Published in final edited form as:

Curr Opin Immunol. 2014 June ; 0: 58–63. doi:10.1016/j.coi.2014.02.003.

Novel vaccine approaches for protection against intracellular pathogens

Kristin L Griffiths and Shabaana A Khader*

Department of Molecular Microbiology, Campus Box 8230, 660 South Euclid Avenue, St. Louis, MO 63110-1093.

Abstract

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 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.

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 disease 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 [7–9]. 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

© 2014 Elsevier Ltd. All rights reserved.

*Correspondence to Shabaana Khader, PhD, Phone: (314) 286-1590 Fax: (314) 362-1232, khader@wustl.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

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 are in the form of a 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 in humans 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- α (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 & 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, BCG ureChly⁺ 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, BCG ureChly⁺ 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 *M.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, which 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 protection over parenteral delivery [7,8], suggesting that the site of T cell induction for optimal responses may differ between pathogens. Provided there is no risk of reversion, thus, live attenuated vaccines represent a valid approach for T cell-inducing vaccines given the potential for induction of an immune response to a broad range of antigens.

Subunit vaccines

A final approach to vaccination against intracellular pathogens is subunit vaccines, composed of peptides, proteins or non-protein components of the pathogen. In order for subunit vaccines to be successful, they must be targeted to the correct APC to ensure efficient uptake and presentation. Cells such as dendritic cells (DC) can be targeted through liposomes, which surround the antigen, escorting its delivery inside the cell. The outcome of the immune response to the subunit alone depends on the inherent immunogenicity of the subunit itself, however the immunogenicity of the vaccine can be improved by co-administration with an adjuvant. Currently, the only two adjuvants licensed for use in humans are alum and monophospholipid-A (MPL), although several are in development; alum is known to be beneficial for antibody-inducing vaccines and stimulates a Th2-skewed response, whereas MPL induces a Th1 bias. It should be noted that the choice of adjuvant may adversely affect the desired outcome; indeed, the alum included in several Expanded Programme on Immunisation (EPI) schedule vaccines has been shown to reduce the immunogenicity of MVA85A when MVA85A was delivered concurrently with the EPI vaccines [42].

Most novel vaccines in development against leishmaniasis are protein subunit vaccines targeting various antigens. Kaur *et al.* demonstrate protective efficacy in the mouse model of *L.donovani* infection of a subunit vaccine comprising gp63 with hsp70 as the adjuvant, enhancing IFN- γ and reducing IL-4 and IL-10 levels [43]. Similarly, recent studies using gp63 delivered in cationic liposomes with the adjuvant combination MPL and trehalose dimycolate showed protective efficacy and IFN- γ induction in the mouse model following challenge with *L.donovani* [44]. Results from these experiments highlight the adjuvanting effect of targeting APCs with liposomes. Another leishmania vaccine administered with MPL includes the polypeptide LEISH-F1, which is composed of three conserved leishmanial proteins: LeIF, TSA and LmSTI1. LEISH-F1 has been shown to be safe and immunogenic

in humans, inducing IFN- γ responses above background in naïve individuals, those with a history of *Leishmania* exposure, and infected individuals undergoing chemotherapy [45–48].

Subunit vaccines against tularemia are also in development. Here, the heat shock protein DnaK was combined with the *F.tularensis* surface protein Tul4 and administered to mice intranasally along with the adjuvant GPI [47]. The vaccine was shown to induce antigen-specific IFN- γ and IL-17 responses, as well as antibodies, and afforded 80% efficacy following challenge with a lethal dose of LVS, and reduced dissemination to liver and spleen. Another approach used to adjuvant subunit vaccines is the use of proteosomes – vesicles formed through protein-protein interactions that have been shown to be immunogenic in a similar manner to liposomes. Such an approach has been used for vaccination against *Chlamydia spp* using the major outer membrane protein (MOMP). In mice, MOMP formulated as a proteosome and delivered intramuscularly and subcutaneously, induces both antibody and IFN- γ production, and protection following challenge with *C.muridarum* [50].

