Enhancing dendritic cell activation and HIV vaccine effectiveness through nanoparticle vaccination

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SUMMARY
Novel vaccination approaches are needed to prevent and control human immunodeficiency virus (HIV) infection. A growing body of literature demonstrates the potential of nanotechnology to modulate the human immune system and generate targeted, controlled immune responses. In this Review, we summarize important advances in how ‘nanovaccinology’ can be used to develop safe and effective vaccines for HIV. We highlight the central role of dendritic cells in the immune response to vaccination and describe how nanotechnology can be used to enhance delivery to and activation of these important antigen-presenting cells. Strategies employed to improve biodistribution are discussed, including improved lymph node delivery and mucosal penetration concepts, before detailing methods to enhance the humoral and/or cellular immune response to vaccines. We conclude with a commentary on the current state of nanovaccinology.

Introduction

Opportunities offered by nanovaccinology

The fields of nanotechnology and materials science are playing an increasingly important role in the rational design of vaccines for the future. This convergence has allowed the engineering of size, shape, charge, and surface functionality into experimental vaccines. Nanoparticle vaccines (nanovaccines) can target antigen-presenting cells (APCs), codeliver adjuvant with antigen, and control antigen processing within APCs to elicit desired immunogenic outcomes. At least 50 nanomedicines are approved and commercially available, with more than 90 in preclinical or clinical testing [1,2]. Although the major focus to date has been on the treatment of cancers, the investigation of nanovaccines to treat and prevent infectious disease is collecting momentum.

Human immunodeficiency virus type 1

HIV/AIDS remains a major global health challenge. The development of combination antiretroviral therapy (cART) transformed the morbidity and mortality associated with HIV to a chronic, manageable disease [3]. However, lifelong adherence to cART is associated with potentially harmful side effects, drug resistance, financial burden, and stigma [4,5]. Moreover, current cART strategies cannot eliminate the virus from infected individuals. The success of cART has resulted in increased prevalence owing to the reduction in mortality. By the end of 2014, an estimated 36.9 million people were living with HIV, with 2.0 million new infections and 1.2 million HIV-related deaths each year [6].

Vaccines

Vaccination is widely viewed as the most effective medical intervention in the control of infectious disease [7]. However, many challenges stand in the way of a successful HIV vaccine. Historically, conventional vaccines stimulate a humoral immune response and provide protection through induction of antibodies (Ab) with neutralizing capabilities. However, efforts to generate broadly neutralizing antibodies (bNAb) against HIV have been largely unsuccessful [8]. Reasons include the immense genetic diversity within and between HIV subtypes, largely inaccessible neutralizing epitopes that reside beneath a glycan shield, and development of sufficiently immunogenic antigens [7,9–11]. Beyond the challenges of eliciting an effective humoral response, it is widely believed that a highly effective HIV vaccine will also require induction of cellular immunity to kill virally infected cells. The recent success of cytotoxic T lymphocyte (CTL) strategies to combat previously untreated cancers by ‘releasing’ immune checkpoints that otherwise stop T cell-mediated immune responses, such as programmed cell death protein 1 and CTL antigen 4 [12], offers renewed hope for T cell strategies to treat and prevent infectious diseases. Immune responses require antigen processing by, and activation of, professional APCs such as dendritic cells (DCs). Importantly, for cell-mediated immune responses, antigen must be shuttled into the ‘cytoplasmic’ major histocompatibility complex (MHC)-I pathway before it can be presented to naïve CTLs.

