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How advances in immunology provide insight into improving vaccine efficacy

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Abstract

Vaccines represent one of the most compelling examples of how biomedical research has improved society by saving lives and dramatically reducing the burden of infectious disease. Despite the importance of vaccinology, we are still in the early stages of understanding how the best vaccines work and how we can achieve better protective efficacy through improved vaccine design. Most successful vaccines have been developed empirically, but recent advances in immunology are beginning to shed new light on the mechanisms of vaccine-mediated protection and development of long-term immunity. Although natural infection will often elicit lifelong immunity, almost all current vaccines require booster vaccination in order to achieve durable protective humoral immune responses, regardless of whether the vaccine is based on infection with replicating live-attenuated vaccine strains of the specific pathogen or whether they are derived from immunization with inactivated, non-replicating vaccines or subunit vaccines. The form of the vaccine antigen (e.g., soluble or particulate/aggregate) appears to play an important role in determining immunogenicity and the interactions between dendritic cells, B cells and T cells in the germinal center are likely to dictate the magnitude and duration of protective immunity. By learning how to optimize these interactions, we may be able to elicit more effective and long-lived immunity with fewer vaccinations.

Keywords

Vaccination; Immunological Memory; Antibody; Protection

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1. Introduction: Success of current vaccines

It is difficult to over-emphasize the role that vaccines have played in public health by controlling infectious disease, improving the quality of life and increasing life expectancy. For example, analysis of the impact of immunization with 7 of the 12 vaccines administered during routine childhood immunization in 2001 revealed that within a single US birth cohort, these vaccines prevent 33,000 deaths and 14 million cases of disease [1]. In addition to reducing morbidity and mortality, this program of routine childhood vaccination is estimated to result in societal cost savings of nearly $33 billion that would otherwise be lost due to hospitalization, disability, and loss of productivity. The 2001 US immunization schedule involved 12 vaccines, including ones against measles, mumps, rubella (collectively known as MMR), tetanus and diphtheria toxoids, acellular pertussis, *H. influenza* B, polio, hepatitis B virus, a pneumococcal conjugate, influenza, and varicella zoster virus. In addition to these vaccines, the 2013 immunization schedule (http://www.cdc.gov/mmwr/preview/mmwrhtml/su6201a2.htm) also includes vaccines against rotavirus, hepatitis A virus, human papilloma virus, and meningococcal disease. The addition of these vaccines will undoubtedly further increase the cost-benefit ratios of routine childhood immunization as well as providing further reductions in disease and mortality.

From a historical perspective, vaccines have dramatically changed the landscape of infectious disease. Polio, measles, and rubella are no longer endemic in the US and smallpox, once arguably the most feared global threat among infectious diseases, is now extinct worldwide. Comparisons between the levels of disease in the pre-vaccine era [2] and the most recent reports on morbidity and mortality for vaccine-preventable diseases [3] show the dramatic influence that routine vaccination can have on human health (Figure 1). In 2012, there were no reports of polio in the US and cases of measles, mumps, rubella, and *H. influenza* B, have dropped by >99% from the pre-vaccine era. The number of tetanus cases has dropped by 94% and there have been only two cases of diphtheria reported in the US in the last 10 years. Cases of hepatitis A and hepatitis B have declined by 99% and 96%, respectively. Pneumococcal disease has been reduced by 94% and the incidence of varicella (i.e., chickenpox) has been reduced by >90%. In contrast, *Bordetella pertussis*, the causative agent of whooping cough, represents a continuing and potentially growing concern. While vaccination has reduced the incidence of whooping cough by approximately 79% between the pre-vaccine era and 2012, the outbreaks have become progressively larger since the whole-cell pertussis vaccine was replaced by acellular pertussis vaccines consisting of only 2–4 bacterial antigens [4, 5]. Although neither natural infection nor vaccination elicit lifelong immunity, the resurgence of this vaccine-preventable disease may be linked to rapidly waning immunity against pertussis despite a 5-dose vaccination regimen [6–8]. The whole-cell pertussis vaccine was self-adjuvanted (i.e., containing bacterial LPS) and induced long-term protection and antibody responses to a broad range of bacterial proteins. However, it was eventually determined to be too reactogenic for routine use and was replaced by acellular pertussis vaccines that are safer, but have recently been found to induce shorter-lived immunity. An interesting study in Australia shows a clear difference in the duration of protective immunity when comparing the whole-cell vaccine to the acellular pertussis vaccine [7]. Moreover, they found that in cases of mixed vaccination regimens,

children who first received the whole-cell vaccine had long-lived immunity regardless of whether booster vaccination was performed with whole-cell or the acellular pertussis vaccine. In contrast, if the acellular vaccine was administered first, then booster vaccination with the whole-cell vaccine was much less efficient at eliciting long-term protection, suggesting that initial priming to only a few bacterial antigens may suppress immune responses to a broader array of bacterial antigens upon subsequent exposure [9]. Together, these results show that our overall current vaccine program is effective (Figure 1), but development of new and improved vaccines that induce durable protective immunity will be essential to the continued success of these vaccination efforts.

2. Duration of immunity depends on the characteristics of the vaccine or infection

Studies describing the duration of immunological memory following acute viral infection date back to the time of Panum, who in 1847, reported that the maintenance of long-term immunity against measles could be sustained for up to 65 years in the absence of re-exposure to the pathogen [10]. Studies by our group [11] previously showed that infection with measles virus results in stable serum antibody responses that are largely maintained above a protective threshold for life (95% confidence interval of antibody half-life; 104 years – infinity). This does not mean that all antibody responses are equally long-lived; indeed, antibody responses to tetanus and diphtheria toxoids showed an 11-year and 19-year half-life, respectively, which is much shorter than that observed for viral infections [11]. An 11-year half-life of tetanus-specific antibodies was also identified by an independent group studying humoral immunity among HIV+ patients [12]. This group not only examined antibody responses to tetanus, but they also performed longitudinal analysis of serum antibody responses to HIV gp120, gp41, and p55 Gag for 7 years after HIV suppression following the initiation of antiretroviral therapy. Interestingly, the antibody responses to HIV envelope antigens, gp120 and gp41, declined rapidly during this course of time (half-life of 81 weeks and 31 weeks, respectively), whereas antibody responses to HIV p55 Gag were more stable, with an approximate half-life of 12.5 years [12]. The shorter antibody half-life to HIV envelope antigens may be linked to their immunosuppressive characteristics [13]. On the other hand, unlike gp120 and gp41 monomers, p55 Gag forms multivalent particulates containing 1500–1800 p55 Gag molecules [14] and these types of complex structures may be involved with the induction of more long-lived humoral immune responses (discussed further in section 7). Chronic antigenic stimulation may also result in more short-lived plasma cells and fewer long-lived plasma cells [15, 16], but this alone would not explain the differential long-lived response to HIV p55 Gag in comparison with HIV gp120 and gp41. Instead, this work indicates that the duration of serum antibody responses may not only differ between pathogens (e.g., HIV vs. measles) or between specific vaccine antigens (e.g., tetanus vs. diphtheria toxoid), but may also differ between individual antigens within a single pathogen (e.g., HIV gp120 vs. HIV p55 Gag). This suggests that there are antigen-specific characteristics that influence the magnitude and durability of the antibody response to each particular antigen. Further studies are needed and analysis of antibody responses to other chronic infections such as hepatitis C virus after successful antiviral therapy would be particularly interesting to compare to the results