Finally, subunit vaccines can also be used as a boost for vectored or live vaccines in heterologous prime-boost strategies. Such approaches have the advantage of inducing an immunogenic response at the time of primary vaccination, whilst overcoming the problem of anti-vector immunity in the boost. Indeed, the subunit TB vaccine H56, composed of three proteins expressed during both active and latent *Mtb* infection has shown both efficacy and immunogenicity in mice and non-human primate (NHP) disease models when used as a boost for BCG delivered along with the novel adjuvant IC31 (NHP) or CAF01 (mice) [51,52].

Concluding remarks

From the literature reviewed here, it seems clear that the overriding hurdle is the ability to induce a protective T cell phenotype that localizes to the correct organ and translates from animal studies to humans. Strategies for developing vaccines against intracellular pathogens aim to deliver antigen to APCs in order to induce antigen presentation (Figure 1). The key variable is the type of adaptive response required for pathogen clearance, which can be modulated by the mechanism of antigen delivery. The choice of mechanism should be based on the type of immune response required; if a mixed cellular and humoral response is required, then adenoviruses could be considered. Alternatively, vectors that naturally induce the cell phenotype of interest or subunit vaccines administered along with a T cell-skewing adjuvant could be employed if a particular phenotype of T cell is required. If the pathogen of interest itself induces a protective immune response, then live attenuated vaccines are a viable option. Deletion mutants targeting immunosuppressive mechanisms in the infecting pathogen could be used in vaccination against pathogens that modulate the host response. In all mechanisms discussed here, route of delivery should be carefully selected in order to localize the immune response in the most effective manner.

Acknowledgments

This work was supported by Washington University in St. Louis and NIH grant HL105427 to S.A.K.

