Published in final edited form as:
Expert Rev Vaccines. 2009 May ; 8(5): 543–546. doi:10.1586/erv.09.26.

How to design effective vaccines: lessons from an old success story

Nabil Ahmed[1,2,3] and **Stephen Gottschalk**[1,2,3,4]

[1]Center for Cell and Gene Therapy, Baylor College of Medicine, Texas Children's Hospital, The Methodist Hospital, Houston, Texas, USA

[2]Texas Children's Cancer Center, Baylor College of Medicine, Texas Children's Hospital, The Methodist Hospital, Houston, Texas, USA

[3]Department of Pediatrics, Baylor College of Medicine, Texas Children's Hospital, The Methodist Hospital, Houston, Texas, USA

[4]Department of Immunology, Baylor College of Medicine, Texas Children's Hospital, The Methodist Hospital, Houston, Texas, USA

Abstract

Despite the successful development of vaccines that are able to elicit potent and protective immune responses, the majority of vaccines were developed empirically and the mechanistic events leading to protective immune responses are often poorly understood. This impedes the development of new prophylactic as well as therapeutic vaccines for infectious diseases and cancer. Gaucher *et al.* took advantage of the effective yellow fever (YF) vaccine 17D (YF17D) to prospectively identify key immunological responses elicited by the vaccine using functional genomics and flow cytometric analysis. The results of the study clearly indicate 'that the immune response to a strong vaccine is preceded by the coordinated induction of master transcription factors that lead to the development of a broad, polyfunctional and persistent immune response integrating all effector cells of the immune system'.

Keywords

vaccine; functional genomics; immune response; yellow fever

Background

Modern vaccination dates back to the 18th century when Edward Jenner used the cow pox virus to protect humans against small pox [2]. After successful vaccination campaigns throughout the 19th and 20th centuries, the WHO certified the eradication of smallpox in December 1979 highlighting the potential potency of vaccines [3]. However, many vaccines have been less successful - especially therapeutic vaccines - and at present it is unknown how vaccines have to activate the immune system to induce protective adaptive immune responses in humans [4-8]. In addition, the interplay of the innate immune system and adaptive immune responses post vaccination remains poorly understood [9]. Clearly, the desired property of a vaccine depends on the targeted disease [7, 8, 10, 11]. For example,

Corresponding author: Stephen Gottschalk, Phone: 832-824-4179, Fax: 832-825-4732, smg@bcm.edu, Center for Cell and Gene Therapy, Baylor College of Medicine, 6621 Fannin Street MC 3-3320, Houston, TX 77030.

Evaluation of: Gaucher D, Therrien R, Kettaf N *et al.* Yellow fever vaccine induces integrated multilineage and polyfunctional immune responses. *J Exp Med.* 2008 Dec 22;205(13):3119-31 [1].

humoral immunity is required to protect individuals from most bacterial infections, where as the protection from viruses and therapeutic vaccines require the induction of humoral as well as cellular immune responses [7, 10, 12-14].

To elucidate some of the pathways involved in vaccine induced long-lasting protective immune responses, *Gaucher et al.* took advantage of the yellow fever (YF) vaccine 17D (YF17D). Developed empirically more than 70 years ago, the life attenuated yellow fever vaccine is considered one of the most successful vaccines ever made, inducing protective immunity for up to 35 years in almost all vaccinated individuals [15]. Rather than focusing on standard readouts such as antibody titers and Elispot assays [12, 13, 16, 17], Gaucher *et al.* preformed gene expression array analysis from whole blood and conducted an analysis of YF-specific Th1/Th2 cell responses in 40 healthy volunteers post vaccination. In addition, they evaluated the YF17D vaccine using a novel *in vitro* system of primary immune responses (modular immune in vitro construct [MIMIC] system). Results obtained form the clinical study and MIMIC system correlated well and indicate that the activation of all arms of the immune system is most likely required for the induction of long-lasting protective immune responses.