Problems with conventional vaccines for HIV

HIV has proved incredibly challenging for vaccine scientists, with no effective vaccine since the discovery of the virus...
more than three decades ago. Two recent clinical trials, STEP and RV144, suggest that a paradigm shift is required in the rational design of an effective HIV vaccine. The STEP trial examined the efficacy of a cell-mediated vaccine strategy \[13\]. Using an adenovirus 5 (Ad5) vector, HIV-seronegative participants were immunized with Ad5 encoding HIV-1 Gag/Pol/Nef or placebo. The vaccine was highly immunogenic, with HIV-specific CTLs identified in 73% of vaccinees \[14\], and provided the first evidence of T cell-selective pressure by an HIV vaccine \[15\]. However, the vaccine failed to prevent infection or control viral load in infected subjects. In fact, HIV infection was higher in both men with preexisting Ab to the adenovirus vector serotype and those who were uncircumcised. This trial raised concerns about the safety of viral vectors and their utility in inducing cell-mediated immunity \[16,17\]. The only clinical trial to report a significant reduction in HIV infection, the RV144 trial, explored a strategy aimed at inducing both cellular and humoral immune responses \[18\]. This vaccine regimen involved a recombinant canarypox virus prime containing subtype B Gag, Pro, and subtype E gp120, followed by a subtype B/E bivalent gp120 boost in alum adjuvant. The RV144 trial demonstrated a modest 31% protection, but provided some hope for an effective HIV vaccine. RV144 illustrated the importance of inducing both T cell immunity (as illustrated by the correlate of CD4+ T cell immunity \[19,20\]) and functional nonneutralizing Ab \[21,22\]. Nanotechnology offers opportunities to tailor the immune response for improved vaccination against HIV (Figure 1).

Specifically, these are focused around (1) enhancing DC activation, (2) improving biodistribution and pharmaokinetics, and (3) tuning the immune response to better reflect HIV vaccine correlates of protection.

Increase in nanovaccinology research

In this review, we highlight the central role of DCs in the immune response to vaccines before describing how nanoparticles can enhance both vaccine uptake by and activation of DCs. We then discuss strategies employed by nanotechnology to improve biodistribution, drainage to lymph nodes, and mucosal penetration of vaccines. The ability to modulate humoral versus cellular immune responses is covered, before discussing the current state of approved nanovaccines. We conclude with a comment on the opportunities and challenges offered by nanovaccinology for the development of an effective HIV vaccine. The potential for nanotechnology to rationally improve the current state of HIV vaccinology is reflected in a substantial increase in research over the last 5 years (Figure 2).

Activating dendritic cells using nanoparticles

Critical role of DC activation in generating immunity

As the most efficient APCs, DCs play a central role in mounting effective antigen-specific immunity. These cells exhibit functional heterogeneity and both reside in and travel across human tissues, where they process pathogenic and self-antigens. When activated DCs present antigen in the context of MHC-I or MHC-II, they induce the clonal expansion of antigen-specific CD8+ or CD4+ T cells, respectively. DCs are, therefore, a crucial player in combating virally infected cells, and DC-targeted vaccines have been the focus of >100 preclinical studies \[23\].

Human DCs can be broadly divided into myeloid CD11c+ ‘conventional’ DCs (cDCs) and CD123+ ‘plasmacytoid’ DCs (pDCs) \[24,25\]. cDCs are further defined as blood (CD1c/
BDCA-1+ or CD141/BDCA-3+), dermal (CD103- or CD103+), and epidermal Langerhans cell (CD207+) subsets, while the pDC subset is present in blood, secondary lymphoid organs, and peripheral tissues. In the mouse, DCs are similarly divided into cDC and pDC subsets, with CD8α+ and CD11b+ cDCs the most likely equivalents of human CD141+ and CD1c+ subsets, respectively [26–28]. In the course of natural infection by HIV, cDC subsets and pDCs have different susceptibilities to HIV infection and possess distinct functions regarding antiviral activity (reviewed in [25]). Importantly, vaccines can be targeted to DC subsets for enhanced cellular and/or humoral immune responses (see section ‘Enhancing vaccine effectiveness by activating humoral and/or cellular immunity’). For example, by targeting antigen to appropriate cell surface receptors on the ‘cross-presenting’ CD8α+ (mouse)/CD141+ (human) subset, both cellular and humoral immune responses can be improved [29].

Adjuvant effects of nanoparticles

For optimal vaccine efficacy, the DC receiving antigen must be activated by a maturation stimulus known as an adjuvant. Historically divided into danger-associated molecular pattern (DAMP) and pathogen-associated molecular pattern (PAMP), these stimuli activate DCs and upregulate costimulatory surface molecules. In the absence of any activation signal, the result is either immune tolerance or suboptimal immunity.