observed after HIV infection/antiretroviral therapy. An effective HIV vaccine will most likely need to elicit high affinity, broadly neutralizing antibodies with substantial somatic hypermutation [17, 18] and unlocking the secret(s) to long-term maintenance of these types of immune responses will be key to the success of a protective HIV vaccine. Moreover, a better understanding of the underlying mechanisms responsible for inducing long-lived antibody responses will be important for improving other suboptimal vaccines (e.g., acellular pertussis) as well.

Several models have been proposed to explain the mechanisms underlying the durability of serum antibody responses, including the polyclonal stimulation model, the plasma cell niche competition model, and the plasma cell imprinted lifespan model ([19–21], reviewed in [22]). According to the polyclonal stimulation model, successive rounds of infection or vaccination will induce polyclonal B cell activation that results in repopulation of the pre-existing plasma cell pool and increased antigen-specific antibody levels. With the plasma cell niche competition model, multiple rounds of infection or vaccination will trigger new plasma cells that compete with pre-existing plasma cells for finite space in survival niches, resulting in the displacement of older plasma cells for newly generated plasma cells/plasmablasts and a subsequent loss of pre-existing antibody titers. The imprinted lifespan model postulates that plasma cells are “imprinted” with a certain lifespan during the initiation of the humoral immune response and are unlikely to be altered by subsequent infections or vaccinations.

A recent study examined plasma cell homeostasis in mice by vaccinating animals with model antigens and then subjecting them to further vaccination with unrelated antigen or infection with a vaccine strain of *Salmonella typhi* [15]. At 5 weeks after booster vaccination with NP-KLH (nitrophenol conjugated to keyhole limpet hemocyanin) to establish a pre-existing antibody response, animals were immunized intraperitoneally with FITC-labeled ovalbumin adjuvanted with 2×10^8 killed *Bordetella pertussis* bacteria. This resulted in a transient spike in splenic NP-specific plasma cells (thus supporting the polyclonal stimulation model), but also triggered the sustained loss of approximately 80% of NP-specific plasma cells in the bone marrow in addition to lowering the pre-existing levels of NP-specific serum IgG by 75 days post-vaccination. This suggests that although polyclonal B cell activation had occurred, the final outcome was that competition for plasma cell survival was an overriding mechanism and that serological memory was lost following this antigenic insult. Infection with *Salmonella* also resulted in a transient spike in the total number of splenic NP-specific plasma cells by 16 days post-infection but was followed by a trend showing lower NP-specific plasma cell numbers by 75 days post-infection. Again, pre-existing NP-specific serum antibody levels were lower in *Salmonella*-infected animals, indicating that bacterial infection reduced pre-existing vaccine-mediated antibody levels.

In terms of clinical studies, direct competition between plasma cells for finite space does not appear to be a major issue for children undergoing routine vaccination as each new combination of vaccines must be tested to ensure adequate antibody responses to each of the individual vaccine components before the new combination of vaccines is accepted by the ACIP (Advisory Committee on Immunization Practices) and other regulatory bodies for inclusion in childhood immunization schedules. For instance, the individual live attenuated

measles, mumps, and rubella vaccines have been combined into the MMR vaccine. This vaccine has since been combined with the live attenuated varicella vaccine (i.e., MMRV). The seroconversion rates and levels of antibody to measles, mumps, rubella and varicella were not altered when the MMRV was co-administered with a booster dose of tetanus, diphtheria, acellular pertussis, hepatitis B virus, inactivated polio, and *H. influenza* B [23]. Likewise, the immune responses to these latter vaccine antigens were not altered by co-infection/co-administration with measles, mumps, rubella, and varicella (MMRV). This is fortunate from a vaccinology perspective because it shows that carefully designed combinations of vaccines can be successfully administered together on the same visit without “overwhelming” a child’s immune system, which has been a concern among some parents [24, 25] and even a small number of physicians [26], resulting in unnecessarily reduced vaccine uptake and increased risk for unvaccinated or under-vaccinated children.

Less is known about how multiple vaccinations/infections might alter pre-existing serum antibody responses to vaccines/infections encountered in the distant past. We performed a small study to determine if infection or vaccination might alter pre-existing serum antibody responses in adult human subjects [27]. Four subjects received booster smallpox vaccination (i.e., vaccinia virus infection), and pre-existing immunity to 9 antigens (vaccinia, tetanus toxoid, diphtheria toxoid, pertussis toxoid, measles, mumps, rubella, varicella, and Epstein-Barr virus) was measured for up to 1 year after vaccination. Although vaccinia-specific antibody titers were boosted by 8- to 80-fold, there was no significant increase or decrease in pre-existing antibody levels specific for the 8 other vaccine or virus antigens. Although one infection may be insufficient to elicit an appreciable change in other pre-existing antibody titers, it is possible that multiple infections/vaccinations might cumulatively augment an increase or decrease in pre-existing antibody levels. In this regard, a case study was performed on an individual who received multiple immunizations including tetanus/diphtheria booster vaccination, primary smallpox and yellow fever vaccinations, and vaccinations against hepatitis A virus (primary and booster vaccination), polio (booster vaccination) and *Salmonella typhi* (primary vaccination administered as four oral doses given two days apart) [27]. Similar to the subjects who received smallpox vaccination, no statistically significant increase or decrease in pre-existing serological memory to unrelated antigens was observed following successive rounds of vaccination or infection. There may be several reasons why these results differ from that observed in mice [15]. In addition to the anticipated caveats associated with using different vaccine antigens with different doses and routes of vaccination, one possibility is that there may be differences between mouse and human immunology. On the other hand, a more interesting possibility for the difference between experimental results is that the timing of heterologous infection or vaccination could play a role in determining the fate of pre-existing plasma cells. In the human study, the pre-existing antibody responses and the underlying plasma cell population had been established for months or years prior to the heterologous antigenic challenge whereas in the mouse study, the pre-existing antibody response had been established just 5 weeks prior to immune activation through heterologous vaccination/infection. Perhaps there is a window of vulnerability in which newly generated plasma cells or plasmablasts are susceptible to inflammatory signals that result in decreased survival rates. This is an important question and further studies are needed to increase our basic understanding of this aspect of adaptive

immunity, especially since it could play a role in determining the most appropriate vaccination schedules for conferring protective immunity in the clinical setting without potentially altering pre-existing serological memory.