Methods and results

YF17D vaccination rapidly induces master transcription factors upstream of genes involved in multiple arms of the immune response

Gaucher *et al.* used gene expression arrays (Illumina BeadChips V2) to study gene expression profiles in whole blood 3, 7, 10, 14, and 60 days post YF17D vaccination in 40 healthy individuals. They found significant changes in the expression of 594 genes, which peaked on days 3 and 7 post vaccination. To identify the transcription factors upstream of these genes, independent component analysis followed by gene set enrichment was performed. Interestingly, three major nodes of transcriptional regulation of downstream target genes were identified as early as day 3 post vaccination: interferon regulatory factor (IRF)7, signal transducer and activator of transcription (STAT)1 and v-ets erythroblastosis virus E26 oncogene homolog 2 (avian) (ETS2). Functionally, these transcriptional nodes and many of the identified genes were associated with Toll-like receptor (TLR), interferon-γ induction, complement factors, macrophage/dendritic cell (DC), natural killer (NK)- and B-cell activation. Results were validated by quantitative real-time PCR in a subset of 7 donors.

Upfront stimulation of the innate immune arm: YF17D vaccination activates components of the inflammasome complex upregulating IL-1β production shortly after inoculation

The inflammasome is a cytoplasmic multiprotein complex that activates caspase-1 and - 5, leading to the processing and secretion of the pro-inflammatory cytokines, interleukin(IL)-1β, IL-18, and IL-33 [18]. Inflammasomes interact with TLR to mediate appropriate responses to pathogenic triggers constituting a substantial part of the innate immune response. Gaucher *et al.* showed that caspase-1 and −5 were upregulated at the RNA level as early as 3 days after YF17D vaccination. Moreover, other genes involved in the production of IL-1β, namely IL-1R1 and -1RN, were also modulated by the vaccine. To functionally validate these findings, they incubated DCs with live and UV-inactivated YF17D vaccine and showed a marked increase in IL-1β production confirming that YF17D vaccine activates components of the inflammasome complex, and that viral replication was not required for this activation.

YF17D vaccination induces proliferation and expansion of various leukocyte subsets

Does the up-regulation of genes associated with activation of various leukocyte subsets result in their proliferation? To answer this question, peripheral blood mononuclear cells

(PBMCs) from a number of individuals were stained pre- and post vaccination for the proliferation marker Ki67 in addition to lineage and activation specific markers. The frequencies of both the CD4- and CD8-positive lymphocyte subsets increased, along with NK cells, monocytes and B cells. Cell proliferation peaked within 7 to 14 days post vaccination and then declined to baseline. These results confirmed the widespread mobilization of all major cellular subsets early after YF17D vaccination.

A durable activation of the adaptive immune arm: YF17D vaccination induces an early YF-specific mixed Th1/Th2 response that appears early and is persistent

To further define which part of the adaptive immune arm is activated by the vaccine, PBMCs, obtained day 28 or day 60 post vaccination, were activated with YF17D-derived peptide pools. The pattern of cytokine secretion was determined using cytometric bead assays (CBA) and intracellular cytokine staining. Although the cytokine patterns varied substantially among individuals, most had mixed Th1/Th2 responses with high levels of TNF; CD4+ T cells produced IL-2 or IFN-γ, confirming the Th1 component of the immune response. In addition, YF-specific central memory responses could be detected in PBMCs one year post vaccination. Thus, the vaccine not only induced a strong innate immune response early after vaccination, but also brisk and persistent Th1/Th2 adaptive responses.

Recapitulation of the central immune response to YF17D vaccination in vitro

The authors then took advantage of the MIMIC system, which simulates the human immune response in a high-throughput method enabling rapid, clinically relevant predictions about the efficacy of vaccines. This system uses pheresis-derived PBMCs and purified leukocyte sub-populations (monocytes, T cells, and B cells) from healthy donors to simulate vaccination site and lymphoid tissue interactions between cells in response to stimulation with a vaccine of interest. Using this system, Gaucher *et al.* validated the multi-lineage response to YF17D vaccination (and UV-inactivated vaccine) including Th1/Th2 responses. Moreover, gene expression analysis identified the same central nodes of transcription as those described after vaccination with YF17D in healthy individuals.

Discussion, expert commentary and five-year view

How can we best assess the potency of vaccines? For the systematic development of a successful vaccination strategy, it is mandatory to develop ‘read out systems’ or ‘correlates of immune-mediated protection’ (COP) that would reliably predict immune responses to novel vaccines *in vivo* [19]. Fortunately, there is an expanding knowledge on the molecular mechanisms of immune responses and on the immunological parameters that correlate best with lasting protection [8]. As expected, this has not been a simple task and COPs have continued to lag behind the development of novel vaccines [4, 5, 7, 8, 14, 19, 20]. This is exemplified by the development of vaccines for Rotavirus: prompted by the emergence of severe deadly disease, intense work on vaccine development for this virus started in the early 1980’s and since 2006 two vaccines have been licensed in many countries. Nevertheless, to date, in spite of the intense work in this area, COPs for these two vaccines are still not well defined [21].