Incorporating adjuvants

Nanoparticles can encapsulate adjuvants to ensure codelivery of antigen and adjuvant. By incorporating toll-like receptor (TLR) agonists within poly(lactic-co-glycolic acid) (PLGA) nanoparticles and targeting these to DC surface receptors, CTL responses were induced at 100-fold lower adjuvant dose compared with adjuvant administered in vivo. Encapsulating ssRNA into the coat protein of a plant virus (PapMV) protected the natural ligand and generated TLR7-dependent effector and memory CTL responses that provided protection in a bacterial challenge model [33]. Nanoparticles delivered to specific targets can also prevent the toxicity associated with systemic exposure to many adjuvants such as TLR agonists, which can elicit dangerous systemic cytokine production [30]. Hanson and colleagues used lipid nanoparticles coated with poly(ethylene) glycol (PEG) to encapsulate cyclic di-GMP (cdGMP) adjuvants and avert systemic toxicity [34]. More recently, Lynn et al. compared the local and systemic cytokine responses generated by unconjugated TLR2/6, TLR4, and TLR9 agonists versus those encapsulated within particulate carriers [35]. Particulate delivery of the TLR agonists enhanced DC activation and lymph node cytokine production, while alleviating systemic cytokine production and morbidity.

Nanoparticles as adjuvants

Intrinsic adjuvant properties can be engineered into nanoparticles by virtue of their component materials, and activate APCs through induction of (1) autophagy, (2) complement, and/or (3) inflammasomes [36,37]. Moreover, studies suggest that engineering increased hydrophobicity into nanomaterials can increase DC uptake and activation [38,39]. Materials science allows manipulation of nanoparticle properties such as size, shape, and surface chemistry to modify their intrinsic adjuvant properties, and hence the ensuing immune response [38–41].

**Figure 2.** Number of HIV vaccine publications using nanoparticles. We performed a search using “nanoparticle* and HIV* and vaccin*” on Web of Science (search performed on 9 Nov 2015). A substantial year-on-year increase is observed.
The properties of gold nanoparticles are easily controlled and these have been extensively studied in vivo [42]. As carriers for an HIV Env DNA vaccine, gold nanorods administered by intradermal injection demonstrated adjuvant activity by promoting DC maturation [43]. By functionalizing nanorod surfaces, endosomal escape could be promoted with polyethyleneimine (PEI) or toxicity minimized with poly(diallyldimethyl ammonium chloride). These surface modifications resulted in high transfection efficiencies and significantly enhanced cellular and humoral immunity in vivo. More recently, le Guével and colleagues investigated the effect of size on DC maturation and lymphocyte proliferation in vitro [44]. Gold nanoclusters of 2 nm were readily internalized by DC, but did not induce DC maturation or proliferation of cocultured lymphocytes. However, 12 nm nanoparticles led to DC maturation, T cell proliferation, and induction of inflammatory natural killer cells. In contrast, Tomič et al. found no human DC activation following incubation with 10 nm or 50 nm gold nanoparticles [45]. Furthermore, these nanoparticles demonstrated immunosuppressive effects, with both sizes significantly impairing lipopolysaccharide-induced DC activation and stimulation of allogeneic CD3+ T cells. Nanoparticles of 10 nm size demonstrated the greatest immunosuppression; however, both sizes were administered at the same mass concentration, resulting in a higher nanoparticle to DC ratio for the smaller particles. These conflicting results regarding the effect of nanoparticle size on immunological outcomes highlight the urgent need for the ‘bio-nano’ research field to elucidate the urgent need for the ‘bio-nano’ research field to define and adopt standards for (1) the production and quality control of nanoparticles and (2) the experimental models used to investigate their properties.

Physicochemical properties mediate DC uptake

The function of nanoparticle properties in mediating DC uptake, such as size and surface charge, has been well reviewed elsewhere [46–48]. In contrast to macrophages that favor the uptake of larger microparticles, the optimal particle size for DC uptake is below 500 nm [49], typically spanning 200 nm [50] to 300 nm [51]. The role of surface charge in mediating DC uptake is somewhat controversial. Many studies report an increased uptake of positively charged nanoparticles by DCs of human origin [49,52,53], largely owing to enhanced interaction with negative membrane potentials. In contrast, Lunov et al. reported preferential internalization of negative nanoparticles by phagocytic cells [54]. Our own data suggests a preferential temperature-dependent internalization of negatively charged 8–10 nm hyperbranched polymers by human DCs ex vivo, compared with positive and neutral polymers of the same size (Glass et al. unpublished). However, the benefits of enhanced DC uptake must be balanced against the myriad cytotoxic effects of charged, particularly cationic, nanoparticles (reviewed in [47]).

