Modulation of HIV-1 immunity by adjuvants

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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 adjutant, which itself derives from the Latin adjuvare 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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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...
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

| Publication                  | Animal model | Immunogen            | Adjuvant class | 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 |              | +                 |       |           |         |       |
| Turanek et al. 1994 [23]    | Mouse        | gp41 peptide         |                | ++                |       |           |         |       |
| Sienek et al. 1995 [24]     | Mouse        | HIV-2 split virus    |                | +                 |       |           |         |       |
| Ahlers et al. 1996 [25]     | Mouse        | Cluster peptide      |                | +                 |       |           |         |       |
| Cleland et al. 1996 [26]    | Guinea pig   | Env gp120            |                | +                 |       |           |         |       |
| Perraut et al. 1996 [27]    | Squirrel monkey | Env gp160/peptides |                | +^f               |       |           |         |       |
| Peet et al. 1997 [28]       | Mouse        | Env gp120            |                | +                 |       |           |         |       |
| 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.

*bTwo emulsions tested, one contained muramyldipeptide. Results similar between emulsions.

cZinc-lecithin adjuvant.

dMultiple other adjuvants tested; polymethylmethacrylate microparticles superior.

ePoly-lactic acid microspheres formulated for sustained release; comparable to other adjuvants tested.

fMultiple additive formulations tested with alum and emulsions. Muramyldipeptide formulations superior.

gCompared with protein in a proprietary adjuvant and with DNA immunization.
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 an 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
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
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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REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Reed SG, Orr MT, Fox CB. Key roles of adjuvants in modern vaccines. Nat Med 2013; 19:1597–1608. This is an excellent review that contains timeline of adjuvant development and overview of mechanisms of adjuvant action.
2. Barouch DH, Letvin NL, Seder RA. The role of cytokine DNAs as vaccine adjuvants for optimizing cellular immune responses. Immunol Rev 2004; 205:266–274.
3. Coffman RL, Shiver A, Seder RA. Vaccine adjuvants: putting innate immunity to work. Immunology 2010; 33:492–503. This is an excellent review of adjuvants and the role of PRRs in triggering the immune system. Good figures and tables; has a useful summary about the kinds of innate immune cells recruited by different stimulatory triggers.
4. Lore K, Karlsson Hedestam GB. Novel adjuvants for B cell immune responses. Curr Opin HIV AIDS 2009; 4:441–446. This is an excellent review of adjuvants and the role of PRRs in triggering the immune system. Good figures and tables; has a useful summary about the kinds of innate immune cells recruited by different stimulatory triggers.
5. De Gregorio E, Ulmer J, Caproni E, Ulmer JB. Vaccine adjuvants: mode of action. Front Immun 2013; 3:214.
6. Edelman R, Tacket CO. Adjuvants. Int Rev Immunol 1990; 7:51–66.
7. Morrow MP, Weiner DB. Cytokines as adjuvants for improving anti-HIV responses. AIDS 2000; 22:333–338.
8. Colarato SA, Weiner DB. Enhancement of human immunodeficiency virus type 1-DNA vaccine potency through incorporation of T-helper 1 molecular adjuvants. Immunol Rev 2004; 199:84–99.
9. Gallo RC. A reflection on HIV/AIDS research after 25 years. Retriovirology 2006; 3:72.
10. Zagury D, Bernard J, Cheynier R, et al. A group specific anamnestic immune reaction against HIV-1 induced by a candidate vaccine against AIDS. Nature 1988; 332:726–731.
11. Zagury D, Leonard R, Foucher M, et al. Immunization against AIDS in humans. Nature 1987; 326:249–250.
12. Niedrig M, Gregersen JP, Fultz PN, et al. Immunologic response of chimpanzees after immunization with the inactivated whole immunodeficiency virus (HIV-1), three different adjuvants and challenge. Vaccine 1993; 11:87–74.
13. LaCasce RA, Foulis KE, Trahey M, et al. Fusion-competent vaccines: broad neutralization of primary isolates of HIV. Science 1999; 283:357–362.
14. Nunberg JH. Retraction. Science 2002; 296:1025.
15. Pyle SW, Morein B, Bess JW, et al. Immune response to immunostimulatory complexes (ISCOMs) prepared from human immunodeficiency virus type 1 (HIV-1) or the HIV-1 external envelope glycoprotein (gp120). Vaccine 1989; 7:485–473.
16. Beilke RD, Clements ML, Dolin R, et al. Safety and immunogenicity of a fully glycosylated recombinant gp160 human immunodeficiency virus type 1 vaccine in subjects at low risk of infection. National Institute of Allergy and Infectious Diseases AIDS Vaccine Evaluation Group Network. J Infect Dis 1993; 168:1387–1395.
17. Pitisutthin P, Gilbert P, Gurwith M, et al. Thai HIV Vaccine Evaluation Group; randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. J Infect Dis 2006; 194:1661–1671.
Spectrum of HIV antibodies in vaccine and disease

