of adjuvants to direct the immune response to immunogens suggests they will be critically important in any successful HIV-1 vaccine.

Summary
The parallel development of adjuvants along with better HIV-1 immunogens will be needed for a successful AIDS vaccine. Additional comparative testing will be required to determine the optimal adjuvant and immunogen regimen that can elicit antibody responses capable of blocking HIV-1 transmission.

Keywords
adjuvants, antibodies, antigens/peptides/epitopes, B cells, HIV-1, vaccines

INTRODUCTION

Many hurdles remain for the development of a globally deployable HIV-1 vaccine. Elicitation of a durable immune response that can prevent HIV-1 infection or disease will likely require the use of an adjuvant for some or all immunizations. At present in the USA there are only two licensed adjuvants, although other adjuvanted vaccines are licensed in other parts of the world, and many more have been tested in human and animal trials. This review will highlight recent work in adjuvant development for HIV-1 vaccines with particular emphasis on antibody responses.

The word 'adjuvant' derives from the French *adjuvant*, which itself derives from the Latin *adjuvate* that can be translated to 'helper'. The term was first used in a modern vaccine context by Gaston Ramon of Institut Pasteur in a series of papers in the 1920s (e.g., [1▪▪,2,3▪▪]) that established the use of adjuvants for eliciting high-titer antitoxin responses. Since that time, many compounds and formulations have been tested for their ability to adjuvant a vaccine response, with the development of new adjuvants paralleling an increased understanding of pattern recognition receptors (PRRs) and their role in recruiting and directing the immune system.

An adjuvant is a compound, formulation, preparation, or delivery system that enhances or modifies the immunogenicity of the primary antigen in a vaccine. Adjuvants perform this function in a variety of ways, but nearly all involve the triggering of PRRs to stimulate the innate and adaptive arms of the immune system. This is accomplished in one of two ways – through the incorporation of active compounds in a vaccine formulation (e.g., formulating a protein immunogen in a liposome containing a TLR4 agonist) or by incorporating elements in the vaccine that result in the production of immune stimulants (e.g., addition of plasmids expressing cytokines in a DNA vaccine regimen).

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KEY POINTS

- A wide variety of adjuvants have been tested for HIV-1 vaccines, but recent comparative data are limited.
- There are no 'universal' adjuvants, but adjuvants must be selected based on the desired response and carefully paired with the immunogen being tested.
- Heterologous adjuvant strategies may be needed to balance efficacy and side-effects.

These distinctions are not absolute, and some formulations incorporate elements of both approaches.

The development of adjuvants has accelerated in the last 25 years and has to some degree paralleled the development of HIV-1 vaccine candidates. During that time, a number of excellent reviews have been published [1▪▪,2,3▪▪,4–8] that the reader may find useful. This review will focus on the historical context of adjuvant development since the discovery of HIV-1, recent developments, and finally will highlight the lack of comparative data currently available.

HISTORICAL CONTEXT

Shortly after the discovery of HIV-1, then Secretary of Health and Human Services Margaret Heckler held a 1984 press conference in which she predicted that vaccine trials against HIV-1 would be possible within 2 years [9]. The first vaccine trial began in 1986 [10,11], and was followed by a series of attempts to develop an effective HIV-1 vaccine. Early vaccine studies focused on leveraging strategies that had been successful for other vaccines including virus inactivation [12–14] and subunit immunogens [15] along with novel strategies such as recombinant viral constructs [11]. Although early subunit vaccine candidates were immunogenic [16], none of the follow-up efficacy trials showed protection [17,18].

Concurrent with the development of vaccine candidates, numerous animal and human studies compared available adjuvants in head-to-head trials. No clearly superior regimen was identified, likely because of the lack of a consistent immunogen across trials along with differing immunization schemes and different outcome measures. For example, Mannhalter *et al.* [19] in 1991 reported the immunization of chimpanzees with a recombinant envelope (Env) gp160 using alum, a water-in-oil emulsion (termed lipid-based adjuvant), or alum plus deoxycholate. T-cell responses were best for the lipid-based adjuvant and were shown to last for months after the final immunization; antibody responses were not reported. Niedrig *et al.* [12] reported in 1993 on another group of chimpanzees immunized with formaldehyde-inactivated HIV-1 adjuvanted with alum, Freund's incomplete adjuvant (an oil-in-water emulsion), or with a zinc hydroxide/lecithin-based adjuvant; in this study, antibody titers were best with the lecithin-based adjuvant, although proliferation and antibody-dependent cell-mediated cytotoxicity (ADCC) responses were similar between lecithin and alum arms. Levi *et al.* [20] reported in 1993 a comparison in rabbits of alum, Iscom, Iscomatrix, muramyl dipeptide (MDP), and Freund's complete adjuvant with a recombinant gp160 as the immunogen. Antibody titers were highest with Freund's complete adjuvant and MDP. During the same time period, numerous mouse studies were published and nearly all demonstrated that one adjuvant was superior. These and other head-to-head studies of vaccines are shown in Table 1 [19–30].

