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Advances in dendritic cell immunotherapies for HIV-1 infection

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Abstract

Augmentation of adaptive immunity via HIV therapeutic vaccination may be a key component of curative strategies. Adoptive dendritic cell (DC) immunotherapies may prove useful in enhancing the success of these approaches by circumventing certain defects in DC function during HIV infection. Thus far, DC immunotherapies that utilize autologous, inactivated virus as an immunogen have provided the most promising results however, are beset with practical constraints. Consequently, alternative forms of immunogens are under investigation, with an emphasis on RNA-based approaches. Here we review the data from DC immunotherapy trials for HIV infection and discuss challenges and future directions in the field.

Keywords

dendritic cell; human immunodeficiency virus; immunotherapy; therapeutic vaccine

1. Introduction

Immunotherapies that successfully expand and enhance HIV-specific adaptive immunity may play an important role in approaches to reduce cellular viral reservoirs that pose the major obstacle to HIV cure. One goal of HIV immunotherapies, also termed therapeutic vaccines, is to augment the cytotoxic capacity of antiviral CD4 and CD8 T cells in antiretroviral therapy (ART) suppressed individuals to more closely resemble those observed in long-term nonprogressors and elite controllers that have been found to be more effective at killing infected cells. As orchestrators of the complex interplay between the innate and adaptive arms of immunity, myeloid dendritic cells (DCs) represent a key target of therapeutic vaccine strategies. DCs have the ability to sense a wide range of pathogens and vaccine adjuvants that results in their enhanced antigen presentation capacity allowing them to induce and modulate T-cell responses. However, DC dysfunction during HIV infection poses a major challenge to the development of successful therapeutic vaccines. DCs from HIV-infected individuals are impaired in their ability to respond to a variety of pathogen-

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associated stimuli, including HIV itself, which limits their ability to generate optimal adaptive responses [1]. Treatment with ART has been shown to ameliorate dysfunctional DC responses to some extent, though DC cytokine secretion in response to certain stimuli is only partially rescued in ART-treated individuals [2]. Therefore, in order for therapeutic vaccines to be effective, they will likely require the incorporation of means to either rescue or circumvent DC dysfunction during HIV infection. One way to potentially bypass defects in DC function is by *ex vivo* manipulation followed by reintroduction of these modified cells to the patient as a form of adoptive immunotherapy. These DC vaccines are generated by leukapheresis of the patient following which large numbers of DCs can be derived *in vitro* from patients' monocytes in the presence of growth factors and cytokines such as GM-CSF and IL-4. The DCs are then loaded with HIV antigens *in vitro* and potentially activated with a maturation stimulus prior to injection back into the patient to enhance their antigen presentation and immunomodulatory function. These monocyte-derived DCs from HIV-infected individuals have been found to be competent antigen presenting cell (APC) with the ability to stimulate strong proliferative and cytotoxic T lymphocyte (CTL) responses [3]. However, some DC defects, such as cytokine secretion, may persist depending on the *in vitro* maturation stimulus used that must be a consideration when devising DC preparations [2,4,5].

Thus far, greater than 10 DC immunotherapy trials have been undertaken for HIV with multiple studies ongoing (summarized by Vanham and Van Gulck; Table 2) [6]. The majority of these trials have employed one of two general approaches to load antigen onto DCs: i) pulsing DCs with autologous inactivated virus and ii) introduction of HIV RNA into DCs via electroporation [7-15]. We will focus our discussion on these approaches, though it should be noted that additional methods have included using HIV peptides to load DCs and infection with recombinant viral vectors [1]. Each of these studies has demonstrated safety, and all have resulted in some degree of enhancement of HIV-specific adaptive immunity. However, only a subset of these trials has evaluated viral control as an outcome, and fewer have accomplished this goal by any measure.