References

1. Conlan JW. Tularemia vaccines: recent developments and remaining hurdles. *Future Microbiol.* 2011; 6:391–405. [PubMed: 21526941]
2. Igietseme JU, Eko FO, Black CM. Chlamydia vaccines: recent developments and the role of adjuvants in future formulations. *Expert Rev Vaccines.* 2011; 10:1585–1596. [PubMed: 22043957]
3. Pitt JM, Blankley S, McShane H, O'Garra A. Vaccination against tuberculosis: how can we better BCG? *Microb Pathog.* 2013; 58:2–16. [PubMed: 23257069]
4. Singh B, Sundar S. Leishmaniasis: vaccine candidates and perspectives. *Vaccine.* 2012; 30:3834–3842. [PubMed: 22475861]
5. Torrado E, Fountain JJ, Robinson RT, Martino CA, Pearl JE, Rangel-Moreno J, Tighe M, Dunn R, Cooper AM. Differential and site-specific impact of B cells in the protective immune response to *Mycobacterium tuberculosis* in the mouse. *PLoS ONE.* 2013; 8:e61681. [PubMed: 23613902]
6. Ray HJ, Cong Y, Murthy AK, Selby DM, Klose KE, Barker JR, Guentzel MN, Arulanandam BP. Oral live vaccine strain-induced protective immunity against pulmonary *Francisella tularensis* challenge is mediated by CD4⁺ T cells and antibodies, including immunoglobulin A. *Clin Vacc Immunol.* 2009; 16:444–452.
7. Gopal R, Rangel-Moreno J, Slight S, Lin Y, Nawar HF, Fallert Junecko BA, Reinhart TA, Kolls J, Randall TD, Connell TD, et al. Interleukin-17-dependent CXCL13 mediates mucosal vaccine-induced immunity against tuberculosis. *Mucosal Immunol.* 2013; 6:972–984. [PubMed: 23299616] This study highlights the mechanisms behind the role for IL-17 in protection against *Mtb* infection, uncovering a possible target pathway for vaccination.
8. Griffiths KL, Stylianou E, Poyntz HC, Betts GJ, Fletcher HA, McShane H. Cholera Toxin Enhances Vaccine-Induced Protection against *Mycobacterium Tuberculosis* Challenge in Mice. *PLoS ONE.* 2013; 8:e78312. [PubMed: 24194918]
9. Khader SA, Bell GK, Pearl JE, Fountain JJ, Rangel-Moreno J, Cilley GE, Shen F, Eaton SM, Gaffen SL, Swain SL, et al. IL-23 and IL-17 in the establishment of protective pulmonary CD4⁺ T cell responses after vaccination and during *Mycobacterium tuberculosis* challenge. *Nat Immunol.* 2007; 8:369–377. [PubMed: 17351619] This study marked a paradigm shift in TB vaccine design, shifting the focus from Th1 to Th17-based immunity.
10. Cruz A, Fraga AG, Fountain JJ, Rangel-Moreno J, Torrado E, Saraiva M, Pereira DR, Randall TD, Pedrosa J, Cooper AM, et al. Pathological role of interleukin 17 in mice subjected to repeated BCG vaccination after infection with *Mycobacterium tuberculosis*. *J Exp Med.* 2010; 207:1609–1616. [PubMed: 20624887]
11. Gopal R, Rangel-Moreno J, Junecko BA, Mallon D, Chen K, Pociask D, Connell TD, Reinhart TA, Alcorn JF, Ross TM, et al. Short Communication: Mucosal preexposure to Th17-inducing adjuvants exacerbates pathology following influenza infection. *American Journal of Pathology.* Accepted. This paper shows the need for a balance of cytokine induction to induce protection vs. pathology, highlighting one of the considerations to be taken into account in developing mucosal vaccines.
12. Graham FL, Prevec L. Methods for construction of adenovirus vectors. *Mol Biotechnol.* 1995; 3:207–220. [PubMed: 7552690]
13. Reyes-Sandoval A, Berthoud T, Alder N, Siani L, Gilbert SC, Nicosia A, Colloca S, Cortese R, Hill AV. Prime-boost immunization with adenoviral and modified vaccinia virus Ankara vectors enhances the durability and polyfunctionality of protective malaria CD8⁺ T-cell responses. *Infect Immun.* 2010; 78:145–153. [PubMed: 19858306]
14. Zhu J, Huang X, Yang Y. Innate immune response to adenoviral vectors is mediated by both Toll-like receptor-dependent and -independent pathways. *J Virol.* 2007; 81:3170–3180. [PubMed: 17229689]
15. Chirmule N, Probert K, Magosin S, Qian Y, Qian R, Wilson J. Immune responses to adenovirus and adeno-associated virus in humans. *Gene Ther.* 1999; 6:1574–1583. [PubMed: 10490767]
16. Colloca S, Barnes E, Folgori A, Ammendola V, Capone S, Cirillo A, Siani L, Naddeo M, Grazioli F, Esposito ML, et al. Vaccine vectors derived from a large collection of simian adenoviruses induce potent cellular immunity across multiple species. *Sci Transl Med.* 2012; 4:115ra112.