Gaucher *et al.* have used functional genomics and flow cytometric analysis to determine the immune response to the very effective YF17D vaccine. YF17D vaccination resulted in a rapid and transient modulation of a large number of genes linked to activation of several components of the immune system spanning the innate and adaptive arms. Multiple unique interactions between the various effector arms of the immune system were observed, which are most likely responsible for the long-term protective immunity induced by the vaccine. However, it remains elusive which of these events post YF17D vaccination are essential and which ones are dispensable. This might become evident in the future when other vaccines

are evaluated using the same approach. Nevertheless, we expect that some of the findings of this study will be either integrated into existing COPs or will facilitate the development of new COPs.

While genomics has been successfully used to identify new vaccine antigens [22], Gaucher's *et al*. study highlights that genomics is also a promising tool for evaluating vaccine induced immune responses. Is it possible in the future to develop and test vaccines using solely genomics? Most likely not, however genomics will help us to better understand vaccine induced immune responses and might prevent failures like the STEP HIV vaccine trial. Another noteworthy result of the study is that the results of the human vaccine study correlated well with the results obtained using the *in vitro* MIMIC system. If confirmed in future studies, the MIMIC system might thus represent an efficient screening system to evaluate vaccines before conducting costly phase I clinical studies in humans.

In summary, Gaucher *et al.* used functional genomics to attempt to answer a question that has occupied a central place in the field of vaccine research since its beginning: How can we best evaluate the potency of a vaccine? Their results are encouraging and should entice the field to develop novel vaccine evaluation strategies. To close with Churchill, Gaucher's *et al.* study 'is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning [23].'

References

1. GAUCHER D, THERRIEN R, KETTAF N, ANGERMANN BR, BOUCHER G, FILALI-MOUHIM A, MOSER JM, MEHTA RS, DRAKE DR III, CASTRO E, AKONDY R, RINFRET A, YASSINE-DIAB B, SAID EA, CHOUIKH Y, CAMERON MJ, CLUM R, KELVIN D, SOMOGYI R, GRELLER LD, BALDERAS RS, WILKINSON P, PANTALEO G, TARTAGLIA J, HADDAD EK, SEKALY RP. Yellow fever vaccine induces integrated multilineage and polyfunctional immune responses. J. Exp. Med. 2008; 205:3119–3131. [PubMed: 19047440]
2. RIEDEL S. Edward Jenner and the history of smallpox and vaccination. Proc. Bayl. Univ Med. Cent. 2005; 18:21–25. [PubMed: 16200144]
3. AMANNA IJ, SLIFKA MK, CROTTY S. Immunity and immunological memory following smallpox vaccination. Immunol. Rev. 2006; 211:320–337. [PubMed: 16824139]
4. LAMBERT PH, LIU M, SIEGRIST CA. Can successful vaccines teach us how to induce efficient protective immune responses? Nat. Med. 2005; 11:S54–S62. [PubMed: 15812491]
5. LI PG, KERN F, GRATAMA J, ROEDERER M, MANCA F. Measurement of antigen specific immune responses: 2006 update. Cytometry B Clin. Cytom. 2007; 72:77–85. [PubMed: 17285633]
6. PLOTKIN SA. New vaccination strategies. Bull. Acad. Natl. Med. 2008; 192:511–518. [PubMed: 18819697]
7. PLOTKIN SA. Vaccines: correlates of vaccine-induced immunity. Clin. Infect. Dis. 2008; 47:401–409. [PubMed: 18558875]
8. QIN L, GILBERT PB, COREY L, MCELRATH MJ, SELF SG. A framework for assessing immunological correlates of protection in vaccine trials. J. Infect. Dis. 2007; 196:1304–1312. [PubMed: 17922394]
9. TRINCHIERI G, SHER A. Cooperation of Toll-like receptor signals in innate immune defence. Nat. Rev. Immunol. 2007; 7:179–190. [PubMed: 17318230]
10. SIBER GR, CHANG I, BAKER S, FERNSTEN P, O'BRIEN KL, SANTOSHAM M, KLUGMAN KP, MADHI SA, PARADISO P, KOHBERGER R. Estimating the protective concentration of anti-pneumococcal capsular polysaccharide antibodies. Vaccine. 2007; 25:3816–3826. [PubMed: 17368878]
11. SITATI EM, DIAMOND MS. CD4+ T-cell responses are required for clearance of West Nile virus from the central nervous system. J. Virol. 2006; 80:12060–12069. [PubMed: 17035323]
12. DENOEL PA, GOLDBLATT D, DE V, JACQUET JM, PICHICHERO ME, POOLMAN JT. Quality of the Haemophilus influenzae type b (Hib) antibody response induced by diphtheria-