2. Autologous, inactivated virus approaches

Notwithstanding significant limitations in comparing results from DC immunotherapy trials discussed below, it has been observed that those utilizing whole, inactivated, autologous virus as an immunogen are among the most successful to date in terms of viral control [9,10,12,16]. HIV can be inactivated for use in clinical settings in multiple ways, including heat inactivation as well as chemical inactivation with aldrithiol-2 (AT-2, 2,2'-dithiodipyridine) or psoralen/UVB irradiation [9,10,13,17]. Chemical inactivation with AT-2 has the advantage of leaving envelope glycoproteins unaltered so they may bind and fuse with target cells normally, but results in covalent modifications in internal nucleocapsid viral proteins to render the virus nonreplicative [17]. AT-2-inactivated SIV-loaded DCs were first evaluated in the nonhuman primate model where immunization of untreated, chronically infected rhesus macaques sustained an impressive > 3 log decrease in viral load compared with animals who received DC alone [16]. When this formulation was studied in humans with untreated HIV infection, again substantial reductions in viral load were observed for greater than 1 year in > 40% of patients following vaccination [12]. Median reduction of

viral load was approximately 80% and correlated with gag-specific, cytotoxic CD8⁺ T cells and HIV-specific CD4⁺ T cells. Multiple other studies have utilized heat-inactivated autologous HIV-pulsed DCs in both treated and untreated individuals and achieved moderate improvements in viral control [9,10]. The most recent of these studies was a blinded, placebo-controlled trial performed in ART-suppressed individuals followed by structured treatment interruption (STI) [10]. Participants who received DC pulsed with heat-inactivated HIV (HIV-DC) sustained decreases in viral load set point of > 1 log more frequently than subjects who received unpulsed DCs postvaccination. Decreases in viral load in the HIV-DC arm were consistent with an increase in HIV-specific T-cell responses via ELISPOT assays. The success of these studies has provided valuable proof of principle that *ex vivo* enhancement of DC function can improve viral control. However, there are many practical limitations involved in autologous viral preparations including *ex vivo* propagation of sufficient virus and rigorous quality assurances to confirm viral inactivation. Therefore, approaches that employ other forms of HIV immunogens may be more feasible for larger-scale applications. Thus far, these alternative approaches have not shown equivalent promise, though investigations remain underway.

3. RNA-based approaches

Recently, several trials have introduced HIV RNA into autologous DCs via electroporation as a means for antigen loading [7,8,11,14,15]. Two trials were carried out using nonautologous sequences of HIV mRNA and were found to be immunogenic [7,15]. Van Gulck *et al.* vaccinated six ART-suppressed individuals with DCs electroporated with mRNA encoding Gag and a chimeric Tat-Rev-Nef and observed increases in HIV-specific immune responses via IFN ELISPOT, and some increase in CD8⁺ T cell polyfunctionality in 50% of donors [15]. A similar formulation was administered to 17 ART-treated participants by Allard *et al.*, which consisted of DC electroporated with HIV mRNA encoding Tat, Rev and Nef [7]. This was also found to be immunogenic via ELISPOT responses; however, a subsequent STI revealed no change in time to viral rebound or viral load compared with historical controls. Under current investigation is a unique, personalized platform that incorporates RNA for Gag, Vpr, Nef and Rev amplified from each donors pre-ART plasma, in addition to RNA encoding an immunostimulatory protein, CD40L, to enhance DC function (AGS-004) [8,11,14]. A Phase I study to evaluate safety and immunogenicity in 10 ART-suppressed individuals revealed an increase in HIV-specific CD8⁺ T-cell proliferative immune responses following vaccination [14], and Phase II studies are currently ongoing. Early reports from a Phase II study of AGS-004 reveal that in patients who underwent STI there was a subset who displayed delayed viral rebound that corresponded with an increase in proliferative responses to HIV antigens and the presence of activated CD4 and CD8 T cell that were low in PD-1, a marker for T-cell exhaustion [8]. In contrast, donors who experienced more rapid viral rebound had effector T cells with high levels of PD-1 expression. More information will be obtained from an ongoing Phase II, double-blinded, placebo-controlled study of AGS-004 in ART-treated patients undergoing STI. Notably, an open-label, single-arm substudy of this larger Phase II study in six acutely infected individuals was preliminarily evaluated and found to enhance CTLs in all patients thus far [11]. Nevertheless, following STI, vaccination did not allow for sustained ART interruption

in this population. The efficacy of this personalized formulation on viral control in chronically infected, ART-treated individuals following STI remains to be elucidated.