Targeting specific DC subsets

Nanoparticles can be further modified to enhance overall DC uptake. For example, by broadly targeting C-type lectin receptors on DCs, the addition of surface carbohydrates increased gold nanocluster uptake by human monocyte-derived DCs by 250% [55]. Functionally distinct DC subsets offer the ability to tailor the ensuing immune response, such as driving cellular versus humoral immunity. Traditionally, the human BDCA-3 (CD141+) and BDCA-1 (CD1b+) blood DCs are regarded as professional ‘cross-presenting’ cells [56]. However, other DC subsets demonstrate the ability to cross-present, including plasmacytoid blood DCs [57] and lymphoid organ-resident DCs [58].

Many studies have investigated Ab targeting of antigen to specific DC surface receptors. Targeting vaccines to specific receptors affords a level of control over the intracellular routing of antigen, such as via early or late endosomal compartments (elegantly reviewed in [23]). Using Ab-targeted ovalbumin (OVA) antigen, Reuter et al. recently investigated the parameters affecting MHC-I versus MHC-II antigen presentation [59]. Receptor expression level, surface turnover, and speed of antigen internalization were not major factors influencing MHC-I versus MHC-II antigen presentation. Moreover, the antigen load delivered by that receptor did not affect the efficiency of antigen presentation. Instead, the authors concluded that enhanced MHC-I presentation required processing by specialized DC subsets, such as mouse CD8+ DCs, which are believed to be the functional equivalents of human BDCA-3 DCs [28]. Importantly, even when two DC subsets express the same targeted surface receptor, they may process the antigen differently. Antigen delivered via DEC205 significantly enhanced MHC-I antigen presentation by cross-presenting mouse CD8+, but not CD8−, DCs. However, Ab-functionalized nanoparticles are internalized differently to Ab–antigen complexes [60] and our understanding of the best DC targets for nanoparticle delivery of antigen is lagging and only now being elucidated.

One potential strategy to increase antigen cross-presentation has been to target cell surface receptors, such as the C-type lectin receptor, Clec9A [61,62]. In humans, Clec9A is selectively expressed on BDCA-3 DCs and a small subset of monocytes. Monoclonal Ab (mAb) targeting Clec9A induced strong humoral responses in the absence of an adjuvant in mice and nonhuman primates [63]. Upon coadministration of an adjuvant, however, a strong CTL response was generated [62]. In the presence of adjuvants, T cell responses to HIV gag p24 were greatly enhanced in mice when p24 was targeted on α-Clec9A mAb compared with fusion to nonbinding control mAb and non-targeted p24 protein [62]. When nanovaccines were Ab-targeted to BDCA-3+ or DC-SIGN+ DCs, antigen-specific T cell responses increased [64]. The immune response was potentiated when both receptors were targeted simultaneously – a process found to rely on cytokine-mediated communication between DC subsets.

Bringing nanovaccines and DCs together

Employing chemistry to control biodistribution and pharmacokinetics

Systemic administration of nanoparticles leads to substantial clearance by mononuclear phagocytes, primarily in the liver and spleen [65]. By altering the surface composition of nanoparticles, we and others have examined strategies for evading nonspecific phagocytic clearance. The most commonly
examined polymer is the US FDA-approved PEG, which imparts hydrophilicity and reduces both protein adsorption and phagocytic clearance [66–68]. We recently examined the role of particle size, PEG molecular weight, and the presence/absence of nanoparticle template cores in evading phagocytic clearance and extending blood circulation. Higher-molecular-weight PEG reduced association with primary phagocytic cells in fresh human blood, while smaller particle diameters and removal of template cores reduced phagocytic association ex vivo and extended in vivo circulation times [68]. These strategies can be utilized to reduce nonspecific clearance, enhance biodistribution, and thereby improve vaccine efficiency.

