Vaccine development: perspectives from life-history traits

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Major breakthroughs in vaccinology arise from our understanding of the fundamentals of immunology and infection biology of the disease. The pathogenicity cycle of a microorganism, from its introduction into the host until the resultant effects of infection, is closely linked to the host's immune response towards the pathogen. Additionally, questions in vaccinology, such as what determines the most efficacious route for vaccine delivery and whether mimicking infection from a different route of entry will generate a better immune response, remain open and may not be fully addressed during vaccine development. This article highlights the importance of the life-history traits of the pathogens and enumerates their relevance in designing vaccines. We revisit the path from life-history traits to vaccine development considering the pathogenicity cycle, which may prove critical in designing effective future vaccines and predicting vaccine behaviour in humans.

Keywords: Immune response, life-history traits, pathogenesis cycle, route of administration, vaccine development.

Significance

THE process of vaccine development includes questions such as what determines the most effective route for vaccine delivery and whether mimicking infection from a different route of entry provides better protection. This article asks many such questions to highlight the importance of the life-history traits of pathogens and their relevance in designing future vaccines.

The current vaccine landscape

Vaccines are among the best tools at the disposal of humankind for long-term and large-scale combat against diseases. The hope of being less susceptible to infectious disease has paved the way for vaccines to be in widespread use. The first instances of inducing immunity against infectious pathogens came from realizations of 15th-century healers of smallpox: infection prevented reinfection, and thus an artificial introduction of cowpox scabs could prevent natural infections. Others took forward these ideas and came up with safer ways to introduce a live pathogen into the human body for 'vaccination' – all during an era when even concepts about the immune system had not emerged[1]. Since then, the scientific community has made leaps and bounds in understanding the biology behind vaccination and has helped improve the methods of vaccine development. This, in turn, has enabled humankind to successfully eradicate many diseases, including smallpox and some strains of poliomyelitis (commonly known as polio), using the 'technology' of vaccines. A variety of vaccines are currently being developed and used against several emerging infectious diseases[2]. Major breakthroughs in our understanding of vaccines come from immunology[3] – a field that deals with the human body's reaction to foreign entities. The response of the human body to pathogens is now well-established to be mediated by various 'arms' of the immune system (described in subsequent sections; Figure 1); and vaccines function by giving glimpse of the infection. In addition to our improved appreciation of vaccinology, the scientific community has also set up a well-functioning pipeline for vaccine development, which takes into account the biological and practical aspects of vaccination[4].

Albeit extensive research on vaccines and state-of-the-art vaccine development strategies, our understanding is incomplete and insufficient. This can be seen from the high rates of conflicting results and unexpected responses during clinical trials and the long duration it takes to design and develop a vaccine[4]. Based on observations from vaccines used in the recent past, one can identify many questions that hold important implications for the future of vaccines. Every vaccine needs to be administered through a particular route of entry in human beings. Why are some vaccines administered through the intravenous (IV) route while others orally? What determines which of these routes results in better vaccine efficacy? Is it dependent on the portal from which the pathogen enters the human body during an infection? Do all modes of vaccine entry elicit the same type of immune response? Additionally, can infection through a different portal of entry cause a different disease manifestation? Are these questions routinely addressed during vaccine development? Would mimicking the typical pathogenesis (while ensuring safety) result in the best vaccines? Should vaccine development focus on addressing transmission, infection or disease? Answering these questions and reintroducing some basic concepts from immunology and infection biology can provide an avenue to improve the success rate

