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Novel vaccine approaches for protection against intracellular pathogens

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Vaccination against intracellular pathogens requires generation of a pool of memory T cells able to respond upon infection and mediate either killing of the infected cell or induce killing mechanisms in the infected cell. T cell-inducing vaccines must aim to target the antigen to antigen-presenting cells (APCs) so that it can be presented on MHC molecules on the cell surface. Methods to do this include making use of vectors such as plasmid DNA or viruses, live attenuated pathogens or subunit vaccines targeted and enhanced using adjuvants. The choice of approach should be guided by the phenotype and localization of the desired T cell response. This review will discuss current approaches in the pipeline for the development of T cell-inducing vaccines, including vectored, live attenuated, and subunit vaccines.

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Introduction

Diseases for which vaccination has been successful are caused by pathogens that are either extracellular, spend a significant part of their lifecycle outside the cell, or whose disease is mediated through toxins. Vaccination against intracellular pathogens, however, including those causing diseases such as tuberculosis (TB), tularemia, chlamydia and leishmaniasis, has proven more difficult [1–4]. Given their intracellular nature, immunity against these pathogens is primarily T cell-mediated, a fact that is well-established. The role of B cells in many of these infections is still debated, however most studies demonstrate that while B cells may contribute to protection, B cell immunity is not central to pathogen control [5,6]. Thus, in the context of vaccine-induced immunity, it is becoming apparent that the phenotype and localization of antigen-specific T cells is essential to vaccine efficacy. For example, there is substantial new evidence supporting a role for T helper-17 (Th17) cells in vaccine-mediated immunity against TB [7•,8,9••]. However, given the propensity for high levels of interleukin (IL)-17 to induce inflammation [10,11••], development of such a regime for use in humans needs to be carefully validated. Thus, one of the major challenges faced in the development of T cell-inducing vaccines is the generation of a persistent pool of appropriate memory T cells localized at the correct anatomical site for optimal pathogen clearance via a safe delivery system. This review will discuss current approaches in the pipeline for the development of T cell-inducing vaccines, including vectored, live attenuated, and subunit vaccines.

Vectored vaccines

Vectored vaccines make use of DNA-based constructs in the form of viruses, plasmids or bacteria to express antigenic genes from the pathogen of interest, for antigen presentation in the host. Furthermore, cell death caused by vector infection promotes antigen presentation through uptake of dead cells by antigen-presenting cells (APCs). Vectors in the form of viruses or bacteria are self-adjuvanting, enhancing antigen presentation by engaging pattern-recognition receptors (PRRs). The most common viral vectors in clinical trials are attenuated adenoviruses and Modified Vaccinia Virus Ankara (MVA). Adenoviruses are able to replicate in human cells, leading to prolonged antigen expression and enhanced exposure of T cells to APCs [12]. Adenoviruses signal through the intracellular CpG-sensing TLR9, inducing both cellular and humoral responses [13,14]. However, one drawback to the use of adenoviruses is human is that pre-exposure to the viruses results in an adenovirus-specific memory response (anti-vector immunity), leading to early viral clearance, loss of prolonged gene expression and lower immunogenicity [15]. In an attempt to overcome anti-vector immunity, novel vectors using chimpanzee-specific adenoviruses are in development, which exhibit low pre-existing anti-vector immunity in humans [16,17]. Importantly, several adenovirus-vectored vaccines are in clinical development. In two separate trials, human Adenoviruses 35 and 5 expressing the TB Antigen 85A (Ag85A) have reached Phase II trials in South Africa (ClinicalTrials.gov identifiers NCT01017536 and NCT01198366) and phase I trials in Canada (NCT00800670), respectively. These vaccines aim to boost BCG immunization and enhance the cytokine Interferon (IFN)-γ in both $CD4^+$ and $CD8^+$ T cells [18•,19•]. In addition, both vaccines induce polyfunctional $CD4^+$ and $CD8^+$ T cells producing T helper-1 (Th1) cytokines such as IL-2, Tumour Necrosis Factor-alpha (TNF-α) and IFN-γ. Results from the

Adenovirus 35 trial showed induction of IL-17-producing cells in the peripheral blood mononuclear cells from vaccinees [18•]. Whilst the role of polyfunctional T cells in vaccine-induced protection is still unclear, evidence suggests polyfunctionality is a beneficial feature of T cell immunity, perhaps in terms of broadening the range of effector functions of responding cells.

