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Commentary

Why and How Vaccines Work

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Vaccines save millions of lives from infectious diseases caused by viruses and bacteria. As the world awaits safe and effective COVID-19 vaccines, we celebrate the progresses made and highlight challenges ahead in vaccines and the science behind them.

Introduction

Vaccines have substantially reduced the burden of infectious diseases. An estimated 103 million cases of childhood diseases were prevented between 1924 and 2010 in the United States through vaccination (van Panhuis et al., 2013). In particular, the eradication of smallpox through vaccination in 1980 is one of the crown achievements of medicine. Until then, smallpox had afflicted humanity for at least 3,000 years, killing 300 million people in the twentieth century alone. Vaccines prevent diseases caused by a large number of viruses and bacteria, and those against parasites are under development. Vaccines are also one of the most effective investments in humanity. Every dollar spent on vaccines yields an estimated \$44 in economic returns, by ensuring children grow up healthy and are able to reach their full potential (Ozawa et al., 2016).

In 2020, we are in a midst of a once-in-a-century pandemic. We discuss the birth and evolution of vaccine science, how vaccinations have changed our world, the current state of vaccines, the remaining challenges, and their future outlook.

Evolution of Vaccine Science

Edward Jenner is duly credited with providing the first scientific description of vaccination when he published his monograph *An Inquiry into the Causes and Effects of the Variolae Vaccinae* in 1798. Although, notably, variolation (i.e., inoculating people with material from

smallpox cases) was practiced in China, India, and Turkey for centuries before it was introduced to the West by Lady Mary Montague—the wife of the British ambassador to the Ottoman court. Vaccination itself, which involved injecting material from cowpox vesicles to healthy individuals was first demonstrated by Benjamin Jesty, a Yetminster, England farmer, approximately a quarter century before Jenner's vaccine demonstration.

While Jenner's technique for vaccination was relatively widely used throughout the nineteenth century, vaccination was conducted from person-to-person or animal-to-animal, i.e., material from a vaccinated individual was used to vaccinate another individual.

The modern science of vaccination was developed by Louis Pasteur. Pasteur developed vaccines in the laboratory using the same agent that caused the disease, starting with chicken cholera vaccine. In 1879/1880, Pasteur used a culture of chicken bouillon to develop a chicken cholera vaccine that could be produced in a lab. Five years later, he followed this with a human rabies vaccine.

The next major innovation came from American scientists Daniel Elmer Salmon and Theobald Smith when they pioneered development of vaccines based on killed pathogens. Others took advantage of techniques developed by Pasteur, Salmon, Smith, and their contemporaries and developed vaccines against typhoid, cholera, and plague before the end of the nineteenth century. Throughout the

twentieth century, more and more infectious diseases—ranging from influenza to rotavirus—became vaccine-preventable. These vaccines were either live attenuated, whole killed pathogens, or alternatively, so-called subunit vaccines that contained antigens (e.g., protein, polysaccharides, or conjugated) but not the rest of the pathogen. A major development happened in 1986 when the first genetically engineered vaccine—the Hepatitis B surface antigen recombinant vaccine—became available. However, until the last couple of decades, vaccines were developed using empirical approaches. More recently, in parallel with increasing availability of sequencing and bioinformatics tools, there has been an increased focus on so-called “rational” vaccine design approaches.

History of Adjuvants

Vaccines were used successfully long before it was understood how they worked. When live-attenuated vaccines were used, they alone were sufficient to induce robust long-lasting immunity. However, in an effort to develop recombinant protein vaccines against diphtheria and tetanus, injection of these proteins in isolation only elicited weak and short-lived antibody responses. Upon trial and error, Gaston Ramon, a French veterinarian and later director of the Pasteur Institute, noticed that horses that received the vaccines developed better immune response if there was inflammation at the site of injection. Later, Ramon



discovered that certain substances (tapioca, lecithin, agar, starch oil, saponin, or breadcrumbs!) can be added to the vaccine to improve the immune response (Christensen, 2016). These observations were followed by the discovery that diphtheria toxoid precipitated with aluminum salts resulted in significant increase of the immune response (Glenny et al., 1926). Since then, Alum (aluminum salts) became the mainstay of adjuvants until about 20 years ago, when the molecular mechanism of adjuvanticity spurred the development of new adjuvants.