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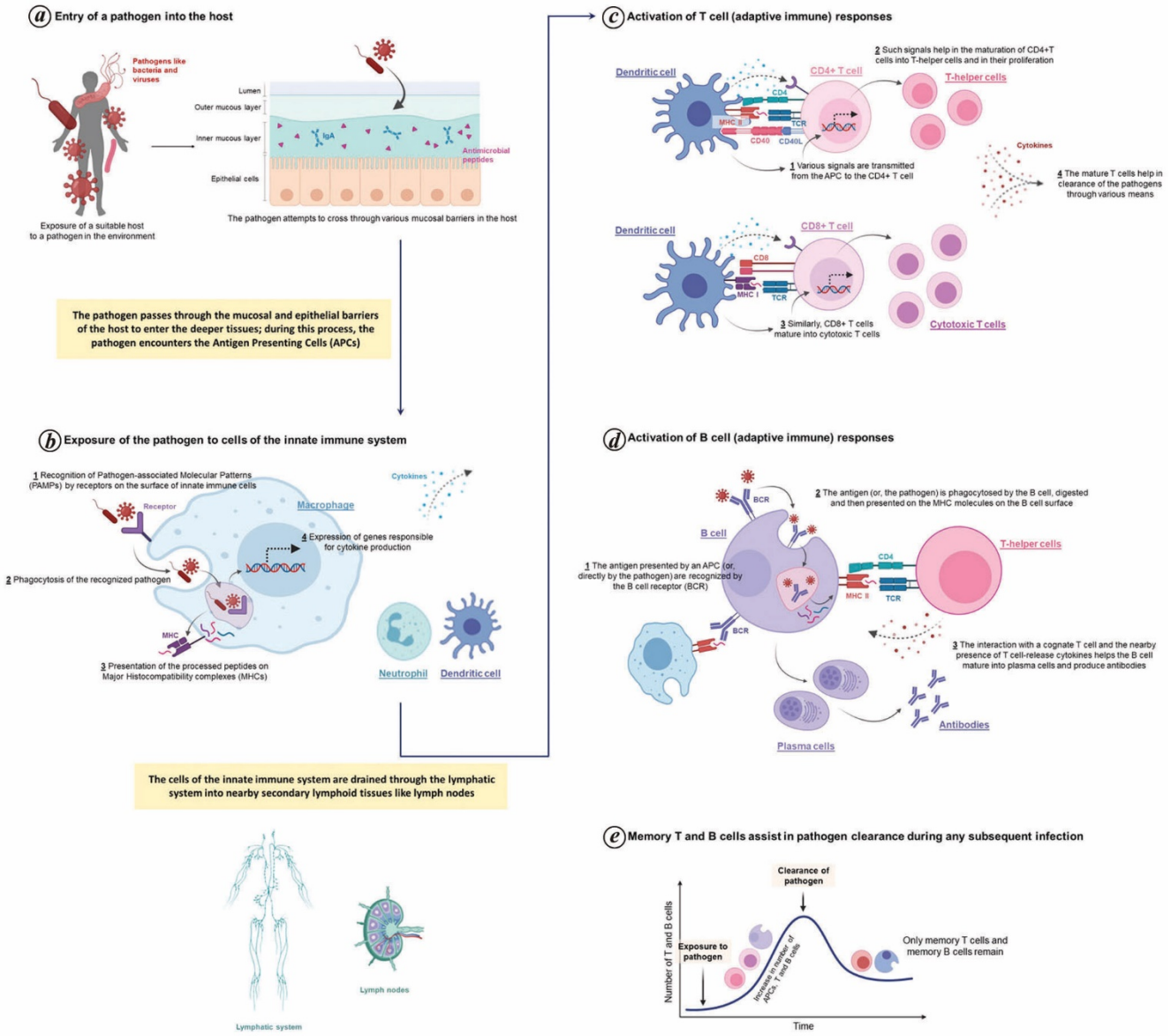

Figure 1. Immunological basis of pathogen clearance. (***a–d***) Exposure of a pathogen to a new host triggers a queue of primary immune responses. ***a***, Entry of a pathogen into the host. Upon entry, the pathogen encounters non-induced, non-specific innate immune responses in the form of anatomical and mucosal barriers, such as the skin, mucosal lining of internal organs, saliva and tears. They act as physical impediments for pathogen transit and produce immunogenic substances. Plasma cells, adjacent to the mucosal lining produce secretory IgA, which interacts with the pathogens and performs immune exclusion. Epithelial cells lining the passageway of the pathogens secrete anti-microbial peptides that disrupt membrane integrity of the pathogens. Crossing these, the pathogen gains access to deeper tissues in the host. ***b***, Exposure of the pathogen to cells of the innate immune system. Pathogen encounters the induced, a non-specific responses in the form of cells of the innate immune system like macrophages, neutrophils and dendritic cells. Each of these antigen presenting cells (APCs) is able to recognize pathogen-associated molecular patterns (PAMPs) using receptors on its surface, leading to digestion of the pathogen and presentation of characteristic peptides on the major histocompatibility complexes (MHCs). This cross-presentation of the exogenous pathogenic antigens also triggers a cascade of changes in gene expression which help in the production of cytokines. Following this, the cells are drained through the lymphatic system into the nearby secondary lymphoid tissues, where a more specific immune response is initiated. ***c***, Activation of T cell (adaptive immune) responses. APCs at the draining lymph nodes interact with the cells of the adaptive immune system called T cells and B cells. The CD4+ T cell contains receptors on its surface, including the T cell receptor (TCR), CD4 and CD40L. They interact with markers on the surface of APCs, such as the antigen-bound MHC-II and with CD40. Such receptor interactions act in combination with cytokine signalling to help in the maturation of CD4+T cells into T-helper cells, and their proliferation. Similarly, signals received by a CD8+T cell from MHC-I and cytokines aid in its maturation to cytotoxic T cells. ***d***, Activation of B cell (adaptive immune) responses. APCs also help in the maturation of B cells. Antigens presented by the MHCs (or directly the pathogen) are recognized by the B cell receptor (BCR). These antigens then interact with the TCRs on T-helper cells, to ultimately aid in B cell maturation into plasma cells and in subsequent antibody production. The responses from APCs, cytotoxic T cells and plasma cells result in clearance of the pathogen and any infected host cells by the production of cytotoxic and inflammatory substances. Antibodies produced by the plasma cell bind to the pathogens, and label them for destruction by immune cells like macrophages. ***e***, Memory T and B cells assist in pathogen clearance during any subsequent infection. During a primary infection, the number of APCs, T cells and B cells steeply increases until the infection has been curbed. Following this, however, some T cells and B cells remain behind as 'memory cells', which act to set up a much faster and more potent immune response during any subsequent (or secondary) infection by the same pathogen.