Vaccine delivery to lymph nodes

The immune response to vaccines is coordinated in the lymph nodes, where activated DCs present vaccine antigen to naïve cognate T cells. Lymph nodes comprise resident DCs as well as migratory subsets that carry antigen from the periphery to induce an immune response [69]. Particulate delivery of vaccine antigen and adjuvant can enhance their ability to target draining lymph nodes, with a suggested ‘sweet spot’ of 20–40 nm [70]. Beyond enhancing immunogenicity, targeting adjuvant to lymph nodes also increases vaccine safety. Nanoparticle encapsulation of the adjuvant cGMP increased lymphatic uptake and reduced systemic toxicity. This led to enhanced antigen-specific CD4+ T cell expansion and germinal center formation following immunization with a liposomal HIV gp41 peptide vaccine [34].

Recently, in an elegant study by Lynn et al., TLR adjuvants were attached to polymer scaffolds of varying physicochemical properties and evaluated for immunogenicity in vivo [35]. The major factor that prolonged adjuvant activity in the draining lymph nodes was the ability of TLR polymers to form particles, which led to enhanced humoral and cellular immune responses. Using a different strategy, Liu et al. enhanced the particulate nature of a CpG-adjuvanted peptide vaccine by ‘hitchhiking’ it on endogenous albumin proteins [71]. This strategy enhanced lymph node accumulation and T cell priming while markedly reducing systemic toxicity.

Nanoparticles for enhanced mucosal immunity

HIV is predominantly transmitted sexually and enters the body through mucosal sites, such as the vagina or rectum. Therefore, induction of HIV-specific memory T or B cells at these sites is likely to afford increased protection [72]. Immunizing at mucosal sites, however, has substantial challenges and the majority of human vaccines are administered parenterally, providing strong systemic responses but weaker mucosal immunity [73]. Regarding vaginal vaccination, the antigen/adjuvant must penetrate high levels of mucosal fluids that undergo bulk flow and prevent deposition, survive the action of proteases in mucosal gels, and cross multiple layers of squamous epithelium [73]. As such, conventional subunit vaccines may induce suboptimal immunity following intravaginal administration. Mucosal vaccination strategies can be aided by nanotechnology, which can employ highly dense PEG surfaces to impart hydrophilicity and facilitate mucosal penetration [74–81]. PEG adsorption onto recombinant adenovirus expressing HIVgag (rAd-HIVgag) significantly increased gag-specific vaginal IgA and IgG, plus IFN-γ and IL-4 secreting splenic CD8+ and CD4+ T cells, following intravaginal vaccination [81].

The transport of OVA across, and within, colorectal cells has been investigated in vitro using Caco-2 human epithelial colorectal cells [82]. Immune cells within the gut mucosa are important targets for a mucosal vaccine strategy as the rectum is an important site of HIV entry and replication [83]. The encapsulation of OVA within cationic trimethylchitosan (TMC) nanoparticles improved its apparent permeability across a Caco-2 monolayer compared with free OVA and other investigated nanoparticles. When fluorescent OVA was loaded into TMC nanoparticles, OVA predominately associated with cell membranes, suggesting accessibility by APCs. In contrast, OVA was delivered within the epithelial monolayer when loaded into PLGA nanoparticles [82]. However, in untreated colon samples exposed to OVA-loaded nanoparticles ex vivo, no statistically significant difference was observed in OVA uptake.

Several novel strategies have recently been investigated to ‘pull’ recirculating T cells into the vaginal mucosa to form a long-lived sessile population of resident memory T cells and thereby enhance vaccine immunogenicity (reviewed in [84]). An increase in HIV-tropic cell types that would also be pulled into the vaginal mucosa, such as CD4+ T cells, has potential drawbacks by increasing susceptibility to infection. However, in theory, such cells might only remain at these sites during the acute phase of the immune response. Ramanathan et al. recently investigated strategies to expand vaginal DCs to enhance the uptake of vaginally administered nanoparticles [85]. In a murine model, vaginal administration of low dose GM-CSF expanded subepithelial CD11b+ DCs, but not Langerhans (CD11b−) cells or macrophages. The phagocytic capacity of mucosal DCs was not affected by GM-CSF treatment, but instead increased the total number of nanoparticle-processing DCs in the vaginal mucosa. Hence, GM-CSF enhanced overall nanoparticle uptake at the vaginal mucosa without pulling HIV-tropic immune cells. While most DCs are poorly infected by HIV, CD1c+ cDCs have been reported to support productive infection of HIV [86]. HIV subverts immune signaling pathways to replicate within DCs [87] and because DCs can efficiently transfuse CD4+ T cells [88], the benefits of DC expansion strategies must be weighed against any potential increase in infectivity.