Vaccine candidates deemed the most promising advanced to phase I and phase II human trials. These studies tested proteins, peptides, and recombinant poxvirus vectors [31], and although none of the candidates produced overwhelming immunity, the vaccines were generally safe and well tolerated. Without a stronger candidate available, a controversial decision was made to pursue a phase III trial of poxvirus prime-gp120 boost vaccine strategy. The proposal had detractors [32] and supporters [33], and ultimately demonstrated a modest and short-lived degree of efficacy [34,35]. The adjuvant used in that trial was alum, the only US Food and Drug Administration (FDA)-approved adjuvant at that time. Studies are now being considered to examine the same immunization regimen using more potent adjuvants to see whether protection can be enhanced or prolonged. The remainder of this review will address more recent developments in adjuvant research.

ADJUVANTS FOR DNA VECTORS

DNA vaccines are attractive for eliciting $CD8^+$ T-cell responses, as protein production and antigen processing can occur without the need for an infectious vector. DNA vaccines are generally not as potent at eliciting antibody responses, although evidence suggests that DNA vaccines can prime for subsequent protein boosts [7,8]. Numerous studies have reported the ability of immune modulators to provide an adjuvant effect for DNA vaccines [36–49]; most of these studies were performed in mice and few compared more than one regimen against an unadjuvanted control. There are no studies

Table 1. Early adjuvant comparisons

Publication	Animal model	Immunogen	Adjuvant class					
			Alum	Oil/water emulsion	Iscom	Liposomes	Saponin	Other
Mannhalter *et al.* 1991 [19]	Chimpanzee	Env gp160	+[a]	+++	—	—	—	—
Ronco *et al.* 1992 [21]	Rhesus	Env gp160/peptides	+	+++	—	—	—	—
Wu *et al.* 1992 [22]	Mouse	Env gp160	+	—	—	—	+++	—
Levi *et al.* 1993 [20]	Rabbit	Env gp160	+	++[b]	++	—	—	—
Niedrig *et al.* 1993 [12]	Chimpanzee	Inactivated whole virus	+	+	—	—	—	+++[c]
Turánek *et al.* 1994 [23]	Mouse	gp41 peptide	++	++	—	++	—	—
Stieneker *et al.* 1995 [24]	Mouse	HIV-2 split virus	+	+	+	+	—	+++[d]
Ahlers *et al.* 1996 [25]	Mouse	Cluster peptide	+	++	—	—	++	—
Cleland *et al.* 1996 [26]	Guinea pig	Env gp120	+	++	—	—	++	++[e]
Perraut *et al.* 1996 [27]	Squirrel monkey	Env gp160/peptides	++[f]	++[f]	—	—	—	—
Peet *et al.* 1997 [28]	Mouse	Env gp120	+	++	—	—	++	++[g]
Sauzet *et al.* 1998 [29]	Mouse	Lipopeptide	+	+++	—	—	+	—
Verschoor *et al.* 1999 [30]	Rhesus	Env gp120	—	+	+++	—	—	—

[a]—, not tested; +, tested in the study; ++, tested and similar to other adjuvants in the study; +++, superior formulation in the study.
[b]Two emulsions tested, one contained muramyldipeptide. Results similar between emulsions.
[c]Zinc-lecithin adjuvant.
[d]Multiple other adjuvants tested; polymethylmethacrylate microparticles superior.
[e]Polylactate microspheres formulated for sustained release; comparable to other adjuvants tested.
[f]Multiple additive formulations tested with alum and emulsions. Muramyldipeptide formulations superior.
[g]Compared with protein in a proprietary adjuvant and with DNA immunization.

comparing all available DNA-encoded adjuvants, but a few smaller-scale studies have been reported. For example, testing of a series of DNA adjuvants in mice suggested that one of the tested adjuvants was superior [e.g., granulocyte/macrophage colony stimulating factor (GM-CSF) [50]], but studies in primates showed a more modest benefit [40]. Work is ongoing, but in the absence of a systematic study, at present, it is not clear whether any DNA-encoded adjuvant is superior in eliciting desirable immunity for an HIV-1 vaccine.