3. Duration of T cell memory and immune correlates of cellular immunity

Many advances in the quantitative analysis and functional characterization of $CD4^+$ and $CD8^+$ T cell memory have occurred over the last two decades. The frequency of antigen-specific T cell responses can be measured by a variety of approaches including MHC Class I tetramers, peptide-induced intracellular cytokine staining, and IFNγ ELISPOT [28]. Moreover, with substantial advances in flow cytometry instrumentation and reagents, a large number of T cell functions and phenotypic markers can be examined simultaneously. These studies, together with sophisticated microarray analysis of T cell gene expression profiles, have led to a wealth of information in understanding memory T cell responses. Despite these great technological advances, there is still much that we don't know about the durability of vaccine-induced human T cell memory or the role that they play in protection against different diseases. Studies have shown that antiviral T cell memory can be maintained for decades after smallpox vaccination [29, 30] and that vaccinia-specific $CD4^+$ T cell memory appears to be preferentially maintained over $CD8^+$ T cell memory [29, 31]. However, the role of vaccine-induced antiviral T cell responses in protection against orthopoxviruses is unclear as an elegant study performed in non-human primates demonstrated that neutralizing antibodies were both necessary and sufficient for vaccine-mediated protection against lethal monkeypox infection, indicating that vaccine-induced T cells play only a minor role in protective immunity to this family of viruses [32]. Likewise, infection with flaviviruses such as yellow fever virus-17D (YFV-17D) [33–36] and dengue virus (DENV) [37, 38] induce strong antiviral $CD4^+$ and $CD8^+$ T cell responses during primary human infection. However, studies involving infection/immunization of YFV-17D-immune human subjects with recombinant vaccine strains of YFV-17D expressing the neutralizing epitopes of other flaviviruses such as Japanese encephalitis virus (JEV) [39] or DENV2 [40] indicate that flavivirus-specific T cells play little to no role in protection against re-infection [41]. Perhaps most perplexing is the failure of the STEP trial in which HIV-specific T cell responses were elicited by a 3-dose vaccination regimen, but failed to prevent HIV infection in field efficacy trials [42]. This latter case symbolizes the many challenges faced with development of T cell vaccines; although vaccine-induced T cells can be quantitatively measured, there is no accepted correlate of immunity that is based on a certain number of pre-existing T cells or type of vaccine-elicited T cell response. Bearing this in mind, it is difficult to know if an appropriate level of T cell memory has been reached or how protective the vaccine-induced T cells can be on their own – especially since there is growing consensus that for the most difficult infectious diseases (e.g., AIDS, malaria, and tuberculosis) it is likely that a combination of effective pathogen-specific humoral and cell-mediated immunity may be needed in order to generate an effective vaccine. More work is needed in this area and identification and validation of T cell-specific correlates of immunity (or co-correlates of immunity if both T cells and antibody are required for vaccine-mediated protection) will be an important step forward in this field.

4. Role of adjuvants in determining the magnitude and duration of immunity

Adjuvants have been used extensively in clinical settings to enhance the efficacy of inactivated or recombinant vaccine antigens [43–45]. In general, adjuvants have been shown to increase the magnitude of vaccine-mediated immune responses, which may manifest as increased seroconversion rates, antigen dose sparing effects, or the ability to use fewer immunizations to achieve comparable levels of immunity [45]. However, there is also the expectation that advanced adjuvant systems, specifically those directed towards innate immune system signaling pathways, may be able to direct the type of immunity elicited following vaccination and better match pathogen-specific immunity requirements (e.g., skewing of cellular vs. humoral immunity) [44, 45]. A wide array of adjuvants have been developed, but due to high reactogenicity and associated safety concerns, only a limited number are currently used in licensed vaccines [44]. In the U.S., only aluminum salts (collectively known as alum) and MPL (monophosphoryl lipid A; a detoxified form of LPS) are currently approved for humans, while European regulators have allowed a more extensive list of human adjuvants, including oil-in-water emulsions (MF-59 and AS03), as well as the use of virosomes for enhanced immunogenicity [44, 45].

Of the adjuvants currently in clinical use, aluminum salts have the longest history, with >70 years of experience in human vaccines [46]. While the true mechanism by which these adjuvants potentiate immune responses remains controversial [47], their ability to enhance the levels of humoral immunity is well established, with pre-clinical reports dating back to 1926 [46]. Due to this history of safety and efficacy, aluminum salts continue to be used frequently in the development of new vaccine candidates. As a recent clinical example, an inactivated Ross River virus (RRV) vaccine candidate was tested at multiple antigen dose levels with or without aluminum hydroxide adjuvant [48]. Following three immunizations at the low dose vaccine level (1.25 μg), serum ELISA titers were nearly 5-fold increased with the addition of this adjuvant, thus demonstrating a clear dose-sparing effect. Although alum is effective at boosting humoral immunity, this adjuvant may be limited with respect to induction of cellular immunity and this is one of the many reasons why it will be important to have additional adjuvant choices [45].

A range of new adjuvants directed towards innate immune system pathways (in particular, Toll-like receptors, TLR) have entered the clinical vaccine setting [44, 45]. For this class of adjuvants, some of the most revealing studies have come from the recent introduction of a licensed HPV vaccine adjuvanted with MPL, a TLR4 agonist. The Cervarix HPV vaccine is comprised of virus-like particles (VLP) for two major serotypes of HPV (HPV-16/18) and is formulated with both aluminum hydroxide and MPL as the combination adjuvant, AS04. The Gardasil HPV vaccine utilizes a similar VLP-technology consisting of four serotypes of HPV (HPV-6/11/16/18), but uses only an aluminum hydroxyphosphate sulfate adjuvant. In a head-to-head comparison, the MPL-adjuvanted Cervarix vaccine elicited antibody responses that were approximately 2- to 9-fold higher than that observed with Gardasil [49]. Interestingly, despite the difference in absolute antibody titers, serum antibody decay patterns for both vaccines were remarkably similar, suggesting that the MPL-based adjuvant