17. Dicks MD, Spencer AJ, Edwards NJ, Wadell G, Bojang K, Gilbert SC, Hill AV, Cottingham MG. A novel chimpanzee adenovirus vector with low human seroprevalence: improved systems for vector derivation and comparative immunogenicity. *PLoS One*. 2012; 7:e40385. [PubMed: 22808149]
18. Hoft DF, Blazevic A, Stanley J, Landry B, Sizemore D, Kpamegan E, Gearhart J, Scott A, Kik S, Pau MG, et al. A recombinant adenovirus expressing immunodominant TB antigens can significantly enhance BCG-induced human immunity. *Vaccine*. 2012; 30:2098–2108. [PubMed: 22296955] These papers demonstrate a revival of the use of adenoviral vectors in development of vaccines against intracellular pathogens.
19. Smail F, Jeyanathan M, Smieja M, Medina MF, Thantrige-Don N, Zganiacz A, Yin C, Heriazon A, Damjanovic D, Puri L, et al. A human type 5 adenovirus-based tuberculosis vaccine induces robust T cell responses in humans despite preexisting anti-adenovirus immunity. *Sci Transl Med*. 2013; 5:205ra134. These papers demonstrate a revival of the use of adenoviral vectors in development of vaccines against intracellular pathogens.
20. McShane H, Pathan AA, Sander CR, Keating SM, Gilbert SC, Huygen K, Fletcher HA, Hill AV. Recombinant modified vaccinia virus Ankara expressing antigen 85A boosts BCG-primed and naturally acquired antimycobacterial immunity in humans. *Nat Med*. 2004; 10:1240–1244. [PubMed: 15502839]
21. Delaloye J, Roger T, Steiner-Tardivel QG, Le Roy D, Knaup Reymond M, Akira S, Petrilli V, Gomez CE, Perdiguero B, Tschopp J, et al. Innate immune sensing of modified vaccinia virus Ankara (MVA) is mediated by TLR2-TLR6, MDA-5 and the NALP3 inflammasome. *PLoS Pathog*. 2009; 5:e1000480. [PubMed: 19543380]
22. Tameris MD, Hatherill M, Landry BS, Scriba TJ, Snowden MA, Lockhart S, Shea JE, McClain JB, Hussey GD, Hanekom WA, et al. Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. *Lancet*. 2013; 381:1021–1028. [PubMed: 23391465] This paper reports the results from the first TB vaccine efficacy trial since BCG. Although the trial showed no difference in protection compared to BCG alone, it highlighted the feasibility of a large-scale efficacy trial in a TB-endemic area.
23. De Cassan S, Pathan A, Sander C, Minassian A, Hill AV, McShane H, Fletcher H. Investigating the induction of vaccine induced Th17 and regulatory T cells in healthy, BCG immunised adults vaccinated with a new tuberculosis vaccine, MVA85A. *Clin Vaccine Immunol*. 2010; 17:1066–1073. [PubMed: 20484570]
24. Griffiths KL, Pathan AA, Minassian AM, Sander CR, Beveridge NE, Hill AV, Fletcher HA, McShane H. Th1/Th17 cell induction and corresponding reduction in ATP consumption following vaccination with the novel Mycobacterium tuberculosis vaccine MVA85A. *PLoS One*. 2011; 6:e23463. [PubMed: 21887254]
25. Scriba TJ, Tameris M, Mansoor N, Smit E, van der Merwe L, Isaacs F, Keyser A, Moyo S, Brittain N, Lawrie A, et al. Modified vaccinia Ankara-expressing Ag85A, a novel tuberculosis vaccine, is safe in adolescents and children, and induces polyfunctional CD4+ T cells. *Eur J Immunol*. 2010; 40:279–290. [PubMed: 20017188]
26. Pamer EG. Immune responses to *Listeria monocytogenes*. *Nat Rev Immunol*. 2004; 4:812–823. [PubMed: 15459672]
27. Jia Q, Bowen R, Sahakian J, Dillon BJ, Horwitz MA. A heterologous prime-boost vaccination strategy comprising the *Francisella tularensis* live vaccine strain capB mutant and recombinant attenuated *Listeria monocytogenes* expressing *F. tularensis* IgIC induces potent protective immunity in mice against virulent *F. tularensis* aerosol challenge. *Infect Immun*. 2013; 81:1550–1561. [PubMed: 23439306] This vaccine represents an example of a vaccine making use of immunogenic characteristics of both the target pathogen, as well as a live vector to induce optimal immunogenicity.
28. McConkey SJ, Reece WH, Moorthy VS, Webster D, Dunachie S, Butcher G, Vuola JM, Blanchard TJ, Gothard P, Watkins K, et al. Enhanced T-cell immunogenicity of plasmid DNA vaccines boosted by recombinant modified vaccinia virus Ankara in humans. *Nat Med*. 2003; 9:729–735. [PubMed: 12766765]
29. Yager EJ, Dean HJ, Fuller DH. Prospects for developing an effective particle-mediated DNA vaccine against influenza. *Expert Rev Vaccines*. 2009; 8:1205–1220. [PubMed: 19722894]