Some studies have examined the effect of adding compounds to DNA vaccines without having them encoded in a vector. Mycobacterial extracts have been shown to enhance T-cell and antibody responses in mice [51] as have TLR9 agonists [52]. Liposomes with mannan as a delivery vehicle for a DNA vaccine enhanced fecal IgA responses and altered subclass responses in mice [53]. Another Toll-like receptor (TLR) agonist, imiquimod, applied topically adjuvanted a DNA vaccine in mice, although the effect was similar to that of GM-CSF [54]. As with DNA-encoded adjuvant molecules, it is unclear which of these strategies is superior.

Recent studies have suggested that physical adjuvants may be beneficial for DNA vaccines. Electrical current as an adjuvant has been tested in mice [55], rhesus macaques [56,57], and humans [58▪]. The results suggest that electroporation alone is as effective as DNA-encoded adjuvants, although side-effects were higher in electroporation groups [58▪]. Electroporation almost certainly acts by increasing uptake of vaccine DNA into cells and through minor tissue damage that stimulates damage-associated PRRs that recruit an inflammatory response. Further testing will be needed to determine whether electroporation can be implemented so as to reduce side-effects yet remain effective.

ADJUVANTS FOR RECOMBINANT VECTORS

Immune stimulatory molecules can be encoded in viral or bacterial vectors that have sufficient room in their genomes (e.g., poxviruses, mycobacteria). As with DNA vaccines, studies have tested different adjuvants with mixed results. For poxvirus vectors, cytokines [59,60], soluble CD40 ligand [61], and CD252 [62] have been tested in mice and each enhanced immune responses compared with controls. Similar strategies have been tested for other viral vectors (e.g., rhabdovirus [63]).

Adjuvants can also be added with the vector but not encoded by it. For example, soluble CD40 ligand added to a DNA-prime/poxvirus-boost strategy enhanced T-cell responses though the effect on antibody was variable [64]. It remains to be seen if

any of these strategies will ultimately prove useful for human trials.

Whether other adjuvant formulations can enhance recombinant vectors is under investigation. Naito *et al.* [65] demonstrated in a mouse model that tethering of liposomes to a poxvirus vector overcame previous immunity and could stimulate humoral and cellular immunity. Many adjuvants, such as oil-in-water emulsions, can disrupt lipid membranes and so would be considered inappropriate for enveloped replicating vectors like poxviruses. In addition, as replicating vectors stimulate the immune system by the transient infection they cause, it is not clear that an adjuvant that is only transiently present at the site of injection would be useful. Future studies will be needed to clarify these questions.

ADJUVANTS FOR SUBUNIT IMMUNOGENS

For HIV-1 vaccine studies, the greatest variety of adjuvants have been tested for subunit/recombinant protein immunogens. As noted above, a large number of head-to-head trials were performed prior to 2000 (Table 1), but since that time, few large-scale direct comparisons have been published.

Older adjuvants continue to be explored to define those parameters critical for efficacy. Alum is one of the most commonly employed adjuvants because of its long history of use in humans and the relative ease for regulatory approval; for this reason, research to optimize its utility is ongoing. Hansen *et al.* [66▪] showed that the ability of alum to adsorb an Env protein was important for immunogenicity, but that binding too tightly reduced immune responses after immunization. Dorosko *et al.* showed that alternative methods of delivering alum can direct the immune response; injection of an alum-based peptide immunogen in the region of the supramammary lymph node of goats resulted in antibody secretion into colostrum [67].

Novel adjuvants continue to be studied in animal models. Lipid-based adjuvants like the AS0x series have been shown to stimulate strong antibody responses in guinea pigs, although responses were similar to those elicited by an oil-in-water emulsion adjuvant [68]. One of the adjuvants in this series, AS01B, elicited high-titered antibodies in rhesus macaques [69] and was also used in a human HIV-1 clinical trial wherein it generated antibody and T-cell responses [70]. Another adjuvant in that series, AS02A, also elicited immune responses in humans [71], but which of the adjuvants in this series is the best for an HIV-1 vaccine is not yet established.