tetanus-acellular pertussis/Hib combination vaccines. Clin. Vaccine Immunol. 2007; 14:1362–1369. I. [PubMed: 17699836]

13. HUBER VC, MCKEON RM, BRACKIN MN, MILLER LA, KEATING R, BROWN SA, MAKAROVA N, PEREZ DR, MACDONALD GH, MCCULLERS JA. Distinct contributions of vaccine-induced immunoglobulin G1 (IgG1) and IgG2a antibodies to protective immunity against influenza. Clin. Vaccine Immunol. 2006; 13:981–990. [PubMed: 16960108]
14. VAN DER ZEIJST BA, DIJKMAN MI, LUYTJES W, VAN ALPHEN AJ, VAN DEN DOBBELSTEEN GP. On the design of national vaccination programmes. Vaccine. 2007; 25:3143–3145. [PubMed: 17293011]
15. REINHARDT B, JASPERT R, NIEDRIG M, KOSTNER C, L'AGE-STEHR J. Development of viremia and humoral and cellular parameters of immune activation after vaccination with yellow fever virus strain 17D: a model of human flavivirus infection. J. Med. Virol. 1998; 56:159–167. [PubMed: 9746073]
16. MICHALIK DE, STEINBERG SP, LARUSSA PS, EDWARDS KM, WRIGHT PF, ARVIN AM, GANS HA, GERSHON AA. Primary vaccine failure after 1 dose of varicella vaccine in healthy children. J. Infect. Dis. 2008; 197:944–949. [PubMed: 18419532]
17. PANCHANATHAN V, CHAUDHRI G, KARUPIAH G. Protective immunity against secondary poxvirus infection is dependent on antibody but not on CD4 or CD8 T-cell function. J. Virol. 2006; 80:6333–6338. [PubMed: 16775321]
18. MARTINON F, MAYOR A, TSCHOPP J. The inflammasomes: guardians of the body. Annu Rev Immunol. 2009; 27:229–65. [PubMed: 19302040]
19. RIMMELZWAAN GF, MCELHANEY JE. Correlates of protection: novel generations of influenza vaccines. Vaccine. 2008; 26(Suppl 4):D41–D44. [PubMed: 19230158]
20. PANCHANATHAN V, CHAUDHRI G, KARUPIAH G. Correlates of protective immunity in poxvirus infection: where does antibody stand? Immunol. Cell Biol. 2008; 86:80–86. [PubMed: 17923850]
21. DESSELBERGER U, MANKTELOW E, LI W, CHEUNG W, ITURRIZA-GOMARA M, GRAY J. Rotaviruses and rotavirus vaccines. Br. Med. Bull. 2009 Epub ahead of print.
22. BAMBINI S, RAPPUOLI R. The use of genomics in microbial vaccine development. Drug Discovery Today. 2009; 14:252–260. [PubMed: 19150507]
23. CHURCHILL, W. Speech at Lord Mayor's Luncheon, Mansion House following the victory at El Alameinin. North Africa; London: November 10. 1942

Key Issues

- Vaccines have the ability to prevent disease by inducing long lasting immune responses. However, at present some of the biological correlates of immune-mediated protection are ill defined.
- Gaucher *et al*. identifies several transcriptional nodes that are induced by the potent FY17D vaccine including IRF7, STAT1, and ETS2.
- Induction of innate immune responses seem to be important for the activation of adaptive immune responses
- The MIMIC system might be a useful screening tool to assess the potency of vaccines