Enhancing vaccine effectiveness by activating humoral and/or cellular immunity

Enhancing combined cellular and humoral immune responses

Many of the strategies already explored above lead to enhanced cellular and humoral immune responses. While we have discussed the role of nanoencapsulating adjuvants, Zhang et al. explored the impact of antigen encapsulation within PLGA nanoparticles [89]. Using the model antigen OVA, they found the combined formulation of encapsulated OVA plus free OVA mixed with empty nanoparticles enhanced both cellular and humoral immune responses compared with encapsulated, mixed, or free OVA alone. The combined
formulation was hypothesized to enhance immunogenicity through an antigen-depot effect at the administration site, providing antigen persistence following the initial antigen exposure.

Liard et al. demonstrated the importance of immunization route in determining the immune response to a nanovaccine [90]. Using HIV-p24 PLA nanoparticles, subcutaneous immunization generated a solely humoral response, transcutaneous administration induced CD8 effector cells in the absence of any IgG, and intradermal vaccination generated a mixed cellular and humoral immune response.

**Enhancing humoral immune responses**

The only HIV antigen amenable to induction of neutralizing Abs is the envelope (Env) glycoprotein, also known as the viral spike. Env comprises a complex trimer of gp120 and gp41. However, instability and molecular heterogeneity have made native trimers suboptimal vaccine immunogens. After decades of research, we do not have a vaccine able to induce bNAbs and generate protective immunity [11,91]. Recent advances have signaled the use of improved native-like trimer immunogens which offer renewed hope for the generation of bNAbs [92,93]. While such trimers are yet to reliably induce bNAbs, they represent new opportunities for nanovaccinology. Moreover, in light of RV144 [21], the induction of functional nonneutralizing Abs may represent another goal of the humoral response against HIV.

Each HIV virion displays an average of just 14 Env spikes, which is believed to hinder neutralization by preventing bivalent binding of IgG [94] and prevent a broad-spectrum Ab response [95]. Nanoparticles can be used for high-copy display of Env immunogens to enhance the humoral response. When anchored to lipid nanocapsules, Env trimers generated broader and significantly enhanced humoral immune responses compared to vaccinating with soluble trimers in a strong oil-in-water adjuvant [96]. Repetitive antigen display of the membrane-proximal external region (MPER) of Env gp41, a site recognized by many bNAbs [97], on a self-assembling protein nanoparticle induced high MPER-specific Ab titers in the absence of any adjuvant [98]. However, neutralizing capabilities were absent in these Abs. More recently, a liposomal vaccine was investigated for the induction of neutralizing Abs against MPER [99]. Humoral responses were maximized by high-density presentation of MPER on liposomes through a presumed increase in B-cell receptor (BCR) clustering. In another study, Leaman et al. stabilized native virion spikes following cross-linking with bis(sulfosuccinimidyl) suberate [100]. When arrayed on proteoliposome nanoparticles, cross-linked Env spikes generated neutralizing Abs against more resistant tier 1b and tier 2 isolates; however, they did so at low Ab titers and at the cost of tier 1a neutralization.

Analyzing the humoral responses from four recent HIV vaccine clinical trials, Chung et al. [101] applied ‘systems serology’ principles to demonstrate the influence of immunogen type in directing distinct humoral profiles. These included various functional nonneutralizing Ab types. Therefore, rational immunogen design might be able to direct nonneutralizing Ab activities, such as Ab-dependent cellular cytotoxicity and Ab-dependent cellular phagocytosis, which are emerging as important in the protection from and control of SIV/HIV [102–104] and influenza [105,106]. Recently, hemagglutinin-stem nanoparticles generated functional nonneutralizing Abs against immunologically subdominant stem regions, completely protecting mice and partially protecting ferrets from lethal challenge with heterosubtypic influenza virus [107]. However, the use of nanoparticles to constrain subdominant/cryptic HIV epitopes and elicit functional nonneutralizing Abs has not been reported and warrants future investigation. The propensity for studies to evaluate humoral responses based solely on their neutralization activities [98] excludes other functions associated with Ab. This ‘neutralization dogma’ must be abandoned before we can gain maximum benefit from nanovaccinology.