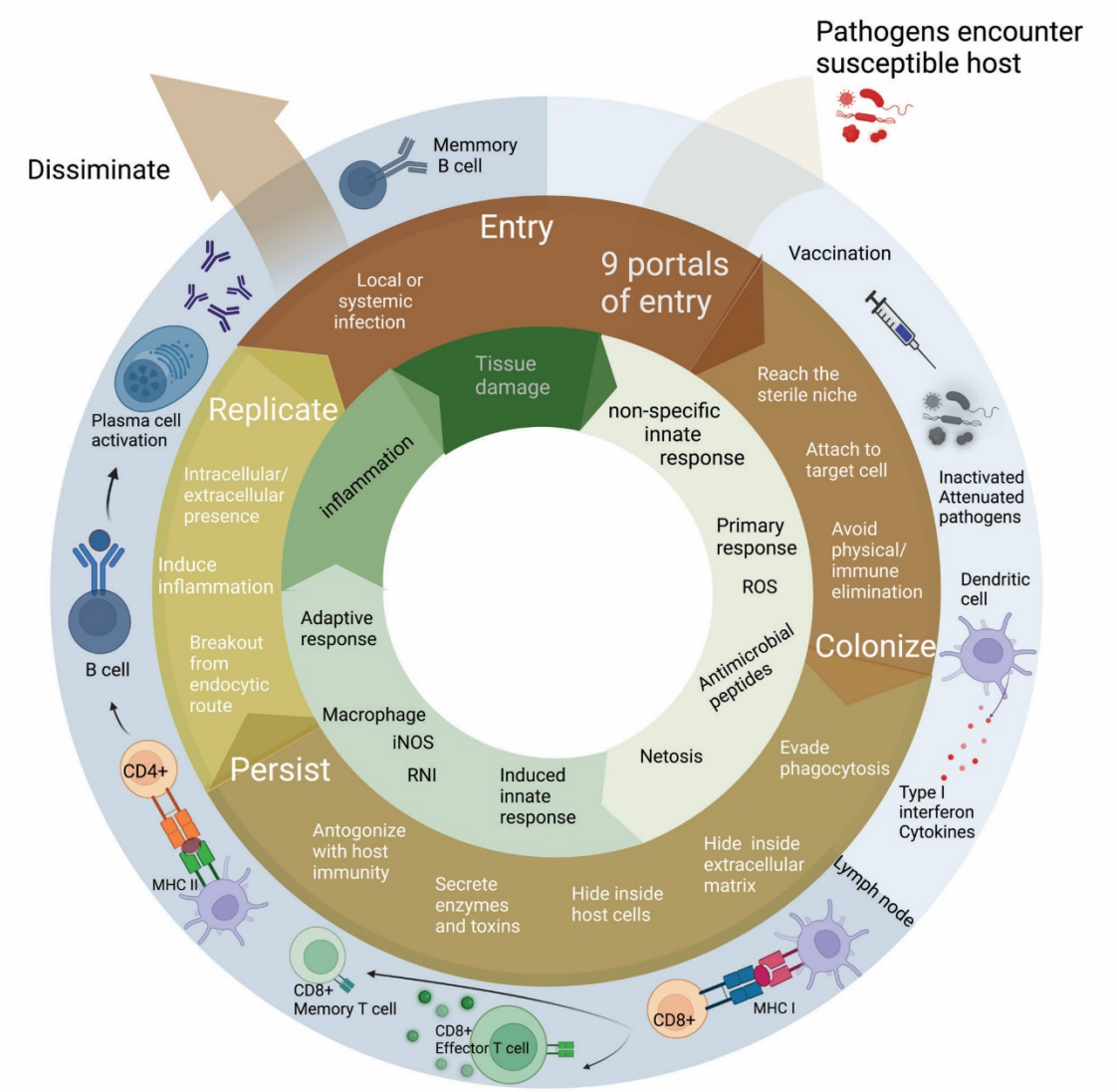

Figure 2. Pathogenicity cycle (intermediate circle) of a human pathogen inside the host shown in conjunction with the host immune response and infection outcome (inner circle). Immune response to an attenuated or inactivated pathogen is represented by the outer circle. Cytokines released upon activation of innate immune response: TNF-α, IL-1, IL-12; Chemokines-CC L2, CCL3, CCL5. Systemic response: Macrophages – TNF, IL-12, IL-6, IL-10, IL-17, IFN-β, IFN-γ[103]. T-cells: IL-2, IL-4, IL-7, IL-15, IFN-γ[104]. B-cells: IL-7, IL-4, IL-6, IL-10, IFN-α, IFN-β, IFN-γ[105]. The nine portals of entry in the pathogenicity cycle include mouth, ears, eyes, nostrils, genitals, urethra, anus, placenta and broken skin. MHC, Major histocompatibility complex. Predominantly MHC-I-restricted CD8+ T cells present viral antigens and MHC-II-restricted CD4+ T cells present bacterial and other exogenous antigens. CD4+ T cells are helper T cells displaying CD4 co-receptor in addition to TCR and help in adaptive immunity. CD8+ T cells produce cytotoxic granules and proinflammatory cytokines.

of vaccines. These studies hope to improve our understanding of pathogenesis while hinting at aspects one should consider during vaccine development. This article enumerates some of the established results on the above topics and highlights some questions for future research, aiming to propel our understanding of vaccine development into a new era.

The biology behind vaccines: a primer

Every pathogenic microorganism broadly establishes infection in a specific host organism via the 'pathogenesis' cycle (intermediate circle in Figure 2). Briefly, the cycle begins when a pathogen encounters and enters a host from the external environment through an appropriate portal. The pathogen navigates itself to its niches within the host, where it attempts to colonize the tissue and persist by evading the immune system. The pathogen subsequently replicates – achieving its ultimate 'goal' and exits out of the infected host to infect other susceptible individuals. These processes, intentionally or unintentionally, damage the host tissues. The features of this cycle are fundamentally connected to the outcome of infection, immune responses and memory, the possibility of reinfection and disease evolution. In each step of the pathogenesis cycle, a pathogen encounters a variety of host immune responses (Figure 1 and innermost circle in Figure 2). During and immediately after the entry of the