had increased the magnitude of antibody responses but had not fundamentally altered the subsequent kinetics of the humoral immune response. In a companion study, HPV-vaccinated subjects were also tested for immunogenicity against heterologous non-vaccine types of HPV (HPV-31/45) [50]. In this study, the MPL adjuvant appeared to offer little to no benefit over the non-MPL-adjuvanted vaccine, suggesting that addition of a TLR-based adjuvant does not necessarily increase the breadth of the antiviral immune response. There are several challenges to making direct comparisons between these different HPV vaccines since a) the vaccine antigens, though similar, were generated through distinct manufacturing processes, b) they contain different aluminum adjuvants (aluminum hydroxide vs. aluminum hydroxyphosphate sulfate) and c) there are also potential caveats to comparing a bivalent vaccine formulation to a quadrivalent vaccine formulation. However, in another report comparing matched vaccine antigens (HPV-16/18) formulated with aluminum hydroxide vs. aluminum hydroxide with the addition of MPL (AS04 adjuvant formulation), the addition of MPL still demonstrated an advantage over aluminum hydroxide alone, with antibody titers enhanced by ~2- to 3-fold during the peak antibody response at one month following the final immunization [51]. Interestingly, by 4-years post vaccination the anti-HPV18 titers were only about 2-fold higher in the MPL/AS04 group and there was no significant difference between groups when measuring total serum antibody responses to HPV16. Together, these clinical results indicate that advanced adjuvant formulations containing TLR agonists can indeed enhance humoral immunity, but extended follow-up is required to determine the duration of this effect since the difference in immunogenicity may be less apparent over time.

5. All vaccines require boosters

One of the most common misconceptions in vaccinology is that inactivated vaccines and subunit vaccines are weaker immunogens and will require booster vaccination whereas live, attenuated vaccines are expected to elicit the same degree of durable protection as that achieved by natural infection. This does not appear to be the case since, with the possible exception of rubella, essentially all common childhood vaccines require booster vaccination regardless of whether they are non-replicating vaccines or live, attenuated vaccines (Table 1). Measles and mumps, for example, are often described as “childhood infections” because a person is typically infected once and then maintains lifelong immunity thereafter. However, unlike wild-type strains of measles and mumps, infection with the attenuated vaccine strains of these viruses is insufficient to provide long-term protective immunity after a single dose and a two-dose regimen is required in order to achieve sustained protection and broad herd immunity. Likewise, although recovery from natural polio infection will provide serotype-specific protection against reinfection, vaccination with the live, attenuated strains of virus used in the oral polio vaccine (OPV) or vaccination with formalin-inactivated polio virus (IPV) require multiple vaccinations in order to achieve sustained protective immunity. Infection with Variola virus, the causative agent of smallpox, induces essentially lifelong immunity [52] and comparisons of antiviral antibody and T cell responses following infection with vaccinia (i.e., smallpox vaccination) indicate that both orthopoxviruses elicit similarly durable antiviral immunity [53]. In a small study examining the U.S. monkeypox outbreak, partial to full protection against monkeypox was observed for decades after

smallpox vaccination [54]. Unlike Variola, which causes a systemic viral infection, vaccinia typically produces a localized cutaneous infection resulting in a pustular lesion described as a “Jennerian vesicle” [52] and despite rare but sometimes serious side effects, vaccinia virus (e.g., DryVax) provided >90% seroconversion after a single dose and was not purposefully attenuated for routine vaccination. In contrast, modified vaccinia Ankura (MVA) represents a highly attenuated strain of vaccinia developed through serial *in vitro* passage. This resulted in a virus that is largely incapable of replication in human cells and typically requires both high doses of virus and at least 3 vaccinations to elicit neutralizing antibody responses similar to one infection with vaccinia virus [55]. If only two MVA vaccinations are administered, then 71.2% seroconversion by $PRNT_{50}$ (plaque reduction neutralization titer-50) is observed at the peak of the immune response at 2 weeks after booster vaccination but this declines to just 28.8% seroconversion within 2 months post-booster vaccination [56]. Another interesting comparison of durable immunity is between natural varicella infection (i.e., chickenpox) and live, attenuated varicella vaccination. Following recovery from primary varicella, strong antiviral immunity is developed and the virus enters latency. Bearing this in mind, there was high optimism that when the first varicella vaccine was introduced, it would likely induce lifelong immunity after a single dose, especially since it is based on active infection with an α-herpesvirus capable of forming latency with the potential for intermittent internal “boosting” of the host through reactivation. In practice, this did not occur since the attenuated live virus vaccine elicits only short-lived immunity, with symptomatic breakthrough of chickenpox cases rising >30-fold from 1 to 9 years after primary vaccination [57]. The problem was not due to a low initial virus infection rate or “take” during primary vaccination since protection is high early after vaccination but then gradually declines as immunity wanes. Based on these studies, the ACIP now recommends a two-dose schedule for varicella.

Yellow fever is a mosquito-borne disease that elicits essentially lifelong immunity among survivors [58] and the live, attenuated yellow fever vaccine is often described as an example of how an effective vaccine can generate life-long immunity after a single dose. However, this is only partially true since lifelong protection is only sustained in a subpopulation of vaccinated individuals. Although virus-specific T cells are not likely to play a major role in controlling yellow fever [41], protective levels of yellow fever virus-specific antibody are achieved in 90–95% of vaccinees during the first year after yellow fever vaccination [59, 60]. However, immunity wanes thereafter and within 5–10 years, only 60–70% of subjects [59, 61] maintain antiviral antibody responses above the protective threshold of >1:10 by $PRNT_{50}$, which is similar to the log-neutralizing index (LNI) of 0.7 that is required for protection of rhesus macaques from lethal yellow fever challenge [62]. Prior to 2013, yellow fever booster vaccination was recommended every 10 years but the World Health Organization recently modified their position, stating that a booster dose is no longer necessary [63]. This viewpoint is not shared by all experts in the field and several groups have questioned the basis for this position [64, 65]. The bulk of evidence used to make the WHO decision on booster vaccination for yellow fever comes from a systematic review on this subject [66] and two of the larger independent studies described therein were performed in non-endemic countries (i.e., with little likelihood of re-exposure/natural boosting by yellow fever or related flaviviruses) and are particularly informative; The first study

identified neutralizing antibody responses for 30–35 years after vaccination in US World War II veterans [61]. However, when PRNT tests were performed, only 63–64% of these subjects (or 70% for those with at least two criteria indicative of prior vaccination) showed evidence of antiviral immunity above the protective threshold of >1:10. Likewise, a second study found that 75% (38/51) of yellow fever vaccine recipients demonstrated PRNT titers of >1:10 from subjects examined from 11–38 years after vaccination [59]. However, if the cross-sectional analysis is broadened to include subjects from 5–38 years after vaccination, then the data appears to show that only 69% (51/74) of subjects had PRNT titers of >1:10 [59]. Together, this indicates that protective antiviral antibody responses can indeed be long-lived after yellow fever vaccination in a subset of the population, but up to 30–40% of vaccinees may be left with a false sense of security due to loss of protective immunity when only a single vaccination is administered.