To minimize off-target Ab responses, nanoparticles can shield T-helper epitopes from BCR engagement. When CD4 + T-helper peptides (such as ‘HIV30’) were restricted to the interior of liposomes, strong Ab responses to MPER were achieved while Ab responses against the helper sequences were minimized [99].

**Enhancing cellular immune responses and cross-presentation**

It is widely believed that a highly effective HIV vaccine will also require induction of cellular immunity to kill virally infected cells. Nanoparticles can be used to induce a Th1 immune response, whereby T-helper cells secrete a collection of proinflammatory cytokines, such as IFN-γ, that drive cellular immunity [31,90,99,108].

Before exogenous antigen can be cross-presented to cognate CD8+ T cells via MHC-I surface proteins, it must be rerouted into the cytoplasmic antigen-processing pathway [56]. Nanotechnology offers various strategies to enhance cross-presentation, including the following examples. To enhance cytosolic delivery of the model antigen OVA, Keller and colleagues employed pH-responsive polymers to form a nanoparticle core, with OVA reversibly attached via disulfide bonds [109]. This strategy increased cytosolic retention and accumulation of OVA in vitro, while subcutaneous administration significantly increased antigen-specific CD8+ T cell responses over soluble antigen, unconjugated physical mixtures of nanoparticle and OVA, and non-pH-responsive control nanoparticles. Polycations, such as PEI, can be engineered within nanoparticles to enhance endolysosomal escape [43,110–112]. By releasing antigen into the cytoplasm, cross-presentation onto MHC-I is increased. However, such molecules are frequently cytotoxic, limiting their therapeutic use. Lastly, cell-penetrating peptides can be functionalized on nanoparticles to facilitate fusion with endosomal membranes [113–115]. These include the highly cationic HIV-derived Tat protein, which can aid enhanced CD8+ T cell immune responses [116].

**Regulatory state of nanomedicines for HIV**

The safety, efficacy, and utility of nanomedicines are evidenced by phase I–IV clinical trials investigating a variety of
disease states and the growing list of FDA-approved products, particularly for the treatment of cancers [1,117,118].

Nanoformulations for improved bioavailability of anti-HIV drug therapies have been thoroughly investigated and have recently entered clinical trials [119]. These include ongoing trials of nanoformulations of the nonnucleoside reverse transcriptase inhibitors MK-1439 [120] and TMC278-LA [121], and the HIV integrase strand transfer inhibitor GSK1265744 [122].

The safety, tolerability, and early immunogenicity of a therapeutic plasmid DNA nanoparticle vaccine for HIV termed ‘DermaVir’ was revealed in a phase I trial [123]. The PEI mannose nanoparticles are applied topically with a DermaPrep medical device to target Langerhans cells that then migrate to lymph nodes. No serious adverse events were reported (0/9 participants) and HIV-specific precursor/memory T cell responses were induced, enabling DermaVir to progress to phase II studies [124]. However, the relative lack of prophylactic or therapeutic HIV nanovaccines entering clinical trials highlights the slow progress in this field.

Expert commentary

The field of nanovaccinology is maturing. Great advances have been made in our understanding of how nanoparticle properties affect their interaction with the immune system. This has informed how materials science can be used to manipulate the immune system, activate APCs, and drive improved immune responses against viral pathogens. Recently, a ferritin-based nanovaccine provided protective immunity against divergent influenza strains by generating nonneutralizing Abs against subdominant hemagglutinin ‘stem’ epitopes [107]. This signaled the possibility of a universal influenza vaccine that could be used to protect against many influenza strains and highlights the potential of this field. Our developing knowledge of correlates of protection from the RV144 trial highlights what an effective HIV vaccine may require and nanotechnology gives us the tools to custom design vaccines that enhance desired humoral and cellular immune responses.

HIV cure strategies are being actively sought to overcome the requirement of lifelong adherence to CART and its inability to clear latently infected cells [125]. The ‘shock-and-kill’ approach is one such strategy, where pharmacological agents are used to induce HIV expression by latently infected cells, thus making them available for clearance by the immune system [126,127]. Therapeutic vaccination can be combined with this latency reactivating approach to ensure that sufficient CTL activity is available to eradicate infected cells. By enhancing the immune response to vaccines, nanotechnology provides new opportunities that should be examined in the context of shock-and-kill strategies.