18. Flynn NM, Forthal DN, Harro CD, et al. gp120 HIV Vaccine Study Group: Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. J Infect Dis 2005; 191:654–665.
19. Mannhalter CV, Pum M, Wolf HM, et al. Immunization of chimpanzees with the HIV-1 glycoprotein gp160 induces long-lasting T-cell memory. AIDS Res Hum Retroviruses 1991; 7:485–493.
20. Liu M, Racaniere U, Birn D, et al. Effects of adjuvants and multiple antigen peptides on humoral and cellular immune responses to gp160 of HIV-1. J Acquir Immune Defic Syndr 1993; 6:855–864.
21. Ronco J, Dedieu JP, Marie FN, et al. High-titer HIV-1 neutralizing antibody response of theses macaques to gp160 and env peptides. AIDS Res Hum Retroviruses 1992; 8:1117–1123.
22. Wu YJ, Gardner BH, Murphy CI, et al. Saponin adjuvant enhancement of antigen-specific immune responses to an experimental HIV-1 vaccine. J Immunol 1992; 148:1519–1525.
23. Turanek J, Toman M, Novak J, et al. Adjuvant effect of liposomes and adlamyloidide dideptide on antigenicity of entrapped synthetic peptide derived from HIV-1 transmembrane region glycoprotein gp41. Immunol Lett 1994; 39:157–161.
24. Skineker F, Kersten G, van Bloois L, et al. Comparison of 24 different adjuvants for inactivated HIV-2 split whole virus as antigen in mice. Induction of titres of binding antibodies and toxicity of the formulations. Vaccine 1995; 13:45–50.
25. Ahlers JD, Dunlop N, Pendleton CD, et al. Candidate HIV type 1 multidentrinated cluster peptide-P18MN vaccine constructs elicit type 1 helper T cells, cytotoxic T cells, and neutralizing antibody, all using the same adjuvant component. AIDS Res Hum Retroviruses 1996; 12:259–272.
26. Cleland JL, Barron L, Daugherty A, et al. Development of a single-shot subunit vaccine for HIV-1.3. Effect of adjuvant and immunization schedule on the duration of the humoral immune response to recombinant MN gp120. J Pharm Sci 1986; 75:1350–1357.
27. Perrault R, Chouette P, Moog C, et al. Immunogenicity of HIV-1LAI gp160 and env peptides in squirrel monkey Saint Sharon: immune response using alumin and experimen tal adjuvants. Clin Exp Immunol 1996; 106:434–441.
28. Peet NM, McKeating JA, Ramos B, et al. Comparison of nucleic acid and protein immunization for induction of antibodies specific for HIV-1 gp120. Clin Exp Immunol 1999; 119:226–232.
29. Sautet JP, Moog C, Krivine A, et al. Adjuvant is required when using Env lipopeptide construct to induce HIV type 1-specific neutralizing antibody responses in mice in vivo. AIDS Res Hum Retroviruses 1998; 14:901–909.
30. Verschoor EJ, Kallings L, Heemskerk C, et al. Comparison of immunity generated by nucleic acid, MF59, and ISCOM-formulated human immuno deficiency virus type 1 vaccines in Rhesus macaques: evidence for viral clearance. J Virol 1999; 73:3292–3300.
31. Keefer MC, Wolf M, Gorse GJ, et al. Safety profile of phase I/II preventive HIV type 1 envelope vaccination: experience of the NIADD AIDS Vaccine Evaluation Group. AIDS Res Hum Retroviruses 1997; 13:1163–1177.
32. Burton DR, Desrosiers RC, Doms RW, et al. Public health. A sound rationale needed for phase III HIV-1 vaccine trials. Science 2004; 303:315–318.
33. Belshé R, Franchini G, Girard MP, et al. Support for the RV144 HIV Vaccine Trial: new series. Science 2004; 305:177–180.
34. Verhoef S, Scholtissek C, Ostermeier H, et al. Vaccination with ALVAC and AIDS/AVS to prevent HIV-1 infection in Thailand. N Engl J Med 2009; 361:2209–2220.
35. Robbins SI, Ferreira NF, Smit S, et al. Combined effects of IL-12 and electroporation enhance the potency of DNA vaccination in macaques. Vaccine 2008; 26:3112–3120.
36. Kajolu SA, Bokoko SD, Elizaia M, et al. Safety and comparative immuno genicity of an HIV-1 DNA vaccine in combination with plasmid interleukin 12 and impact of intramuscular electroporation for delivery. J Infect Dis 2013; 208:818–829.
37. Lu Y, Xin KQ, Hamajima K, et al. Macrophage inflammatory protein-1alpha (MIP-1alpha) expression plasmid enhances DNA vaccine-induced immune response against HIV-1. Clin Exp Immunol 1999; 115:335–341.
38. Kawaguchi SK, Snarsky V, Timini JM, et al. Soluble multimeric TNF superfamily ligand adjuvants enhance immune responses to a HIV-1 Gag DNA vaccine. Vaccine 2012; 30:691–702.