6. The duration of immunity does not necessarily equate to the duration of protection

There are many instances in which natural infection with a particular virus will elicit essentially life-long immunity whereas infection with a closely related live attenuated vaccine strain of the same virus will not provide sufficient levels of immunity to confer long-term protection (Table 1). What is the difference between natural infection vs. vaccination that results in the necessity of booster immunization in order to achieve and maintain protective immunity? Most likely the differences are related to the need for vaccine safety. Most live, attenuated vaccines have been selected on the basis that they replicate less efficiently in their intended host, resulting in reduced pathogenicity and an improved safety profile. However, if the vaccine strain replicates less efficiently and is cleared more rapidly, then the overall antigenic load will be reduced and this is likely to impact the magnitude of the ensuing antiviral immune response (Figure 2). At early time points after vaccination, immunity may reside above the threshold of protection but if it is at or near the protective level, then it could decline to below the protective threshold by the time it reaches the plateau phase. This may explain why vaccines as divergent as the non-replicating, acellular pertussis vaccine [6–8] and the live, attenuated varicella vaccine [57] both initially provide protective immunity that fades over a relatively short period of time. Booster vaccination may further increase the levels of immunity in the short-term (e.g., pertussis) and possibly the long-term (e.g., varicella, measles, mumps, etc.) and the outcome is likely to differ for each particular vaccine and the individual characteristics of the disease it was designed to prevent. Natural acute viral infection, on the other hand, often elicits a higher level of immunity that is more likely to plateau above the protective threshold after a single infection and thereby maintain long-term immunity as well as long-term protection (Figure 2). During longitudinal analysis of 45 subjects followed for up to 26 years (Mean: 15 years, Range; 5–26 years of observation), we found that antibody responses declined rapidly for the first 1–3 years after an infection/vaccination but then plateaued with a more gradual antigen-specific decline thereafter [11]. Similar results have been found in other studies involving vaccination against rubella [67] or yellow fever [59] in which a biphasic response occurs after vaccination or infection. Although representing only anecdotal evidence, when we examined the plateau phase of long-term antibody responses to measles, mumps, or rubella

in a small cohort of vaccinated subjects ([11]; Supplemental Appendix), the antibody responses appeared to be as durable as that observed following natural infection with these viruses. However, similar to previous studies on measles [68] and mumps [69], the set point level of the plateau was lower following vaccination than after natural infection, and resided nearer to the putative threshold required for protective immunity. In other words, following vaccination or infection, detectable antigen-specific immunity may be long-lived, but if it is not maintained above the protective threshold then protective immunity itself may not be long-lived. This may explain the dichotomy between durable protection mediated by natural viral infection and the lack of long-lived durable protection following a single infection with attenuated vaccine strains of the same virus (Table 1).

7. Lessons from vaccines that elicit immunity of differing duration

Vaccines have provided many success stories and even a requirement for a single booster vaccination is a small price to pay for durable immunity without necessitating severe or potentially life-threatening disease from natural infection. However, some vaccines are only partially effective even after multiple vaccinations (e.g., acellular pertussis) and development of better vaccination strategies that require fewer boosters is an important goal, especially in developing countries in which routine vaccination can be complicated by logistical challenges for completing primary vaccination regimens. One way to improve vaccines is to compare successful vaccines and identify potential factors that may be involved with determining long-term vaccine efficacy. In broad terms, vaccines can be categorized into three general classes: multivalent non-protein antigen, monovalent protein antigen, and multivalent protein antigen (Figure 3). According to the imprinted lifespan model for induction of long-lived plasma cells [22], stimulation of T cell-independent antibody responses will be relatively short-lived without T cell help. Although monovalent protein antigens will elicit better, more durable antibody responses (due to acquisition of T cell help), the most long-lived antibody responses are predicted to occur when a multivalent antigen triggers strong B cell activation as well as effective T cell help.

Polysaccharide vaccines such as Pneumovax-23 are able to elicit B cell activation through clustering of the B cell receptor (BCR) but without an associated protein in the vaccine, this occurs in the absence of cognate T cell help by $CD4^+$ T follicular helper cells (T_{FH}). This T-independent antibody response provides only a limited duration of protection in elderly subjects [70] and antibody responses decline to baseline levels within 3–5 years after vaccination [71]. Moreover, T-independent antibody responses fail to establish memory and re-vaccination with purified polysaccharides have been found to lower serum antibody responses, and decrease the frequency of antigen-specific memory B cells [72]. To overcome these limitations, newer polysaccharide vaccines such as Prevnar-13 are conjugated to a carrier protein (e.g., CRM197, a detoxified mutant of diphtheria toxin) and this not only results in an antibody response of higher avidity, but immunological memory is more long-lived [71].

Vaccination with monovalent protein antigens such as tetanus and diphtheria are less likely to trigger BCR clustering than multivalent antigens but since BCR-mediated antigen uptake results in presentation of peptide antigens to $CD4^+$ T_{FH} cells, this facilitates B cell:T cell

contact and the additional costimulation from these interactions elicits stronger and more durable antibody responses than that observed with T-independent antigens such as non-conjugated polysaccharide vaccines. Tetanus and diphtheria toxoid vaccination induce intermediate to long-lived antibody responses that decline with an 11-year and 19-year half-life, respectively [11] and although adult booster vaccination is currently recommended every 10 years in the United States, other countries such as the United Kingdom no longer recommend adult booster vaccination [73] due to the observation of long-lived vaccine-mediated protection in the absence of further immunization. One reason for this is that the duration of protection is a function of the magnitude of the antibody response as well as the half-life of the antibody response and if neutralizing antibody levels begin at a high level, then long-term protective immunity may be maintained potentially for decades even with an 11-year antibody half-life (Slifka, manuscript in preparation).