Science of Adjuvants

We now know that live-attenuated (and to some degree, inactivated) vaccines have worked well because they provide the two requisite signals to induce immunity: the antigen and the natural “adjuvant.” The antigens direct the specificity of the adaptive immune response toward a particular pathogen, while the adjuvants stimulate the innate immune system through pattern recognition receptors (PRRs), which recognize pathogen-associated molecular patterns (PAMPs) (Medzhitov and Janeway, 1997). In order for an antigen to be immunogenic, that antigen must be accompanied by PAMPs that can trigger PRRs in antigen-presenting cells. The reason adjuvants need to be included in vaccine formulations was made clear by the discovery that innate immune recognition via PRRs generates signals required for activation of adaptive immunity (Medzhitov and Janeway, 1997). PRRs include Toll-like receptors (TLRs) that detect structural PAMPs, cytoplasmic viral nucleic acids sensors such as RIG-I and cGAS, and others that detect pathogen activities. We now also understand that dendritic cells are the key cell type responsible for triggering adaptive immune responses based on pioneering work of Ralph Steinman. Engagement of the various PRRs expressed on distinct dendritic cell subsets trigger their activation and migration to draining lymph nodes, where lymphocytes of the adaptive immune system are instructed to take on specialized effector functions suited to combat a given class of pathogens.

Now that we understand the mechanism by which adjuvants stimulate robust immunity, it is possible to design them to

achieve desired outcomes. Currently, there are several licensed adjuvants that are in use for human vaccines (Figure 1). In addition to alum, TLR agonists, monophosphoryl lipid A (MPL—a TLR4 agonist) and CpG 1018 (TLR9 agonist) are approved and used as vaccine adjuvants. In addition, a number of other adjuvants are in current use including virosomes, MF59, ISA51, and a line of adjuvant systems (AS) developed by GlaxoSmithKline—AS01 (liposome with MPL + QS-21), AS04 (3-deacyl-MPL), AS03 (vitamin E/Surfactant polysorbate 80/Squalene). For SARS-CoV-2 vaccines, there are other adjuvant systems in various phases of clinical trials, including Matrix M and Advax (Gupta and Gupta, 2020) (Figure 1). The numerous clinical trials for COVID-19 vaccines provide a rare opportunity for these new adjuvants to be evaluated for safety and efficacy for human use.

Types of Vaccines

Advances in virology, molecular biology, and immunology have created many alternatives to the traditional vaccines (Figure 2A). Modern vaccines include nucleic acid based (mRNA, DNA), viral vectored vaccines, virus-like particles, and recombinant protein (subunit) vaccines. For extracellular bacterial pathogens, conjugate vaccines that elicit antibodies to the carbohydrate moieties unique to the bacterial walls emerged as an alternative to the whole killed bacteria. COVID-19 has brought all vaccine types to the forefront to combat the pandemic (Figure 2B). Because of the speed of cloning and synthesis, mRNA and DNA vaccines were the first to enter the race in the United States.

Depending on the type of vaccines, they either contain PAMPs as endogenous (e.g., genome of the vaccine virus) or intrinsic (inactivated viral genome) components of the vaccine. Others do not contain PAMPs and require adjuvants. Except for some live-attenuated vaccines that produce antigens for an extended time period, most vaccines require booster shots to enhance levels and affinity of antibody responses (Figure 2).