39. Castadelillo A, Spatambli M, Bui CT, et al. Interferon regulatory factor-1 acts as a powerful adjuvant in the DNA based vaccination. J Cell Physiol 2010; 224:702–709.
40. Zou Z, Ye L, Sheen MJ, et al. Enhancement of immune response to an HIV env DNA vaccine by a C-terminal segment of listeriolysin O. AIDS Res Hum Retroviruses 2003; 19:409–420.
41. Calantona SA, Dai A, Trocio RJN, et al. IL-15 as memory T-cell adjuvant for topical HIV-1-DermaVir vaccine. Vaccine 2008; 26:3118–3120.
42. Barouch DH, Sanda S, Tenner-Racz K, et al. Potent CD4+ T cell responses elicited by a bispecific HIV-1 DNA vaccine expressing gp120 and GM-CSF. J Immunol 2002; 168:562–568.
43. Xu R, Megati S, Roopchand V, et al. Comparative ability of various plasmid-based cytokines and chemokines to adjuvant the activity of HIV plasmid DNA vaccines. Vaccine 2008; 26:4816–4829.
44. Sun J, Hou J, Li D, et al. Enhancement of HIV-1 DNA vaccine immunogenicity by BCG-PSN, a novel adjuvant. Vaccine 2013; 31:472–479.
45. Appagani P, Pardey RM, Seth P, et al. Augmentation of HIV-1 subtype C vaccine constructs induced immune response in mice by CpG motif 1826-ODN. Viral Immunol 2005; 18:213–223.
46. Toda S, Iishi N, Okada E, et al. HIV-1 specific-cell mediated immune responses induced by DNA vaccination were enhanced by mannan-coated liposomes and inhibited by antiinterferon-gamma antibody. Immunology 1997; 92:111–117.
47. Zubir AK, Brave A, Engstrom G, et al. Topical delivery of imiquimod to a mouse model as a novel adjuvant for human immunodeficiency virus (HIV) DNA vaccine. Vaccine 2004; 22:1791–1798.
48. Liu J, Kjenke R, Mathiesen I, et al. Anti-HIV-1 CD4+ T cell memory of antigen-presenting cells to the site of inoculation and augmentation of human immunodeficiency virus type 1 DNA vaccine immunogenicity by in vivo electroporation. J Virol 2008; 82:5643–5649.
49. Yin J, Dai A, Leurecux J, et al. High antibody and cellular responses induced to HIV-1 clade C envelope following DNA vaccines delivered by electroporation. Vaccine 2011; 29:6763–6770.
50. Hirao LA, Wu L, Khan AS, et al. Combined effects of IL-12 and electroporation enhances the potency of DNA vaccination in macaques. Vaccine 2008; 26:3112–3120.
51. Calantona SA, Parker SD, Elizaia M, et al. Safety and comparative immunogenicity of an HIV-1 DNA vaccine in combination with plasmid interleukin 12 and impact of intramuscular electroporation for delivery. J Infect Dis 2013; 208:818–829.
52. Only T-cell immunogenicity data are reported, but demonstrates that DNA vaccination with electroporation is effective for eliciting responses in humans.
53. Abalos F, Rodriguez JR, Gazion A, et al. Improving recombinant MVA immune responses: potentiation of the immune responses to HIV-1 with MVA and DNA vectors expressing Env and the cytokines IL-12 and IFN-gamma. Virus Res 2006; 116:11–20.
54. Ghareri MM, Ramirez JC, Rodriguez D, et al. IL-12 delivery from recombinant vaccinia virus attenuates the vector and enhances the cellular immune response against HIV-1 Env in a dose-dependent manner. J Immunol 2009; 182:5351–5357.
55. Liu J, Yu Q, Stone GW, et al. CD40L expressed from the canarypox vector, ALVAC, can boost immunogenicity of HIV-1 canarypox vaccine in mice and enhance the in vitro expression of viral specific epitopes. Vaccine 2011; 29:6763–6770.
56. Naito T, Kaneko Y, Kozbor D. Oral vaccination with modified vaccinia virus Ankara attached covalently to TMPEG-modified cationic liposomes overcomes preexisting poxvirus immunity from recombinant vaccinia vaccination. J Gen Virol 2007; 88:591–600.
57. Hansen B, Mayala P, Singh M, et al. Effect of the strength of adsorption of the HIV-1 516262Vgp140 to aluminum-containing adjuvants on the immune response. J Pharm Sci 2011; 100:3245–3250.
58. A demonstration of the ‘Goldilocks phenomenon’ in which binding to alum must be neither too weak nor too strong for optimal immunogenicity.
59. Grorosko SM, Ayres SL, Connor R. Induction of HIV-1 MPR (649-684) specific IgA and IgG antibodies in caprine colostrum using a peptide-based vaccine. Vaccine 2008; 26:5416–5422.