Unlike monovalent vaccine antigens such as tetanus and diphtheria that require a 3- to 5-dose primary vaccination regimen in order to achieve long-term protective immunity, multivalent vaccine antigens and many natural acute viral infections elicit long-term immunity after only one or two antigenic exposures. For example, after contracting measles, long-term protective immunity is often sustained for life and according to this model (Figure 3) this is likely due to multiple factors including increased crosslinking of the BCR and enhanced antigen presentation to $CD4^+$ T_{FH} cells, resulting in longer T cell:B cell “dwell time” interactions that provide the signals that may be necessary for imprinting a longer lifespan in daughter cells destined to become long-lived plasma cells. Measles vaccination is likely to induce the same immunological signaling interactions since it represents a closely related live paramyxovirus infection. However, with the milder infection and reduced antigen load associated with vaccination vs. natural infection, the level of long-term immunity may require booster vaccination to reset the plateau phase of antibody production to a new set point that resides above the threshold required for protection (Figure 2). Immunization with live, attenuated hepatitis A vaccine or inactivated hepatitis A vaccine are both capable of eliciting long-term antiviral protection [74] but interestingly, a 2-dose vaccination series with an inactivated whole virus hepatitis A vaccine has been estimated to provide more than 30 years of protective immunity in >95% of vaccinees [75]. A recent study examining the duration of antibody responses following 1, 2, or 3 doses of Cervarix, a highly repetitive non-infectious virus-like particle vaccine against human papilloma virus (HPV), is also particularly informative [76]. In this study, subjects were followed for up to 4 years after primary vaccination and the data shows that a single immunization induces a biphasic response that peaked at the 1 month time point, declined sharply by the 12 month time point, but then showed a stable plateau phase of prolonged antibody production from 1 to 4 years after vaccination that was higher than that observed after natural HPV infection. This shows that a single vaccination with an optimized multivalent protein antigen is capable of eliciting persistent antibody responses. A 2-dose or 3-dose vaccination schedule resulted in about a 5-fold higher antibody level during the plateau phase compared to the 1-dose schedule but the durability of the long-term antibody response was notably similar. A recent report has also now shown that a two-dose schedule of Gardasil is non-inferior to the 3-dose schedule [77] and more studies are needed to determine if the levels of serological immunity following a single dose of HPV vaccine might be sufficient to provide long-term

homologous and heterologous protection. If this is observed, then it could have important implications in the vaccination of vulnerable populations in developing countries with a high burden of HPV-associated disease.

8. Potential mechanisms underlying the development of durable humoral immune responses

One of the most intriguing questions regarding human vaccination is why certain vaccine antigens induce robust lifelong antibody responses, while protective immunity to other antigens fades with time. As discussed earlier, we found that humoral immunity to viruses tends to be long-lived, while immunity to non-repetitive protein antigens declines more quickly [11]. For example, long-term antibody responses to measles, mumps, and rubella demonstrated no significant decline in antibody when followed longitudinally for up to 26 years. Other studies examining the duration of antibody to measles (in addition to mumps and rubella) also indicated essentially lifelong immunity when extrapolated from antibody titers measured longitudinally for up to 2 years after sustained peripheral B cell depletion by anti-CD20 treatment in rheumatoid arthritis patients [78]. This provides mechanistic insight into how long-term antibody responses are maintained since long-lived plasma cells appear to sustain antigen-specific antibody levels despite the loss of peripheral $CD20^+$ B cells for a prolonged period of time. By comparison, antibody responses to tetanus and diphtheria show more rapid decay rates, with average half-lives in the range of 11–19 years [11]. These broad differences in antibody maintenance patterns indicate that antigen-specific plasma cell populations can have distinct lifespans. Since terminally differentiated plasma cells show little to no expression of surface immunoglobulin, MHC Class II molecules or other key B cell signaling receptors [79, 80], it is likely that the lifespan decision is made prior to differentiation into a plasma cell. According to the imprinted lifespan model, those B cells capable of binding antigen through the BCR and presenting to T cells will receive additional signals to allow recruitment into the long-lived plasma cell pool with multivalent protein antigens providing a survival advantage over monovalent protein antigens (Figure 3). In practice, there appear to be some exceptions to this rule. For instance, the RTS, S malaria vaccine is comprised of a repetitive, particulate antigen coupled with a strong adjuvant [81] and based on the imprinted lifespan model this should elicit long-term immunity. However, this vaccine elicits protective immunity in ~36% of subjects at the time of vaccination but protection wanes to 0% after 3 years [82]. This could mean that not all highly repetitive particulate antigens induce long-term immunity or it is possible that this vaccine induces a biphasic antibody response in which the early antibody response resides above a protective threshold but then declines to below the protective threshold at later time points. This would not be surprising and would mimic the results obtained following MMR vaccination in which immunity can be long-lived, but booster vaccinations are needed to elicit antiviral antibody responses that are maintained above a protective threshold. Alternatively, the protective threshold may be relatively high since this is a subunit vaccine against a single *P. falciparum* circumsporozoite antigen and this may be similar to acellular pertussis vaccines in which immunity to a few antigens from a complex bacterial pathogen afford short-term immunity during the first year after vaccination, but does not elicit broad enough long-term immunity once the antigen-specific immune response declines to homeostatic levels.

Although longitudinal analysis of antigen-specific serum antibody responses supports the position that different vaccine classes can induce distinct patterns of antibody maintenance [11, 12, 78], the cellular mechanisms that underlie these differences are less well defined.

The germinal center (GC) reaction is believed to be the central point for antigen-specific B cell fate selection. The GC is a transient structure that forms within B cell zones of lymphoid tissues following antigenic exposure, either through natural infection or immunization, with the first description of this structure dating back more than a century [83]. During its development, the GC segregates into a dark zone (DZ) and a light zone (LZ). The DZ is made up almost entirely of dividing B cells undergoing somatic hypermutation (SHM), while the LZ contains a more limited number of B cells, in addition to follicular dendritic cells (FDC) and specialized $CD4^+$ T_{FH} cells [84]. Based on this segregation, a general model of the GC reaction developed, wherein the DZ represents a region dedicated to antibody diversification achieved through proliferation and SHM of the immunoglobulin variable genes. Following this diversification and expansion, newly formed B cell clones migrate to the LZ and compete for survival signals through interactions with antigen displayed on FDCs, in conjunction with matched $CD4^+$ T cell help [83, 85]. The B cell clones able to successfully compete are recruited into the memory pool (either long-lived plasma cells or memory B cells), while those unable to compete effectively are deleted through apoptosis. In this way, only high affinity B cell clones are selected for subsequent memory responses. Recent advances in the ability to visualize different cell populations and track antigen *in situ* have expanded this standard model, adding important detail regarding the selection and cycling of B cells during this process [83]. In general, there is growing support to the theory that B cells are primarily selected for survival based on obtaining help from a limited number of $CD4^+$ T_{FH} cells in the GC, with other factors including competition for limiting antigen and possibly also Fc receptor signaling playing a role in B cell fate decisions [83].