Progress and Challenges

Vaccines have yet to conquer the world’s most lethal and debilitating infections: malaria, *Mycobacterium tuberculosis* (MTB), and HIV-1. These pathogens are difficult

to tackle, as we still do not understand how to elicit the protective immunity or how to counter pathogens’ evasion mechanisms. Natural infection with these agents does not lead to protection from reinfection, and there are no immune correlates of protection to emulate with vaccines. However, promising progress is being made against these pathogens.

Malaria is caused by plasmodia parasites that have complex life cycles with a myriad of antigens. RTS,S/AS01 is the most advanced vaccine candidate that has completed a Phase 3 trial (Agnandji et al., 2011). The vaccine prevented 39% cases of malaria over 4 years of follow-up and 29% cases of severe malaria, making this the first vaccine to have significantly reduced infection and disease. The vaccine consists of the repeat (antibody target) and T cell epitope in the circumsporozoite protein of the *Plasmodium falciparum* malaria parasite and a viral envelope protein of the hepatitis B virus, given with the adjuvant AS01. Based on these encouraging results, pilot vaccinations began in the three countries in 2019: Malawi, Ghana, and Kenya. The pilot vaccination is expected to continue through 2023, which will inform future widespread use of this vaccine (<https://www.who.int/malaria/media/malaria-vaccine-implementation-qa/en/>).

Another breakthrough was reported for a therapeutic TB vaccine candidate, M72/AS01E. A significant protection against disease was reported in a Phase IIb trial conducted in Kenya, South Africa, and Zambia in individuals with latent tuberculosis infection. M72 is a subunit fusion protein vaccine derived from two MTB antigens (32A and 39A) with AS01E adjuvant. The vaccine provided 54% protection in latently infected adults against active pulmonary tuberculosis disease, over 2 years of follow-up (Van Der Meeren et al., 2018).

Despite much effort and resources dedicated to developing HIV-1 vaccines, many candidates have failed to show efficacy in clinical trials. In addition to HIV-1, there are many neglected tropical diseases that require vaccines.

Where Vaccine Science Is Headed

Systems vaccinology (Pulendran et al., 2010) incorporates systems biology approaches using multidisciplinary high-dimensional datasets to better inform