MVA is a non-replicating virus that infects several cell-types, and is a potent inducer of $CD4^+$ T cells [20]. MVA is recognized by a range of both surface and intracellular PRRs, including TLRs 2 and 6, and the NLRP3 inflammasome [21]. Ag85A expressed by MVA was the first TB vaccine to advance to a Phase IIb efficacy trial, and although results were disappointing in terms of efficacy, the vaccine induced strong IFN-γ responses in vaccinees [22••], highlighting the potential for MVA as a vector. Similar to the Adenovirus 35 vaccine, as well as being a potent IFN-γ-inducing vaccine, MVA85A also induces both polyfunctional T cells and Th17 cells in the blood [23–25]. The failure of this vaccine to induce protection over BCG, however, may suggest that the levels of IL-17 induced in the periphery did not translate to levels sufficient to mediate protection in the lungs. Similar to viral vectors, attenuated *Listeria monocytogenes* has been used as a vector due to its potent $CD4^+$ and $CD8^+$ T cell-inducing capabilities [26]. Attenuated *L. monocytogenes* expressing the *Francisella tularensis* antigen IgIC administered to mice who previously received attenuated *F. tularensis* Live Vaccine Strain (LVS), induces IFN-γ, IL-2 and TNF-α in $CD4^+$ T cells, and IFN-γ in $CD8^+$ T cells [27•]. These studies together suggest that induction of optimal polyfunctional T cell responses is crucial for effective vaccine design against intracellular pathogens.

DNA vaccines in the form of plasmids expressing pathogen-derived genes have been in development for a number of years for several diseases, including malaria and influenza [28,29]. A factor that has stunted their progress through clinical trials, however, is low immunogenicity. A recent approach to overcome this is the use of electroporation, thus increasing passage of the vaccine into the cell, at the same time increasing APC recruitment to the site of vaccination [30]. Currently, no DNA vaccines with electroporation against intracellular pathogens have reached clinical trials, however the approach has been employed in both HIV and influenza vaccines in mice, with results showing increased immunogenicity following electroporation [31,32].

Thus, vectored vaccines represent an efficient method of targeting an immune response to antigens of interest. It is important to note that most of the immune responses documented in the past have considered induction of T cells that produce IFN-γ, IL-2 and TNF-α. However, since IL-17 has recently been implicated in protection against several intracellular pathogens [33], perhaps attenuated strains of IL-17-inducing pathogens, such as *Bordetella pertussis* and *Salmonella* spp., may also be considered as vectors in the future.

Live attenuated vaccines

The advantages of using live attenuated vaccines are the self-adjuvanting effect of the attenuated pathogen and expression of most of the pathogen genome, providing a wide range of antigenic targets. Several live attenuated vaccines against intracellular pathogens are in development. With the aim of developing an alternative to BCG, three attenuated forms of mycobacterial species have shown efficacy in pre-clinical studies. In order to improve the immunogenicity of BCG through inducing $CD8^+$ T cells, BCGΔureChly$^+$ was engineered to express the membrane-lysing agent listeriolysin from *L. monocytogenes*. Urease C was also deleted from the genome, allowing acidification of the phagolysosome for optimal conditions for listeriolysin activity. This allows BCG escape from the phagolysosome, leading to increased antigen presentation. In a mouse model of TB, BCGΔureChly$^+$ confers improved protection over wild-type BCG [34,35]. This is associated with increased production of IFN-γ and IL-17 as well as polyfunctional IL-2$^+$TNF-α$^+$ $CD4^+$ T in the lungs, and increased IL-17 in the spleen compared to BCG immunization. IKEPLUS is an attenuated form of *Mycobacterium smegmatis* in which the endogenous *esx-3* secretion system has been replaced by the *Mtb* equivalent [36•], and when used as a vaccine against *Mtb* challenge in the mouse model, it is both immunogenic and protective [36•]. In adoptive transfer experiments, $CD4^+$ rather than $CD8^+$ T cells were found to confer protection, with IKEPLUS inducing higher numbers of cells producing IFN-γ, IL-2 and TNF-α than BCG [36•]. Finally, SO2 is an attenuated *Mtb* lacking PhoP, which forms part of the two-component system PhoP/PhoR essential to virulence [37]. When used as a vaccine in both mouse and guinea pig models of TB, SO2 induces increased total $CD4^+$ and $CD8^+$ T cell numbers in the lymph nodes, as well as increased IFN-γ production [38,39]. In a challenge model, SO2 confers improved protection over BCG, suggesting that use of a vaccine strain that is genetically similar to the challenge strain and more virulent than BCG may be beneficial to inducing protective responses.

That protection against tularemia requires IL-17 is highlighted in two separate studies using attenuated forms of the virulent SchuS4 *F. tularensis* strain. These were administered either intradermally only [40], or intradermally or intranasally [41•] as live attenuated vaccines. The attenuated SchuS4 strains induced improved protection over LVS and was associated with increased IL-17 production in the lungs of challenged mice [40], however intranasal delivery did not improve on intranasal challenge [41•]. This is in contrast to results from *Mtb* vaccine studies in which intranasal delivery confers improved