30. Mir LM, Bureau MF, Gehl J, Rangara R, Rouy D, Caillaud JM, Delaere P, Branellec D, Schwartz B, Scherman D. High-efficiency gene transfer into skeletal muscle mediated by electric pulses. *Proc Natl Acad Sci U S A*. 1999; 96:4262–4267. [PubMed: 10200250]
31. Chen J, Liu Q, Chen Q, Xiong C, Yao Y, Wang H, Wang H, Chen Z. Comparative analysis of antibody induction and protection against influenza virus infection by DNA immunization with HA, HAe, and HA1 in mice. *Arch Virol*. 2013
32. Wang Q, Jiang W, Chen Y, Liu P, Sheng C, Chen S, Zhang H, Pan C, Gao S, Huang W. In Vivo Electroporation of Minicircle DNA as a Novel Method of Vaccine Delivery to Enhance HIV-1-Specific Immune Responses. *J Virol*. 2013
33. Gopal R, Khader SA. Vaccines against tuberculosis: moving forward with new concepts. *Expert Rev Vaccines*. 2013; 12:829–831. [PubMed: 23944644]
34. Grode L, Seiler P, Baumann S, Hess J, Brinkmann V, Nasser Eddine A, Mann P, Goosmann C, Bandermann S, Smith D, et al. Increased vaccine efficacy against tuberculosis of recombinant *Mycobacterium bovis* bacille Calmette-Guerin mutants that secrete listeriolysin. *J Clin Invest*. 2005; 115:2472–2479. [PubMed: 16110326]
35. Desel C, Dorhoi A, Bandermann S, Grode L, Eisele B, Kaufmann SH. Recombinant BCG DeltaureC hly+ induces superior protection over parental BCG by stimulating a balanced combination of type 1 and type 17 cytokine responses. *J Infect Dis*. 2011; 204:1573–1584. [PubMed: 21933877]
36. Sweeney KA, Dao DN, Goldberg MF, Hsu T, Venkataswamy MM, Henao-Tamayo M, Ordway D, Sellers RS, Jain P, Chen B, et al. A recombinant *Mycobacterium smegmatis* induces potent bactericidal immunity against *Mycobacterium tuberculosis*. *Nat Med*. 2011; 17:1261–1268. [PubMed: 21892180] This vaccine represents an important step in the development of a live/attenuated vaccine against *Mtb* infection, demonstrating improved protection over BCG.
37. Perez E, Samper S, Bordas Y, Guilhot C, Gicquel B, Martin C. An essential role for *phoP* in *Mycobacterium tuberculosis* virulence. *Mol Microbiol*. 2001; 41:179–187. [PubMed: 11454210]
38. Martin C, Williams A, Hernandez-Pando R, Cardona PJ, Gormley E, Bordat Y, Soto CY, Clark SO, Hatch GJ, Aguilar D, et al. The live *Mycobacterium tuberculosis phoP* mutant strain is more attenuated than BCG and confers protective immunity against tuberculosis in mice and guinea pigs. *Vaccine*. 2006; 24:3408–3419. [PubMed: 16564606]
39. Nambiar JK, Pinto R, Aguilo JI, Takatsu K, Martin C, Britton WJ, Triccas JA. Protective immunity afforded by attenuated, *PhoP*-deficient *Mycobacterium tuberculosis* is associated with sustained generation of CD4+ T-cell memory. *Eur J Immunol*. 2012; 42:385–392. [PubMed: 22105536]
40. Shen H, Harris G, Chen W, Sjostedt A, Ryden P, Conlan W. Molecular immune responses to aerosol challenge with *Francisella tularensis* in mice inoculated with live vaccine candidates of varying efficacy. *PLoS One*. 2010; 5:e13349. [PubMed: 20967278]
41. Rockx-Brouwer D, Chong A, Wehrly TD, Child R, Crane DD, Celli J, Bosio CM. Low dose vaccination with attenuated *Francisella tularensis* strain SchuS4 mutants protects against tularemia independent of the route of vaccination. *PLoS One*. 2012; 7:e37752. [PubMed: 22662210] This paper is interesting in the finding that route of vaccination against *F.tularensis* does not impact challenge outcome and highlights the differences in requirements for protection between pathogens.
42. Ota MO, Odutola AA, Owiafe PK, Donkor S, Owolabi OA, Brittain NJ, Williams N, Rowland-Jones S, Hill AV, Adegbola RA, et al. Immunogenicity of the tuberculosis vaccine MVA85A is reduced by coadministration with EPI vaccines in a randomized controlled trial in Gambian infants. *Sci Transl Med*. 2011; 3:88ra56.
43. Kaur T, Sobti RC, Kaur S. Cocktail of gp63 and Hsp70 induces protection against *Leishmania donovani* in BALB/c mice. *Parasite Immunol*. 2011; 33:95–103. [PubMed: 21226722]
44. Mazumder S, Maji M, Ali N. Potentiating effects of MPL on DSPC bearing cationic liposomes promote recombinant GP63 vaccine efficacy: high immunogenicity and protection. *PLoS Negl Trop Dis*. 2011; 5:e1429. [PubMed: 22206029]
45. Chakravarty J, Kumar S, Trivedi S, Rai VK, Singh A, Ashman JA, Laughlin EM, Coler RN, Kahn SJ, Beckmann AM, et al. A clinical trial to evaluate the safety and immunogenicity of the LEISH-F1+MPL-SE vaccine for use in the prevention of visceral leishmaniasis. *Vaccine*. 2011; 29:3531–3537. [PubMed: 21414377]