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Modulation of HIV-1 immunity by adjuvants

68. Li Y, Svehla K, Mathy NL, et al. Characterization of antibody responses elicited by human immunodeficiency virus type 1 primary isolate trimERIC and monomeric envelope glycoproteins in selected adjuvants. J Virol 2006; 80:1414–1426.

69. Möhrner A, Douagi I, Forssel MNE, et al. Human immunodeficiency virus type 1 env trimer immunization of macaques and impact of priming with viral vector or stabilized core protein. J Virol 2009; 83:540–551.

70. Leroux-Roels I, Koutsoukos M, Clement F, et al. Strong and persistent CD4+ T-cell response in healthy adults immunized with a candidate HIV-1 vaccine containing gp120, Nef and Tat antigens formulated in three adjuvant systems. Vaccine 2010; 28:7016–7024.

71. Goepfert PA, Tomaras GD, Horton H, et al. Durable HIV-1 antibody and T-cell responses elicited by an adjuvanted multirecombinant vaccine in uninfected human volunteers. Vaccine 2007; 25:510–518.

72. Burke B, Gómez-Roman VR, Lian Y, et al. Neutralizing antibody responses to subtype B and C adjuvanted HIV envelope protein vaccination in rabbits. Virology 2006; 367:147–156.

73. Lai RPI, Seaman MS, Tonks P, et al. Mixed adjuvant formulations reveal a new combination that elicit antibody response comparable to Freund’s adjuvant. PLoS One 2012; 7:e35083.

74. Moody MA, Santra S, Vandenberg NA, et al. TLR-7/8 and 9 agonists cooperate to enhance HIV-1 envelope antibody responses in rhesus macaques. J Virol 2014; 88:3329–3339.

75. Singh SK, Bisen PS. Adjuvanticity of stealth liposomes on the immunogenicity of synthetic gp41 epitope of HIV-1. Vaccine 2006; 24:4161–4166.

76. MacDonald AJ, Cao L, He Y, et al. Ovalbumin, HIV-1 polypeptide and SARS-CoV peptide antigens. Vaccine 2005; 23:3446–3452.

77. Wintoch J, Chagnat CL, Braun DG, et al. Safety and immunogenicity of a genetically engineered human immunodeficiency virus vaccine. J Infect Dis 1991; 163:219–225.

78. Keefe MC, Graham BS, McElrath MJ, et al. Safety and immunogenicity of Env 2-3, a human immunodeficiency virus type 1 candidate vaccine, in combination with a novel adjuvant, MTP-PE/MF59. NIAID AIDS Vaccine Evaluation Group. AIDS Res Hum Retroviruses 1996; 12:683–693.