Oil-in-water emulsions as adjuvants have been used for many years, and include mineral oil-based formulations (e.g., Freund's adjuvant) and more modern squalene-based preparations. They have also proved to be useful platforms for exploring the addition of immune stimulants and other compounds. TLR agonists like CpG oligodeoxynucleotides mixed with the squalene-based adjuvant MF59 appeared to enhance the adjuvant effect [72]. The addition of Carbopol to MF59 enhanced immunogenicity in rabbits to levels comparable with complete Freund's adjuvant, likely because of the slower release of the immunogen [73]. More recently, we reported that combinations of TLR ligands in a different squalene-based oil-in-water emulsion stimulated higher titers of antibodies and a greater breadth of functional responses, and that the combination of TLR7/8 and TLR9 agonists was optimal in rhesus macaques [74].

Other adjuvant formulations have been studied as well. Liposomes formulated with a modified polyethylene glycol elicited durable antibody responses to an Env gp41 peptide; the proposed mechanism was persistence of the modified liposomes leading to a prolonged immune response [75]. Compounds derived from pathogens have also shown promise in initial studies. A protein derived from the worm *Onchocerca volvulus* enhanced antibody responses in mice [76].

There have been multiple human trials with Env protein immunogens combined with different adjuvant formulations (Table 2) [16–18,34,71,77–80]. Unfortunately, comparative data are lacking, especially head-to-head comparisons of adjuvants using the same immunogen and dosing schedule. The use of adjuvants in humans demonstrates promise; for example, the AS02A adjuvant formulated with a Env gp120 immunogen was able to elicit similar titers of antibodies despite a 20-fold difference in the high and low immunogen dose groups, suggesting that the adjuvant might have a dose-sparing effect [71]. Additional studies will be needed to determine the best adjuvant–immunogen combinations for future large-scale trials.

ADJUVANTS FOR MUCOSAL RESPONSES

For eliciting mucosal responses, cholera toxin (CT) and other bacterial products have been extensively tested in animal models. CT combined with Env gp120 elicited mucosal IgA in rhesus macaques [81]; other studies in rhesus (albeit with a different form of CT) have elicited more mixed responses [82]. CT has also been used to direct responses to the mucosa by combining it with agents that enhance retention at the mucosal surface, and has permitted dose sparing [83]. In addition, modified CT combined with cytokines were able to elicit mucosal antibodies

Table 2. Human studies of Env protein immunogens with various adjuvants[a]

Publication	Adjuvant(s)	Immunogen	Outcome
Wintsch *et al.* 1991 [77]	Squalene + Tween80 + MTP-PE	Env $gp120_{SF2}$	Some binding antibodies elicited
Belshe *et al.* 1993 [16]	Alum-deoxycholate	Env $gp160_{IIIB}$	Binding antibodies elicited
Keefer *et al.* 1996 [78]	MF59 ± MTP-PE	Env $gp120_{SF2}$	Binding and neutralizing antibodies elicited, some dose-sparing effect seen with MTP-PE
McCormack *et al.* 2000 [79]	3D-MPL + QS21	Env $gp120_{W61D}$	Poor overall immunogenicity
Pitisuttithum *et al.* 2004 [80]	Alum	Env $gp120_{A244}$	Binding antibodies elicited
Flynn *et al.* 2005 [18]	Alum	Env $gp120_{A244}$/Env $gp120_{GNE8}$	VAX004 trial: antibodies elicited, no protection observed
Pitisuttithum *et al.* 2006 [17]	Alum	Env $gp120_{A244}$/Env $gp120_{MN}$	VAX003 trial: antibodies elicited, no protection observed
Goepfert *et al.* 2007 [71]	AS02A (has 3D-MPL and QS21)	Env $gp120_{W61D}$	Binding and functional antibodies elicited, dose sparing effect seen
Rerks-Ngarm *et al.* 2009 [34]	Alum	Env $gp120_{A244}$	RV144 trial: short-lived and modest protection against infection

MTP-PE, muramyl tripeptide-dipalmitoyl phosphatidylethanolamine; 3D-MPL, 3-deacylated monophosphoryl lipid A.
[a]Additional information about early vaccine trials summarized in [31].

to a peptide immunogen when given to cynomolgus monkeys [84], suggesting that promising combinations identified by other vaccine strategies (e.g., DNA vaccination) might be useful for mucosal immunization.

Other mucosal strategies are being investigated. Interestingly, intranasal cytokines appear to be as effective as CT in eliciting mucosal antibodies in mice [85]. A soybean oil nanoemulsion delivered intranasally with Env gp120 elicited IgA responses [86]. Other bacterial products, like *Mycoplasma*-derived lipopeptides, have been shown to adjuvant vaccines in mice [87,88]. Cranage *et al.* [89] demonstrated that Env gp140 administered with Carbopol intravaginally resulted in better mucosal responses than systemic immunization. In mice, thymic stromal lymphopoietin has been shown to elicit mucosal antibody at levels similar to CT [90]. Finally, a strategy employing microneedles combined with a TLR4 agonist was able to elicit strong antibody responses including vaginal IgA in mice [91▪].

