Published in final edited form as:

Curr Opin HIV AIDS. 2017 May ; 12(3): 278–284. doi:10.1097/COH.0000000000000365.

Adjuvants – Tailoring Humoral Immune Responses

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Abstract

Purpose of review—The development and availability of new generation adjuvants beyond aluminum and emulsion formulations, together with a deeper understanding of the mechanistic role of adjuvant formulations in stimulating innate immunity, offer opportunities to improve candidate vaccine designs intended to protect against HIV-1 acquisition.

Recent findings—Currently, major efforts in vaccine development focus on improving prime-boost vaccine regimens to enhance efficacy beyond 31% observed in the RV144 phase 3 study, and to develop a pathway to induce broadly reactive HIV neutralizing antibodies. Advances in HIV-1 Env immunogen design and improved adjuvant formulations are moving at a parallel pace. This review highlights steps underway to rationally pair vaccine concepts with improved adjuvant formulations in preclinical and early phase 1 clinical evaluation.

Summary—New adjuvants with immune potentiating properties are currently being tested in combination with recent HIV envelope-containing immunogens in prime-boost and subunit protein-only regimens. Greater emphasis is being applied to formulation science, delivery, and targeted safety and immune evaluation with these adjuvants in clinical trials. The need to develop an HIV vaccine that induces more potent and long-lived protective immunity will necessitate continued efforts to optimize adjuvanted vaccine formulations.

Keywords

Adjuvant; HIV; Env immunogens; neutralizing antibodies; innate immunity

Introduction

A preventive HIV vaccine holds the greatest promise for long-lived immunity against HIV infection and remains a global health priority. After nearly three decades of HIV vaccine development and evaluation in humans, no licensed vaccine exists. Major efforts now focus on improving prime-boost vaccine regimens to enhance efficacy beyond the 31% achieved in the RV144 phase 3 trial (1) and on developing a pathway with HIV-1 envelope immunogens to induce broadly reactive HIV neutralizing antibodies (bnAbs) (2). Advances in HIV-1 envelope (Env) immunogen design and adjuvant formulations are moving at a parallel pace, and when combined, vaccine-adjuvant formulations may overcome current challenges of

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Conflicts of interest: None.

potency and durability. This review highlights steps underway to rationally pair vaccine concepts with improved adjuvant formulations in preclinical and early phase 1 clinical evaluation.

Adjuvants Available and in Use

For nearly a century, adjuvants have been combined with vaccine antigens to enhance protective immunity to pathogens, and adjuvants are now components of more than 30 licensed vaccines from different manufacturers (3) (Figure 1). Aluminum gels or salts serve as adjuvants for most licensed pathogen-related subunit vaccines, as well as for most candidate HIV-1 subunit vaccine formulations undergoing clinical testing. Apart from aluminum adjuvants, only three other adjuvants are licensed in the United States for use as part of a vaccine: MF59 (Novartis) in influenza A and B vaccine (FLUAD), AS03 (GSK) in H5N1 influenza adjuvanted vaccine stockpile, and AS04 (GSK) in the bivalent human papilloma virus vaccine (Cervarix) against subtypes 16 and 18 (3,4). Of note, the malaria vaccine Mosquirix (GSK), also known as RTS,S/AS01 (5), has received European Medicines Agency regulatory approval and will be introduced into malaria endemic regions to prevent infection in children (6).

For HIV vaccines, MF59, a squalene-based oil-in-water emulsion, has been formulated with several HIV-1 envelope proteins for extensive clinical testing and is presently in phase 2b efficacy evaluation (HVTN 702) in South Africa as part of an ALVAC/HIV vector prime and adjuvanted bivalent gp120 boost regimen. In addition to AS03 and AS04, the Adjuvant System family (GSK) (3,7) includes two adjuvants containing MPL (monophosphoryl lipid A) and QS21 (Quillia saponaria fraction 21), AS01 and AS02, both of which have been evaluated in phase 1 HIV vaccine studies (8-10). AS01 is gaining interest for formulations with various new Env immunogens that will soon enter clinical testing. Table 1 summarizes novel adjuvant formulations in ongoing and planned HIV vaccine clinical trials.