It must be acknowledged that the current prognosis of HIV-infected patients with access to therapy is very good. In the context of a therapeutic vaccine, these patients have very different cost–benefit scenarios to those with less favorable outlooks, such as terminally ill cancer patients. Nanovaccines can enhance the safety profile of vaccine adjuvants. However, the toxicity of complex, novel materials must be thoroughly evaluated to minimize immediate and long-term side effects. Reporting safety in small animal models is a logical starting point. However, better characterization of acute and long-term nanotoxicology in larger animals is required before we can confidently examine the immunogenic properties of novel materials in humans.

Nanovaccinology offers myriad opportunities in the hunt for an effective HIV vaccine. Nonetheless, challenges do face the manufacture and regulatory approval of any nanovaccine. These include (1) costs of production, which for complex formulations can be substantial and might quickly make an effective vaccine unfeasible; (2) batch-to-batch variation, which must be eliminated; and (3) the potential need to dramatically alter manufacturing processes when expanding production from laboratory to industrial scales. These issues must be solved before any nanovaccine can enter advanced clinical trials. From a regulatory perspective, the increasingly complex pipeline of novel nanomedicines poses challenges to regulatory approval agencies that must update regulations and policies as appropriate [2,128].

Five-year view

The next 5 years will see an increase in basic science focused on understanding fundamental bio-nano interactions, particularly, how physicochemical properties affect biological outcomes. These investigations will benefit from increasingly cross-disciplinary research teams and collaborations that merge the skills of materials science, biology, immunology, and bioinformatics, among others. Improving our knowledge of basic bio-nano science will allow increased rational design of de novo nanoparticle vaccines.

The substantial number of commercially available nanomedicines (see Table 2 in [2]) demonstrates the ability to overcome the manufacturing and regulatory hurdles listed above. As regulatory agencies keep pace with technological advances and our understanding of nanotoxicology improves, we expect safer versions of nanovaccines to begin entering phase I/II trials.

Traditional vaccine strategies have not yielded a successful HIV vaccine despite three decades of effort. Nanotechnology offers an unparalleled ability to modulate the human immune system and generate targeted, controlled immune responses. At present, the improved anti-HIV arsenal provided by nanotechnology is balanced by the complexities associated with producing novel and complex materials that are reproducible, safe, and able to enter clinical testing. With time, nanovaccinology may play a significant role in the development of an effective HIV vaccine.

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Nanoparticles can encapsulate adjuvants or display intrinsic adjuvant properties to ensure codelivery with antigen. The systemic toxicity of adjuvants can be markedly reduced following formation of, or encapsulation within, nanoparticles. Nanovaccines can be targeted to DCs by altering physicochemical properties such as size and charge, by modifying their surface with natural ligands such as lectins, or by functionalizing with more specific targeting moieties such as Abs. Biodistribution can be improved by controlling nanoparticle chemistry. For example, PEGylation can reduce phagocytic clearance and substantially enhance blood circulation times. Nanoparticles can increase vaccine delivery to lymph nodes and enhance immunogenicity. Hydrophilic nanovaccines display enhanced mucosal penetration and may facilitate improved mucosal vaccination strategies. Nanotechnology offers various approaches to enhance cellular and/or humoral immune responses. The improved anti-HIV arsenal afforded by nanovaccinology is balanced by the complexities associated with producing novel and complex materials that are reproducible, safe, and able to enter clinical testing.

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Papers of special note have been highlighted as:
• of interest
  • of considerable interest
Nanovaccines were engineered to bind endogenous albumin, and delivered antigen load were not major factors driving MHC-I versus MHC-II antigen presentation.

The cellular immune response to a nanovaccine was increased by targeting multiple DC subsets in a process that involves cytokine-mediated DC communication.

• By encapsulating T-helper epitopes, strong Ab responses were focused on the membrane proximal external region of gp41, while competing Ab responses against helper epitopes were reduced.


• Nanoparticles were used to display the immunogenically subdominant stem region of influenza hemagglutinin, which generated nonneutralizing Abs capable of protecting animals from heterosubtypic influenza infection.