MIXED STRATEGIES

It is possible that a successful HIV-1 vaccine strategy may involve heterologous immunizations as was used in the RV144 ALVAC-prime/AIDSVAX-boost trial [34]. Such strategies continue to be investigated in animal models. A regimen containing peptides adjuvanted with imiquimod and an oil-in-water emulsion was able to prime for viral vector boosts in rhesus macaques, eliciting strong T-cell and modest antibody responses [92]. Similarly, an alphavirus-based particulate vaccine prime combined with a protein boost using MF59 in rhesus macaques resulted in a better response that was also somewhat protective against infectious challenge [93].

Side-effect considerations may also drive heterologous prime-boost regimens. A study in rabbits using an oil-in-water emulsion for the prime and alum for the boost showed that antibody responses were highest with the mixed regimen [94]. The authors suggested that the regimen could be used to overcome the undesirable side effects of strongly adjuvanted vaccines by using less reactive adjuvants in subsequent steps.

PRACTICAL CONSIDERATIONS

As Edelman and Tacket [6] aptly stated in 1990, 'The best adjuvant will never correct the choice of the wrong epitope.' For now, the AIDS vaccine field has not identified the best immunogen(s) and so work continues to find a strategy that will elicit durable and broad protection against infection. The work to find an optimal adjuvant strategy will continue to parallel these efforts.

At present, the data to drive rational choices of adjuvants for an AIDS vaccine are lacking. This is partly because of the lack of a robust immunogen, but it is also because of the paucity of comparative data being published. In the last decade, few head-to-head comparisons of adjuvant formulations using the same HIV-1 immunogen have been reported, especially when compared with the first 20 years of the AIDS pandemic (Table 1). A partial

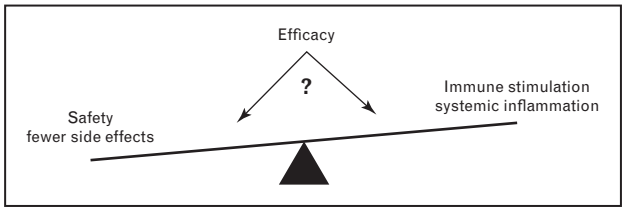

FIGURE 1. Adjuvant activity balance. Adjuvants need to be both well tolerated and effective, but the ability to stimulate an immune response is often associated with side effects. Further investigation will be needed to determine whether efficacy can be achieved with a low side-effect profile or if some degree of inflammation will be necessary.

reason for this is that adjuvants are not licensed by themselves but only as part of the licensure of a vaccine product. That is an entirely appropriate regulatory hurdle, but it does mean that an adjuvant licensed or on track for licensure combined with a vaccine for a non-HIV pathogen could be put at risk. If an adjuvant is found to be superior for an HIV-1 vaccine candidate, by definition other adjuvants will be inferior for that HIV-1 vaccine. This does not mean that an adjuvant inferior for an HIV-1 vaccine is inferior for all other vaccines, nor would it render a licensed vaccine ineffective. However, it would create a perception that one company's adjuvant is 'better' than the others, putting vaccines using the 'inferior' adjuvants at risk. Given that the vaccine market is small compared with blockbuster drugs [95], companies appear to be appropriately reluctant to put their investments at risk.

In addition, other hurdles face adjuvant development. As preventive measures, vaccines should be well tolerated for the general population and ideally cause no side-effects to anyone. As vaccines require stimulation of the immune system, establishing a balance between stimulation and side-effects is paramount (Fig. 1). However, no medical intervention is without risk and it is likely that a successful vaccine will cause some degree of side-effects in some recipients, and it will be important to determine the level of acceptable risk that balances with vaccine efficacy. Public judgment of acceptable risk will depend on vaccine efficacy, that is, a highly effective vaccine against a present threat that has some side-effects will likely be more acceptable than a vaccine that is less effective or is against a pathogen perceived to be less of a threat. Until an effective HIV-1 vaccine is available, work to find better adjuvants should continue.

CONCLUSION

A wide variety of adjuvant formulations are available to enhance the response to HIV-1 immunogens and exciting new work suggests that formulations with better balances between safety and efficacy may be possible. However, there is much work remaining to determine the optimal adjuvant immunogen combination that will be effective in controlling the AIDS pandemic.

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Conflicts of interest

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