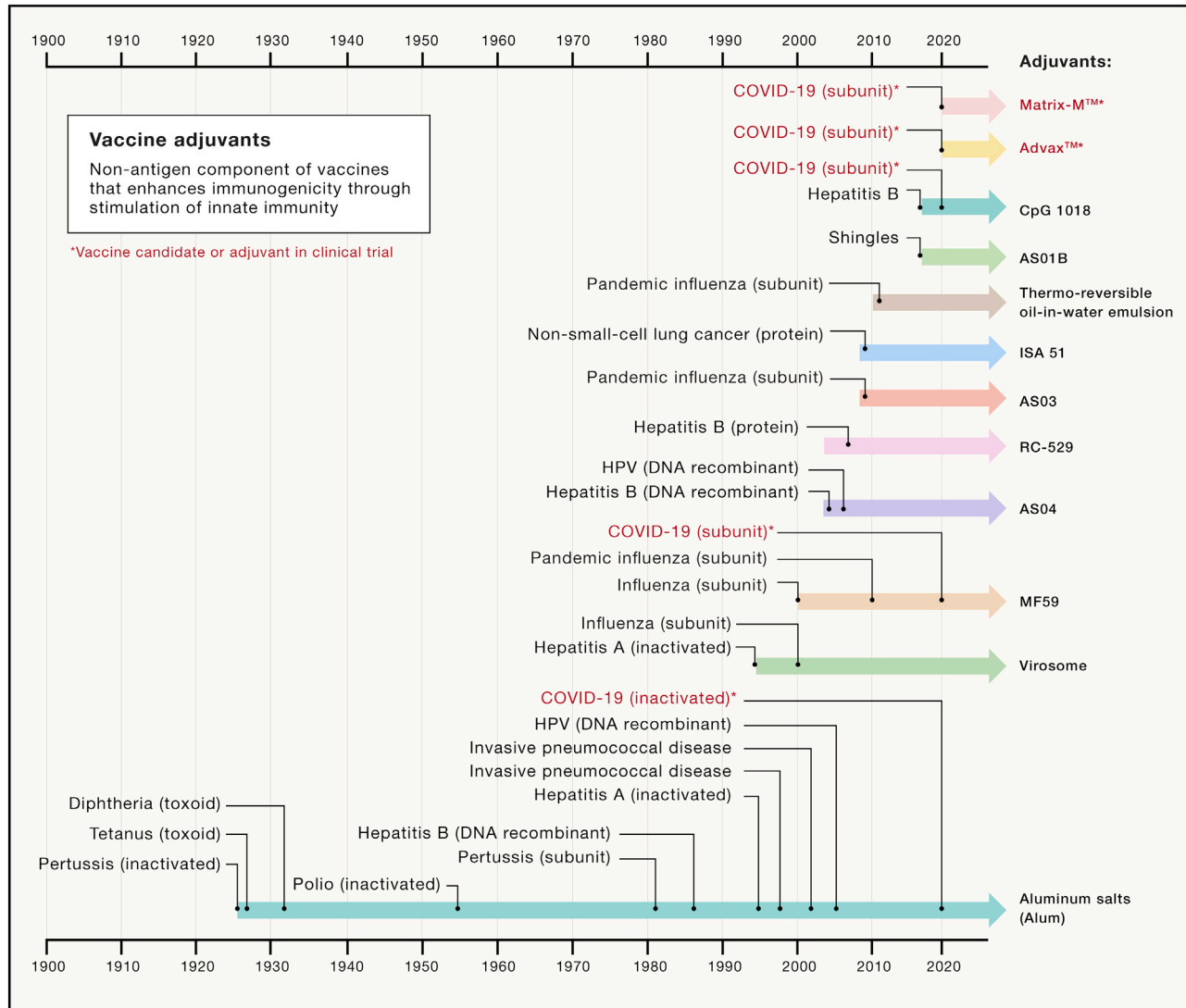


Figure 1. Timeline of Adjuvant Used in Human Vaccines

Adjuvants are non-antigen components of vaccines that stimulate the innate immune system. Adjuvants are indicated by thick arrows from the time of introduction. Vaccines that use the adjuvants are indicated as dots on the arrow at the earliest time of use. Image was made by BioRender.

vaccines—from the discovery phase of design of the vaccine all the way to predicting responses in clinical trials and improving on implementation strategies. Systems vaccinology has been applied to multiple vaccines, including influenza viruses and yellow fever, and revealed an unexpected correlation between gene signatures and vaccine efficacy (Pulendran et al., 2010). Further, the systems serology approach has been applied to HIV-1 vaccines (Chung et al., 2015) that reveal potential antibody correlates of protection.

Another emerging area of vaccinology is T cell vaccines. While antibodies are

the focus of almost all vaccines, and currently levels of antibodies raised against the vaccine antigens are used as correlates of protection, not all viruses are amenable to antibody-dependent immunity. Some viruses have circumvented the ability of antibodies to control them, including HIV-1 (through rapid in-host mutation and escape from antibody recognition), influenza virus (through antigenic drift to avoid previous season's antibody recognition), and herpes simplex virus (through the expression of evasin molecules on virion surface that render antibodies useless). For these

types of antibody-evasive viruses, we need a different approach to vaccination. Fortunately, there are conserved epitopes that can be used to generate T cell immunity through vaccines. A key aspect of T cell immunity is that it works best if the T cells are already present at the site of entry, i.e., the mucosal surface. However, vaccines injected into muscle often fail to induce mucosa-resident memory T cells. A two-step vaccine strategy, prime and pull, can overcome this distribution problem by recruiting and establishing tissue-resident memory T cells in a tissue of choice (primary route