Adjuvant Effects on Innate Immune Responses

Molecular immunostimulants and carrier systems comprise the two main adjuvant categories (4). Most immunostimulants act as ligands for pattern recognition receptors. These include toll-like receptors (TLRs), NOD-like receptors, helicases and C-type lectin receptors. Current novel adjuvants primarily act as agonists targeting TLR 3, 4, 7/8 and 9. Technologic and computational advances over the last decade have brought new insights into how adjuvants mediate immune effects; these are highlighted in several excellent reviews (4,11,12). Adjuvants can stimulate, increase or regulate immune responses to the antigen, which could be critical for poorly immunogenic antigens such as HIV-1 envelope. Desirable adjuvant properties may include increasing the breadth of immunity, enhancing responses in persons with advanced age or immune impairments, extending the durability of vaccine-induced immunity, reducing the need for repeated booster immunizations, sparing antigen dose, and targeting specific types of immune responses.

Of note, new understanding has emerged in defining the mechanism of action of the Adjuvant System family primarily targeting TLR4 (7,13,14), particularly regarding the

cellular and molecular mechanisms of QS-21, a key component in the liposomal AS01 adjuvant (15). Following intramuscular injection, liposomal QS-21 rapidly accumulates in CD169⁺ resident macrophages of draining lymph nodes (LN). These macrophages exert critical functions through initiation of innate cell recruitment into the LN and resulting DC activation and maturation. The adjuvant effect of QS-21 depends on the integration of caspase-1 and MyD88 pathways in the draining LN, at least in part through the local release of HMGB1. Thus, these mechanistic studies provide insight into how this complex adjuvant system can engage the innate immune system to drive the adaptive response.

One challenge in understanding adjuvant effects in humans relates to species differences in innate cells, receptor expression, tissue distribution, and regulation of innate signaling responses, which may limit translation of preclinical experimental findings to clinical applications. TLR expression on antigen-presenting cells (APCs) varies between mouse and human. In inbred mice, functional TLR8 is not expressed on APCs, whereas in humans, myeloid DCs (mDCs) express both TLR4 and TLR8. Human plasmacytoid DCs (pDCs) and B cells express intracellular TLR7 and TLR9, which recognize viral and bacterial nucleic acids and mediate the production of type I IFNs, whereas in mice, TLR7 and TLR9 are primarily expressed by CD8⁺ mDCs (11). Less is known about how responses to TLR ligand stimulation compare between rhesus macaques and humans. *In vitro* stimulation studies (16) indicate that the response pattern of human and rhesus macaque pDCs and B cells to TLR7/8 and TLR9 is comparable, despite some differences in surface phenotype of responding cells. A comprehensive evaluation of rhesus macaque innate responses in blood and draining LN following TLR agonist intradermal injection highlights the distinct signatures of early innate responses both locally and systemically, and provides a major step toward addressing species differences in recognizing TLR agonists now being used in non-human primate vaccine models and advanced to clinical studies (17). These insights into species differences in innate responses can influence the selection of suitable animal models for preclinical immunogenicity and toxicity studies in developing candidates for clinical testing.

Recent Preclinical Investigations with HIV Vaccine-Adjuvant Formulations

An effective HIV vaccine will likely elicit antibodies with sufficient potency and breadth to overcome the genetic diversity of circulating HIV-1 strains. Presumably these antibodies will require the capacity to neutralize HIV, thereby preventing entry into target cells. Antibodies that can mediate anti-viral Fc effector functions such as ADCC and ADCP may also play a protective role, as was suggested from the RV144 trial immune correlates analysis (18-21) and in more recent non-human primate SIV/SHIV challenge studies following vaccination (22). Several recent investigations have made technological advances to evaluate these vaccine effects in preclinical models and a few key studies merit emphasis here.

Li and Shaw (23) identified the residue at position 375 of Env in SHIV strains as a critical location in the CD4-binding pocket. Substitution of bulky or basic amino acids for serine at position 375 appears to enhance HIV Env binding affinity for the rhesus macaque CD4 receptor. The resulting SHIVs demonstrate persistent *in vivo* replication in macaques and elicit predictable patterns of autologous sequence changes associated with neutralization, similar to HIV-1 in humans. These breakthrough findings enable researchers to expand the

panel of SHIV challenge strains to model vaccine-adjuvant effects with diverse circulating and transmitted founder viruses.