79. McCormack S, Tilley A, Carmichael A, et al. A phase I trial in HIV negative healthy volunteers evaluating the effect of potent adjuvants on immunogenicity of a recombinant gp120W61D derived from dual tropic R5X4 HIV-1ACH9320. Vaccine 2000; 18:1166–1177.

80. Pitsouliithim P, Berman PW, Phornrat B, et al. Phase I/II study of a candidate vaccine designed against the B and E subtypes of HIV-1. J Acquir Immune Defic Syndr 2004; 37:1160–1165.

81. Yoshino N, Lu F-X, Fujishahi K, et al. A novel adjuvant for mucosal immunity to HIV-1 gp120 in nonhuman primates. J Immunol 2004; 173:6850–6857.

82. Sunding C, Schön K, Möhrner A, et al. CTLA1-DD adjuvant promotes strong immunity against human immunodeficiency virus type 1 envelope glycoproteins following mucosal immunization. J Gen Virol 2008; 89:2954–2964.

83. Nordone SK, Peacock JW, Kiwan SM, Staats HF. Capric acid and hydroxypropylmethylcellulose increase the immunogenicity of nasally administered peptide vaccines. AIDS Res Hum Retroviruses 2006; 22:558–568.

84. Egan MA, Chong SY, Hagen M, et al. A comparative evaluation of nasal and parenteral vaccine adjuvants to elicit systemic and mucosal HIV-1 peptide-specific humoral immune responses in cynomolgus macaques. Vaccine 2004; 22:3774–3788.

85. Bradney CP, Sempowski GD, Liao H-X, et al. Cytokines as adjuvants for the induction of antihuman immunodeficiency virus peptide immunoglobulin G (IgG) and IgA antibodies in serum and mucosal secretions after nasal immunization. J Virol 2002; 76:517–524.

86. Bieńkowska AU, Jarczak KW, Landers JJ, et al. Nasal immunization with a recombinant HIV gp120 and nonemulsion adjuvant produces TH1 polarized responses and neutralizing antibodies to primary HIV type 1 isolates. AIDS Res Hum Retroviruses 2006; 24:271–281.

87. Boruszyn S, Fiorelli V, Ebensen T, et al. Efficient mucosal delivery of the HIV-1 Tat protein using the synthetic lipopeptide MALP-2 as adjuvant. Eur J Immunol 2003; 33:1548–1556.

88. Boruszyn S, Ebensen T, Link C, et al. Efficient systemic and mucosal responses against the HIV-1 Tat protein by prime/boost vaccination using the lipopeptide MALP-2 as adjuvant. Vaccine 2006; 24:2049–2056.

89. Cranage MP, Fraser CA, Cope A, et al. Antibody responses after intranasal immunisation with trimeric HIV-1 CN54 clade C gp140 in Carbopol gel are augmented by systemic priming or boosting with an adjuvanted formulation. Vaccine 2011; 29:1421–1430.

90. Van Roey GA, Arias MA, Tregoning JS, et al. Thymic stromal lymphopoietin (TSLP) acts as a potent mucosal adjuvant for HIV-1 gp140 vaccination in mice. Eur J Immunol 2012; 42:353–363.

91. Pattani A, McKay PF, Garland MJ, et al. Microneedle mediated intradermal delivery of adjuvanted recombinant HIV-1 CNS-4gp140 effectively primes mucosal boost inoculations. J Control Release 2012; 162:529–537.

92. Rosario M, Bothwick N, Stewart-Jones GB, et al. Prime-boost regimens with adjuvanted synthetic long peptides elicit T cells and antibodies to conserved regions of HIV-1 in macaques. AIDS 2012; 26:275–284.

93. Barnett SW, Burke B, Sun Y, et al. Antibody-mediated protection against mucosal simian-human immunodeficiency virus challenge of macaques immunized with aliphavirus replication particles and boosted with trimeric envelope glycoprotein in MF59 adjuvant. J Virol 2010; 84:5975–5985.

94. Raya NE, Quintana D, Carrazana Y, et al. A prime-boost regime that combines Montanide ISA720 and Alhydrogel to induce antibodies against the HIV-1 derived multiepitope polypeptide TAB9. Vaccine 1999; 17: 2646–2650.

95. Cohen J. Bumps on the vaccine road. Science 1994; 265:1371–1373.