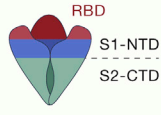



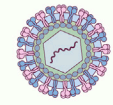

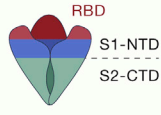
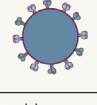



















A Licensed for use					B In clinical trial for COVID-19						
Vaccine type	PAMP	Examples (route if not IM/ID)	Adjuvant	Booster	Vaccine type	PAMP	Examples (route if not IM/ID)	Adjuvant	Booster		
 Live attenuated	 Endogenous	Measles	None	Yes	 mRNA	 RNA	Spike mRNA	None	Yes		
		Mumps	None	Yes			RBD mRNA	None	Yes		
		Rubella	None	Yes			 DNA	 DNA	Spike DNA	None	Yes
		Rotavirus (oral)	None	Yes							
		Yellow Fever	None	No							
		Chicken pox	None	Yes							
		Polio Sabin (oral)	None	Yes							
		Live zoster	None	No							
		BCG	None	No							
Influenza (nasal: FluMist)	None	Annual									
 Killed	 Intrinsic	Whole cell pertussis	None	Yes	 Recombinant protein	 None	Spike	Matrix-M	Yes		
		Polio Salk	None	Yes			Novavax	CpG 1018	Yes		
 Split	 Intrinsic	Seasonal influenza	None	 Viral vector	 Endogenous	 S1-NTD S2-CTD	Novaxin	Advax	Yes		
		Flud for > 65 yr.	MF59				Annual	University of Queensland	MF59	Yes	
 Virus like particles	 Incorporated*	HPV Guardasil 9	Alum	Yes	 Inactivated	 Intrinsic	RBD	Alum	Yes		
		HPV Cervarix	AS04	Yes			Sinovac	CpG	Yes		
 Toxoid	 None	Diphtheria	Alum	Yes	 Wuhan/Sinopharm	 None	Beijing/Sinopharm	Alum	Yes		
		Tetanus	Alum	Yes			Institute of Medical Biology, Chinese Academy of Medical Sciences	Alum	Yes		
 Recombinant subunit	 None	Hep A Havrix	Alum	Yes	 Research Institute for Biological Safety Problems, Rep of Kazakhstan	 None	Biological Safety Problems, Rep of Kazakhstan	None	Yes		
		Hep A Vaqta	Alum	Yes							
		Hep B Engerix-B	Alum	Yes							
		Hep B Recombivax	Alum	Yes							
		HepA/Hep B Twinrix	Alum	Yes							
		Hep B Hepisav-B	CpG	Yes							
		Acellular pertussis	Alum	Yes							
		Zoster Shingrix	AS01B	Yes							
Influenza Flublock	None	Annual									
 Conjugate	 None	MenB Bexsero	Alum	Yes	 None	 None	None	Yes			
		MenB Trumenba	Alum	Yes							
		Pneumococcal Pneumovax	Alum	Yes							
		Pneumococcal Prevnar 13	Alum	Yes							
 Polysaccharide	 None	Pneumococcal polysaccharide PPSV23	None	Yes	 None	 None	None	Yes			

Figure 2. Vaccines, PAMPs, and Adjuvants

(A) Vaccines that are approved for use in humans and their PAMPs. For vaccines that are devoid of PAMPs, adjuvants are required to induce robust immunity. Booster vaccines are required for many vaccines to achieve protective levels of antibodies.

(B) Vaccines that are in clinical trials for COVID-19. Image was made using BioRender.

*While virus like particles lack viral genomes, during the assembly process, some nucleic acids (RNA) may become packaged, serving as PAMPs.

‡This vaccine is a two-dose vaccine: rAd26-S injection followed by rAd5-S injection.

of viral entry) using chemokines or chemokine-inducing agents (Iwasaki, 2016). T cell-based vaccines hold promise for antibody-evasive pathogens and cancer vaccines in which no surface antigens can be targeted.