Francica and Seder performed a longitudinal analysis of adjuvant effects and HIV Env-specific B cell receptor (BCR) responses in rhesus macaques following Env protein vaccination with eight different adjuvants (24). An attractive feature of this study was the selection of clinical protein-adjuvant combinations. The immunogens were HIV clade C Env gp140 (Novartis) alone, or formulated with alum or MF59 with or without agonists of TLR 4 or 7, or with poly IC:LC or ISCOMs. Increased anti-Env binding titers and neutralization titers to tier 1A viruses were observed among the adjuvanted groups in contrast to the unadjuvanted group after four immunizations; notably the alum/TLR7, MF59 and ANE/TLR4 recipients produced the highest mean IgG end point titers. Moreover, the MF59, poly IC:LC and ISCOM formulations elicited marked increases in Env-specific memory B cells after two or four immunizations, although these contracted by 12 weeks after the fourth immunization. Various adjuvant formulations did not modulate changes in overall somatic hypermutation levels and CDR H3 length, properties consistent with but not sufficient for development of bnAbs, and distinct from previous reports with use of MF59-adjuvanted influenza vaccine (25) and CpG-adjuvanted hepatitis B vaccine (26). This investigation provides an excellent framework for assessing B cell and antibody responses with HIV vaccines that can be applied in preclinical and clinical models.

Franchini and collaborators (27-29) conducted extensive investigations in a rhesus macaque SIV vaccine challenge model to elucidate and validate correlates of protection associated with the mildly efficacious RV144 Thai trial regimen (1,18) using a recombinant canarypox vector (ALVAC)-SIV and gp120 formulated either in alum or MF59. Vaccine efficacy against SIVmac251 intrarectal challenge was associated with alum-induced, but not with MF59-induced, Env-dependent mucosal innate lymphoid cells producing IL-17, gp120 V2-specific mucosal IgG, and activation of the RAS pathway (27). Follow-up studies have pointed to the potential importance of mucosal anti-V2 antibodies at the site of virus challenge (29) and the ability of vaccine-induced antibodies to inhibit infectivity of virus that it binds and captures (28). Larger confirmatory studies are in progress to understand the impact of these adjuvants on SIV protection, particularly at mucosal sites, which may inform future human trials with similar regimens.

Additional progress is being made with adjuvant carrier systems to heighten vaccine-induced immunity. Kasturi and Pulendran (30) investigated nanoparticles that encapsulated TLR4 and TLR 7/8 agonists and combined with SIV Env and Gag expressed as soluble proteins or VLPs. The nanoparticle-TLR formulation, in comparison to alum adjuvant, significantly enhanced the magnitude and persistence of antigen-specific antibody responses. Francica and Seder (31,32) have designed thermo-responsive polymer nanoparticles to co-deliver pathogen antigens. This platform holds promise for structure-based vaccine designs potentially including HIV-1 Env neutralizing epitopes. Weiner and others have addressed ways to improve adjuvanted SIV and HIV DNA vaccines with cytokine or chemokine plasmids delivered by electroporation. They recently reported that rhesus macaques co-administered SIV DNA and CCR9L or CCR10L adjuvants by electroporation were protected from multiple low-dose intravaginal SIVsmE660 challenge by over two-fold versus DNA

alone (33). Taken together, these animal studies support the further study of novel adjuvanted vaccines and their potential for advanced evaluation in clinical trials.

Examination of early events in vaccine antigen-adjuvant priming in animal models are lending insight into new methods to induce desired immunity and to measure their effects (34-37). Irvine and colleagues reported heightened antibody responses by sustained delivery of HIV antigens through either repeated injections or osmotic pumps over 1-2 weeks (37). Exponentially increasing dosing was superior to a single full dose priming; findings were consistent with antigen retention in draining lymph nodes and expanded germinal center B and Tfh cell responses. Thus, regulating the kinetics of antigen and adjuvant delivery may stimulate increased vaccine potency. Havenar-Daughton and colleagues have employed repeated direct probing of germinal center responses by fine needle lymph node aspirates in NHP studies and found that induction of neutralizing antibodies against tier 2 autologous HIV strains best correlated with germinal center B cell magnitude and Env-specific CD4+ Tfh cells (35). Furthermore, based on murine, NHP and human data, Havenar-Daughton and Crotty (36) found that significantly higher levels of plasma CXCL13 were associated with heightened germinal center reactivity, attesting to the potential use of CXCL13 as an easily accessible biomarker in blood to follow germinal center activities promoting functional antibody responses and persistence. Similar sampling strategies are under evaluation in early phase human vaccine studies (Figure 2).