4. Expert opinion

There are many challenges in attempting to tease out which variables have determined the greater success of certain DC vaccine trials. There have been no randomized studies to compare various DC formulations and many studies have been uncontrolled; therefore, it is difficult to definitively determine which HIV immunogen(s) and *in vitro* DC maturation stimuli are most effective. Immunologic readouts vary significantly among studies, and it remains unclear which immune responses induced following vaccination are the best measures of viral control. Data from natural HIV controllers have underscored the association of certain CTL functions including measures of cytotoxicity and viral inhibition, as well as proliferation and polyfunctionality as potentially important immunological end points (reviewed by Walker and Yu) [18]. Additional differences in trial design including vaccination schedule, number of DCs injected per dose and patient population (untreated vs ART-suppressed individuals) add further to difficulties in comparing results across studies. Therefore, deciphering which factors underlie the seeming advantage of whole virus approaches versus alternative forms of HIV immunogens, including RNA-based approaches, calls for some speculation, while highlighting the need for well-designed comparative trials. It stands to reason that autologous virus sequences may be preferable by resulting in presentation of personalized epitopes; however, this is also achieved with individually tailored RNA-based immunogens such as AGS-004 [14]. Additional virologic data from Phase II trials of AGS-004 will be helpful in establishing whether this autologous RNA-based approach confers benefits over previous mRNA approaches utilizing consensus sequences. In the future, the use of mosaic HIV immunogens that have been bioinformatically optimized for improved coverage of HIV-1 diversity and shown promise in preclinical studies may be a cost-effective alternative to autologous immunogens (reviewed by Hanke *et al.*) [19].

In addition to the autologous nature of presented epitopes, there may be both qualitative and quantitative differences in the HIV-specific T cells formed. Despite the notable difficulties in comparing the diverse immunological end points among trials, an apparent distinction and possible benefit of whole virus vaccines is the strong stimulation of HIV-specific CD4 T-cell responses compared with RNA-based approaches [7,12,14,20]. Although the major focus in therapeutic HIV vaccine design has been placed on eliciting HIV-1 specific CD8⁺ cytotoxic T-cell responses, the generation of high-quality anti-HIV CD4⁺ T cells may be critical due to direct effects on viral control and through indirect effects on generation of lasting CD8⁺ T-cell responses [21-23]. HIV controllers have been found to possess polyfunctional Th1 effector CD4 cells that produce IFN- γ and express cytotoxic mediators (perforin/cd107a), suggesting that immunotherapies that induce strong secretion of pro-Th1 cytokines are favorable [24]. Other immune correlates that may be important to consider but have been underevaluated in existing trials include various innate immune cell functions, antibody-dependent cell-mediated cytotoxicity and changes in T regulatory and Th17 cell balance. In patients who undergo vaccination while on suppressive ART, assessment of viral reservoirs

is an important end point to measure as it may correlate with subsequent viral control and has implications for cure research [25].

Ultimately, given the practical limitations of manufacturing DC immunotherapies for large-scale use, interventions that directly target DC *in situ* to simultaneously activate and deliver antigen will be more feasible in the future. This may be achieved with a variety of strategies currently under investigation, including vector-based approaches, protein antigens coupled to antibodies against DC endocytic receptors, nanotechnology and coformulations of antigens with DC adjuvants [1]. Eventually, these therapies may serve as a component of prime boost strategies that may be necessary to achieve optimal adaptive and humoral responses. The ongoing works in the field of adoptive DC immunotherapies represent important proof-of-principle endeavors to establish and guide research in this area with the eventual goal of lowering barriers to HIV cure.

Declaration of interest

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