Since activated B cell clones compete for interactions with T_{FH} cells, we speculate that these interactions may be key to imprinting plasma cells with different lifespans and multivalent or aggregated antigens may offer a competitive advantage in this regard. It is well established that soluble monomeric proteins are poorly immunogenic but chemical or heat-induced aggregation greatly increases immunogenicity [86]. Recent studies have provided further mechanistic insight into this phenomenon by showing that aggregated proteins trigger enhanced antigen uptake and migration by dendritic cells, resulting in stronger $CD4^+$ and $CD8^+$ T cell responses than that observed with homologous monomeric antigen [87]. This indicates that particulate antigens (e.g., virus particles or aggregated proteins) are more efficiently transported to the lymph node to initiate the adaptive immune response. I*n vitro* studies, using chimeric BCR constructs specific for different antigens have demonstrated that the quality of the BCR-antigen complex also has a substantial impact on the ability of B cell clones to present antigen and interact with cognate T cells [88]. B cell clones with higher affinity for antigen are able to better maintain the BCR-antigen complex, resulting in more efficient peptide presentation, and likely better competition for limited T cell help in addition to potentially more prolonged T cell:B cell interactions. Immunoelectron microscopy studies support this model, demonstrating that antigen remains bound to the BCR following internalization and transport to the MHC Class II peptide-loading

compartment, and this process is separate from the much less efficient uptake of antigen through pinocytosis [89]. Multimeric proteins with repetitive B cell epitopes will be bound by multiple BCR and the resulting avidity of these interactions is expected to be stronger than that observed with the affinity of a single BCR binding to a single epitope on a monovalent protein. Bearing this in mind, particulate multivalent antigen presented on microbeads is up to 100-fold more effective at triggering B cell uptake and peptide presentation to T cells than soluble antigen [90]. From a vaccine perspective, these data suggest that multivalent protein antigens could provide an advantage in antigen acquisition, downstream antigen processing/presentation, and the recruitment of $CD4^+$ T_{FH} cell help. Although further experimental studies are needed, we postulate that increased dwell time between antigen-specific B cells and $CD4^+$ T_{FH} cells and the associated increase in co-stimulation and local cytokine production [84, 91] could together provide a mechanism for imprinting dividing B cells specific for multivalent antigens with the ability to generate plasma cells with a longer lifespan (Figure 3).

Successful vaccines have changed modern medicine by reducing the morbidity and mortality associated with many infectious diseases. Despite these successes, some vaccines require improvement (e.g., acellular pertussis) and there are many more diseases to which no commercial vaccines currently exist. Analysis of vaccine efficacy and the duration of protective immunity are beginning to provide new details on the mechanisms underlying long-term immunity and several trends are beginning to emerge. In the absence of T cell help, polysaccharide vaccines elicit only short-lived immunity and no immunological memory. In contrast, T cell-dependent protein antigens elicit long-lived immunity but most vaccines require booster vaccination in order to raise the plateau level of long-term immunity above a protective threshold. In general, particulate antigens are more immunogenic than soluble antigens and highly repetitive multivalent proteins may have an advantage in triggering increased BCR clustering and improved antigen presentation to $CD4^+$ T_{FH} cells. More studies are needed to determine how these interactions can be optimized to induce long-lived protective immunity with the fewest number of vaccinations and further analysis of the mechanisms underlying successful vaccines will be important for improving future vaccine design.

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Highlights

- We discuss the importance of vaccines in preventing a number of infectious diseases
- We examine the general requirement for booster vaccination
- We describe how the duration of immunity may differ from the duration of protection
- We present potential mechanisms underlying the development of durable immunity

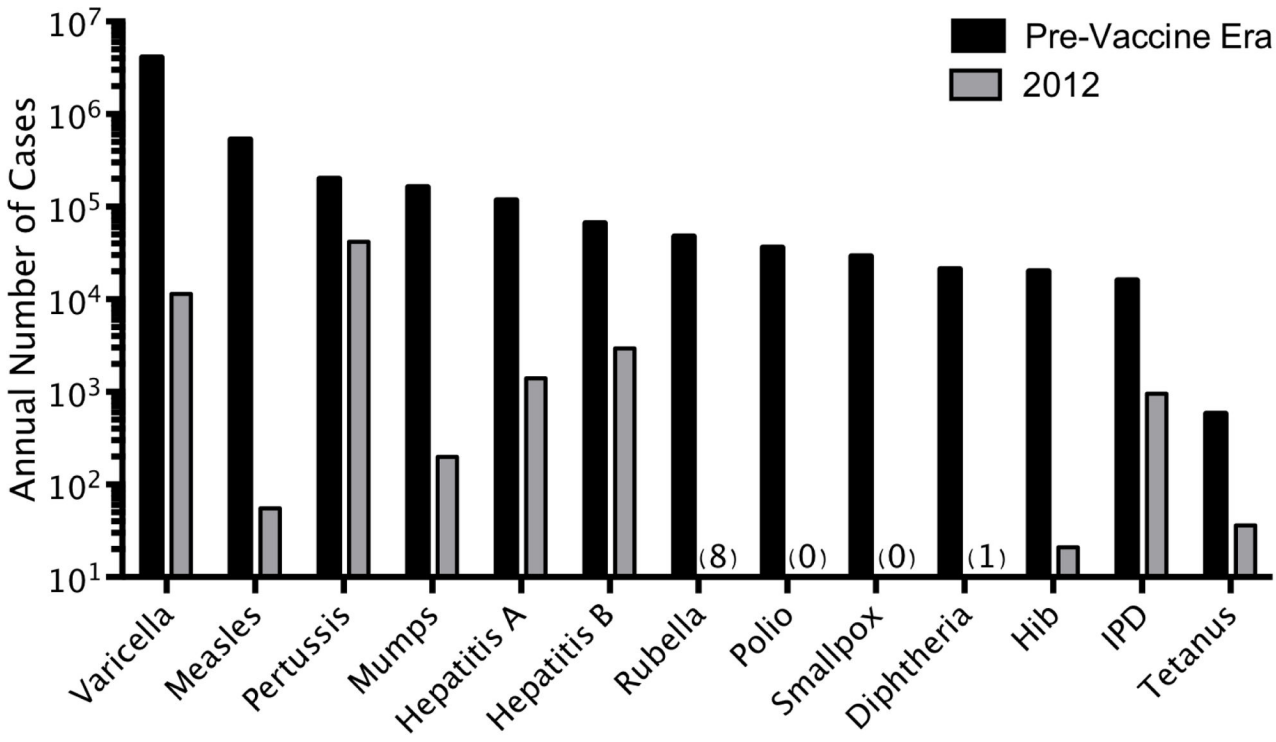

Figure 1. Vaccination reduces the incidence of infectious disease

Values represent the number of annual cases of disease that occurred in the United States during the pre-vaccine era (adapted from [2]) compared to the number of cases for each disease reported to the CDC in 2012 [3]. Invasive pneumococcal disease (IPD, *Streptococcus pneumoniae*) and *Haemophilus influenzae* type b (Hib) case numbers refer to children <5 years of age. Case numbers for polio include both paralytic and non-paralytic forms of the disease. For varicella, the reported incidence in 2012 was 11,477 cases, but this is likely to be underreported due to challenges in clinical diagnosis of milder vaccine-modified cases [92]. For diseases with an incidence of ≤10 cases in 2012, the number of total cases is indicated in parentheses.