Progress in Rational Design of Human HIV Immunogen and Adjuvant Combinations

While it is assumed that newer adjuvant-vaccine formulations induce superior potency to alum-vaccine formulations, formal evaluation in the same clinical study with newer generation Env immunogens have not yet been reported. However, two recent reports comparing two or more adjuvant formulations with subunit proteins in clinical studies merit emphasis. In one report (38), two phase 1/2 studies (RV135 and RV132) related to the RV144 phase 3 trial evaluated the same priming ALVAC-HIV vector (vCP1521) and two different Env protein-adjuvant combinations as booster immunizations. Comparable rates of serum anti-Env IgG antibody decline were noted following vaccination in the two studies, and the MF59 adjuvant formulation did not elicit higher IgG responses relative to alum. As the authors note, limitations of this comparative analysis include different bivalent HIV gp120/B/E proteins and doses in the two studies. In the second report (39), immune responses were compared in a five-arm clinical study using either alum or one of four Adjuvant Systems (AS01B, AS01E, AS03A, or AS04) formulations with hepatitis B surface antigen immunogen. Functional profiles of antigen-specific CD4+ T cells were similar among all arms, dominated by CD40L and IL-2 expression. The AS formulations, particularly AS01 and AS03, elicited more rapid and higher titer antibody responses and CD4+ T cell response rates when compared to the alum arm. Clearly, new well-designed clinical trials comparing adjuvant effects with Env immunogens are critically needed in the HIV vaccine field. Clinical trials are ongoing and planned to evaluate alone, or in some cases to compare, different adjuvant formulations (e.g., alum, MF59 and AS01B) with subunit boosts in prime-boost regimens (Table 1). Additionally, future studies comparing

various TLR agonist formulations with alum adjuvants using a single envelope immunogen are planned and will be key to advancing the field.

Improved understanding of the structural and immunological determinants needed to elicit bnAbs is progressing at a rapid pace in animal models (40). Well-ordered soluble Env trimers have been stably produced and provide a platform for immunogen designs and evaluation to proceed along a pathway for inducing bnAbs (41-44). In addition, germline targeting antigens that can specifically bind and activate B cells of a single bnAb lineage can serve as immunogens to initiate this process. Establishing one to two novel adjuvant formulations (Table 1) with Env trimers and germline targeting antigens will be beneficial for testing in NHP and human investigations to enhance affinity to targeted epitopes and improve potency. Evaluation of the distinct innate and adaptive immune pathways and response kinetics that drive the induction of bnAbs will ideally include selective sampling of draining lymph nodes and bone marrow to characterize germinal center reactivities, long-lived plasma cells, extensive B cell repertoire sequence analysis and functional antibody studies. Figure 2 highlights the key elements of a potential study schema for these first-in-human studies to comprehensively interrogate these responses.

Significant challenges lie ahead in conducting these studies. A given adjuvant is licensed as a component of the adjuvanted vaccine, not separately. Hence, each novel adjuvant must be tailored to a given candidate HIV vaccine, which typically requires new formulation, toxicity, preclinical immunogenicity studies, and extensive safety monitoring in humans for local and systemic adverse events, including longer study follow-up than typical vaccines lacking adjuvants (45-47). These steps slow the pace of moving new HIV vaccine concepts into first-in-human studies.

Conclusion

Since the initiation of HIV-1 preventive vaccine studies in 1987, at least 11 adjuvants have been evaluated with HIV-1 Env protein or peptide immunogens. With heightened interest in developing HIV vaccines that can induce antibodies with protective anti-viral responses, particularly bnAbs, new adjuvant formulations will play a key role in improving Env immunogen designs.