46. Llanos-Cuentas A, Calderon W, Cruz M, Ashman JA, Alves FP, Coler RN, Bogatzki LY, Bertholet S, Laughlin EM, Kahn SJ, et al. A clinical trial to evaluate the safety and immunogenicity of the LEISH-F1+MPL-SE vaccine when used in combination with sodium stibogluconate for the treatment of mucosal leishmaniasis. *Vaccine*. 2010; 28:7427–7435. [PubMed: 20851080]
47. Nascimento E, Fernandes DF, Vieira EP, Campos-Neto A, Ashman JA, Alves FP, Coler RN, Bogatzki LY, Kahn SJ, Beckmann AM, et al. A clinical trial to evaluate the safety and immunogenicity of the LEISH-F1+MPL-SE vaccine when used in combination with meglumine antimoniate for the treatment of cutaneous leishmaniasis. *Vaccine*. 2010; 28:6581–6587. [PubMed: 20688040]
48. Velez ID, Gilchrist K, Martinez S, Ramirez-Pineda JR, Ashman JA, Alves FP, Coler RN, Bogatzki LY, Kahn SJ, Beckmann AM, et al. Safety and immunogenicity of a defined vaccine for the prevention of cutaneous leishmaniasis. *Vaccine*. 2009; 28:329–337. [PubMed: 19879995]
49. Ashtekar AR, Katz J, Xu Q, Michalek SM. A mucosal subunit vaccine protects against lethal respiratory infection with *Francisella tularensis* LVS. *PLoS One*. 2012; 7:e50460. [PubMed: 23209745]
50. Tifrea DF, Pal S, Toussi DN, Massari P, de la Maza LM. Vaccination with major outer membrane protein proteosomes elicits protection in mice against a *Chlamydia* respiratory challenge. *Microbes Infect*. 2013
51. Aagaard C, Hoang T, Dietrich J, Cardona PJ, Izzo A, Dolganov G, Schoolnik GK, Cassidy JP, Billeskov R, Andersen P. A multistage tuberculosis vaccine that confers efficient protection before and after exposure. *Nat Med*. 2011; 17:189–194. [PubMed: 21258338]
52. Lin PL, Dietrich J, Tan E, Abalos RM, Burgos J, Bigbee C, Bigbee M, Milk L, Gideon HP, Rodgers M, et al. The multistage vaccine H56 boosts the effects of BCG to protect cynomolgus macaques against active tuberculosis and reactivation of latent *Mycobacterium tuberculosis* infection. *J Clin Invest*. 2012; 122:303–314. [PubMed: 22133873]

Highlights

- Summary of goals of vaccines against intracellular pathogens
- Overview of T cell-inducing vaccine strategies currently in use
- Outlook for future of T cell-inducing vaccine approaches

Figure 1.

T cell inducing vaccines, (a) vectored, (b) live attenuated and (c) subunit, aim to induce the generation of a pool of memory T cells. Upon infection, antigen presentation by the infected cells induces activation and expansion of the memory T cells and production of effector molecules such as cytokines or cytotoxic mediators depending on the responding cell phenotype. Effector molecule production leads to either T cell-mediated killing of the infected cell or induction of microbicidal mechanisms within the infected cell.