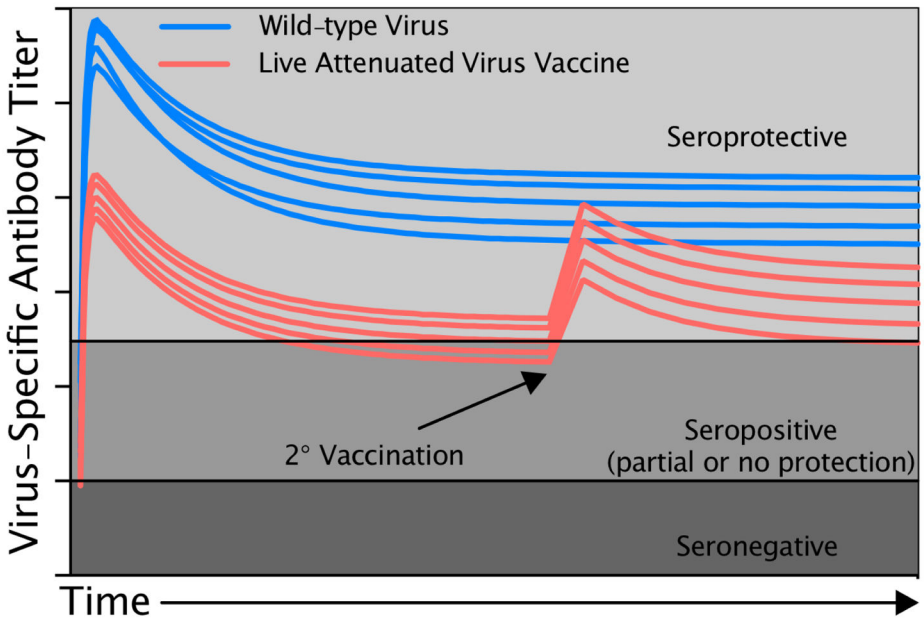

Figure 2. Relationship between long-term immunity and long-term protection

In this illustration, natural infection with a wild-type virus (e.g., measles virus) elicits immunity that peaks shortly after infection, declines for a period of time, and then reaches a plateau phase of long-term maintenance. Following infection with a live, attenuated vaccine (e.g., MMR), the kinetics of the antiviral immune response mirror that observed following natural infection but the level of immunity during the peak and plateau phase may be lower and the plateau may reside at or below the protective threshold and therefore provide only partial protection despite the maintenance of a measurable seropositive immune response. Following booster vaccination, antiviral immunity is increased and if the set point of the new plateau phase resides above the seroprotective threshold, then long-term immunity as well as long-term protection is maintained.

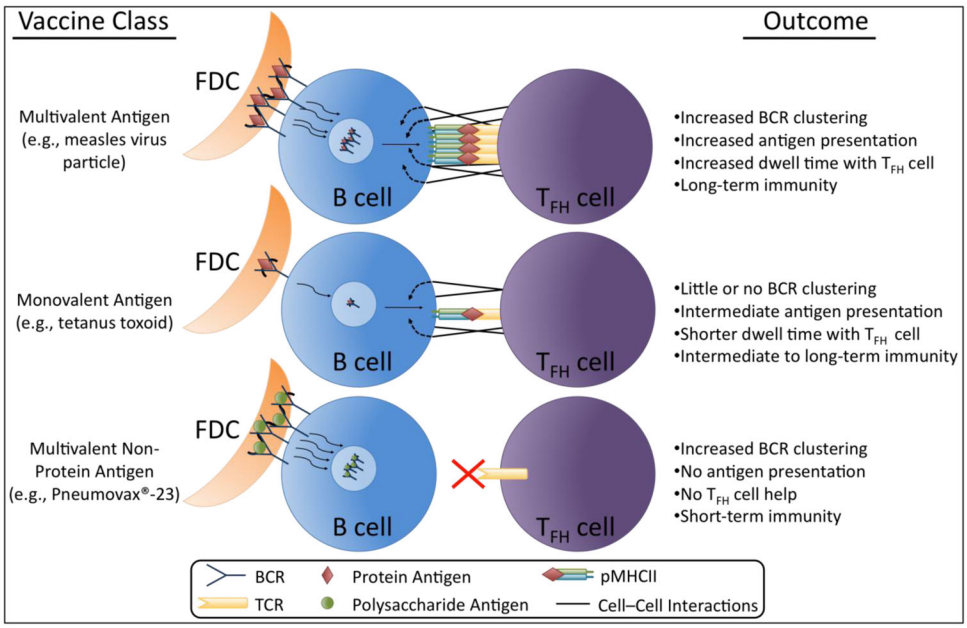

Figure 3. Characteristics of a vaccine antigen determine subsequent levels and duration of immunity

A putative multivalent protein antigen is shown in comparison to a monovalent protein or non-protein antigen. In this example, B cell clones extract antigen from follicular dendritic cells (FDC), followed by processing (protein antigens only) and presentation to T follicular helper (T_{FH}) cells. Several lines of evidence indicate that multivalent interactions increase B cell receptor (BCR) clustering and improve the ability of B cells to secure antigen from antigen presenting cells [88–90, 93–95]. This multivalent interaction also stabilizes the antigen-BCR complex and leads to an increase in peptide-loaded MHC Class II complexes (pMHCII). Increased presentation of pMHCII to cognate $CD4^+$ T_{FH} cells, in addition to other cell-to-cell interactions (e.g., CD40-CD40L, etc.), can lengthen the dwell time between B cells and T cells, improving the survival for a particular B cell clone. Although more studies are needed, the combination of increased BCR clustering and increased antigen presentation to T_{FH} cells by multivalent proteins may play a role in imprinting an increased plasma cell lifespan and sustained antibody production.

Table 1

Artificially attenuated virus vaccines requiring booster vaccination.

Wild-type virus	Live, attenuated vaccine counterpart	Virus family
Measles	MMR	*Paramyxoviridae*
Mumps	MMR	*Paramyxoviridae*
Polio	OPV	*Picornaviridae*
Smallpox, Vaccinia*	MVA	*Poxviridae*
Varicella Zoster Virus	VZV-Oka	*Herpesviridae*
Yellow fever	YFV-17D**	*Flaviviridae*

In this Table, examples of wild-type viruses that are known to elicit long-term protective immunity are compared to their vaccine counterparts, all of which require booster vaccination in order to achieve long-term protective immunity.

*Vaccinia virus was not purposefully attenuated for routine smallpox vaccination.

**The World Health Organization recently changed yellow fever booster vaccination recommendations from once every 10 years to requiring only one vaccination [63].

Abbreviations; MMR; measles, mumps, rubella, OPV; oral polio vaccine, MVA; Modified Vaccinia Ankura, VZV; varicella zoster virus, YFV; yellow fever virus