Acknowledgments

Funding: The author kindly thanks Stephen Voght and Mary Gross for critical reading of the manuscript.

Financial support and sponsorship: The author is supported by the National Institutes of Health-NIAID, Bill and Melinda Gates Foundation and Joel D. Meyers Endowed Chair.

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Key Points

- New HIV vaccine-adjuvant formulations are needed to induce antibodies with greater potency and persistence.
- Accelerating new HIV Env immunogen-adjuvant combinations in humans requires optimal formulation and coordination between preclinical and phase 1 studies.
- Probing the dynamics and molecular effects of complex adjuvant formulations with novel HIV-1 Env immunogens is critical for advancing candidate adjuvanted vaccine regimens that induce desired immune response profiles.

1920-1940	1960-1980	1980-2000	2000-2010	2010-present
Aluminum salts* Freund's complete adjuvant	Muramyl di-peptide (MDP) Poly-IC:LC Monophosphoryl lipid A (MPL)	Virosome* MF59* CpG ODN AS01* Flagellin R837/R848* QS-21	AS02 AS03 AS04*	GLA-SE CpG1018 3M-052 GLA LSQ

*Licensed adjuvants Adjuvants tested (or soon to be tested) in HIV prophylactic vaccine trials

Fig 1.

Heading: 100 years of vaccine adjuvant discovery and development.

Legend: Included among these are six adjuvants licensed for use as vaccine components or therapy (denoted by asterisk), and nine evaluated in HIV prophylactic clinical vaccine trials (red). Three additional adjuvants (blue) are scheduled to be tested in upcoming prophylactic HIV clinical vaccine trials.

Source: original work

Fig 2.

Heading: Probing HIV vaccine-adjuvant immune effects longitudinally to maximize induction of protective immunity.

Legend: Interrogating relevant innate and adaptive immune responses longitudinally in blood, draining lymph nodes and bone marrow following immunization with HIV vaccine-adjuvant combinations. This approach will optimize identification of adjuvant-related innate signaling patterns and key steps and bottlenecks on a path to bnAb development.

Source: original work

Table 1
Novel adjuvant formulations for evaluation in candidate HIV-1 vaccine clinical trials

Adjuvant (Source [*])	Vaccine	Study
MF59 (GSK): Squalene-in-water emulsions	ALVAC-HIV (vCP2438) and bivalent subtype C gp120/MF59	Pivotal phase 2b/3 trial (HVTN ^{**} 702), prime boost regimen in South Africa
	HIV-DNA and bivalent gp120 (all subtype C)	Phase 1/2a trial (HVTN 108), 2 adjuvant arms
	ALVAC-HIV (vCP2438) and bivalent subtype C gp120/MF59	2 adjuvant arm study (<i>in planning</i>)
AS01B (GSK): Liposome-based QS-21 + monophosphoryl lipid A	HIV-DNA and bivalent gp120/AS01B (all subtype C)	Phase 1/2a trial (HVTN 108), 2 adjuvant arms
	eOD GT8 60-mer nanoparticle	Germline targeting Env immunogen (<i>in planning</i>)
	BG505 SOSIP.664 trimer	Well-ordered native-like soluble Env trimer (<i>in planning</i>)
	ALVAC-HIV (vCP2438) and bivalent subtype C gp120/AS01B	2 adjuvant arm study (HVTN 120) (<i>in planning</i>)
GLA-SE (IDRI): glucopyranosyl lipid A in stable emulsion	polyvalent gp120	Phase 1 trial (HVTN) (<i>in planning</i>)
	CH505 gp120	Phase 1 trial (HVTN), B cell lineage (<i>in planning</i>)
GLA-LSQ (IDRI): liposome-based glucopyranosyl lipid A and QS-21	BG505.665 SOSIP trimer	Phase 1 trial (HVTN) (<i>in planning</i>)
3M-052 (3M): TLR 7/8 agonists	BG505.665 SOSIP trimer	Phase 1 trial (HVTN) (<i>in planning</i>)

^{*} Source abbreviations: GSK, GlaxoSmithKline; IDRI, Infectious Disease Research Institute.

^{**} HVTN, HIV Vaccine Trials Network.