Another frontier in vaccine science is to develop mucosal vaccines. Immune effector mechanisms present at the site of vaccine entry offers superior protection. Most pathogens, except for vector-borne, enter the human body through mucosal surfaces. Unlike the skin, mucosal epithelial layers are vulnerable to pathogen entry due to the lack of cornification. There are two types of mucosal surfaces: type 1 surface is simple columnar epithelia (example: gut, lung, endocervix), whereas type 2 surface consists of stratified squamous epithelia (example: eyes, nose, vagina, ectocervix). These two types of epithelial layers use distinct adaptive immune mechanisms of protection, and thus a vaccine must elicit type-appropriate effector responses (Iwasaki, 2016). Of note, type 1 surface epithelium expresses polymeric immunoglobulin (Ig) receptor (pIgR) capable of transporting dimeric IgA to the lumen, whereby it can neutralize incoming pathogens or toxins. Type 2 surface lacks pIgR and relies on IgG for protection. In the respiratory tract, IgA provides protection in the nasal cavity, whereas IgG provides protection in the lung. Both types of mucosal can host tissue-resident memory T cells. Mucosal immunity provides opportunities to block infection altogether or sterile immunity. Vaccines delivered via mucosal surfaces (intranasal, oral) are more potent in establishing local immune memory and effector responses than those delivered parenterally, due to the ability of the dendritic cells to imprint T cell migration to the mucosal tissues (Lencer and von Andrian, 2011). However, based on the mechanism by which mucosal dendritic cells promote mucosa-homing T cells, even a parenteral vaccine can be designed to elicit mucosal immunity (Lencer and von Andrian, 2011). Yet, a vast majority of approved vaccines are injected into muscle without any designs to promote mucosal immunity (Figure 2). Safe and effective vaccines that establish robust mucosal immunity at the site of pathogen entry will transform vaccine landscape.

How to Increase Vaccine Acceptance

Finally, a big barrier to establishing and maintaining herd immunity with vaccines is the lack of vaccine uptake in some subgroups due to misinformation and mistrust. Vaccines have been a victim of their own success. With the decline in the burden of many vaccine preventable diseases, successive cohorts of parents are less familiar with once common diseases such as measles. At the same time, individuals hear about real or perceived vaccine adverse events—often through information propagated on social media platforms. A decline in awareness of severity of vaccine-preventable diseases accompanied by concerns about vaccine safety has been associated with an increase in vaccine hesitancy. Lower trust in government and healthcare providers has also been associated vaccine skepticism. New evidence suggests an association between vaccine hesitancy and values of liberty and purity (Amin et al., 2017). Concerningly, survey data suggest that a substantial proportion of US adults are unlikely to accept the upcoming COVID-19 vaccine.

Fortunately, there has been some progress in developing and deploying interventions to improve vaccine acceptance. First, presumptive communication has been reported to be an approach that relies on verbal defaults by presuming vaccination and announcing that the child (or the adult) will be vaccinated (e.g., “It’s time for you to receive your flu shot.”) versus communicating vaccination as a non-routine, optional procedure (e.g., “Would you like to receive your flu shot?”) (Opel et al., 2015). Motivational interviewing—which elicits behavior change by helping individuals to explore and resolve ambivalence—has shown promise as another technique healthcare providers can use to communicate about vaccines (Gagneur et al., 2018). Moreover, structural interventions that make vaccination accessible and convenient have been shown to increase immunization uptake.

Conclusion

Vaccines continue to be one of the most effective tools to prevent morbidity and mortality from endemic and emergent threats. Recent advances may herald

another golden age of vaccines. However, misinformation and the consequent mistrust of vaccinations pose a threat to their success and positive impact on global human health. More than ever, it is important for scientists to communicate scientific truth and to educate the public about the safety and benefits of vaccination using traditional and social media. Therefore, in the future, vaccine science must not only draw from disciplines such as virology, immunology, bioinformatics, and systems biology but also from social sciences.

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