pathogen into the host, it experiences the innate and induced innate immune response. This non-specific and fast response includes anatomical and physiological barriers, antimicrobial molecules and phagocytic cells that try to neutralize and engulf pathogens upon recognizing pathogen-associated molecular patterns (PAMPs). This initial wave of innate immune reactions helps set up adaptive immune responses – commonly in the form of T cells and B cells. T cells are first activated when they encounter the cognate antigen, processed and presented by the dendritic cells in the lymphoid tissues. Then they rapidly proliferate to build an 'army' against the pathogen. This T cell army, through the action of CD4+ and CD8+ cell types, allows the recognition of intra- and extracellular pathogens, aids in B cell activation and helps eliminate the infected host cell. The B cells result in antibody production; this, in combination with the T cell responses, contributes to pathogen clearance. Once a load of invading pathogens decreases, the number of activated T cells and B cells also decreases. However, many long-living cells remain as 'memory cells'. During any subsequent exposure to the same pathogen, the memory cells mount a faster and more robust immune response and aid in removing the pathogen more efficiently with far less damage to the host.

In this respect, most vaccines function by allowing the first encounter of the host to a pathogen to occur with a weakened or inactive form of the latter, which can only result in mild disease. This enables the memory cells to develop while minimizing the chances of a lethal infection. When a competent pathogen from the environment subsequently attacks, the immune system is primed to fight back in full vigour. In addition to this well-known mechanism, the potential long-term antibodies generated by B cells following vaccination also aid in combating natural infection. Therefore, studying the pathogenesis cycle of the infection and identifying the immune correlates at each step of this cycle can enable one to better understand some of the questions that remain open in the field.

Life-history traits and their relevance to vaccines

Some aspects of vaccines remain incompletely understood

Let us rewind and consider two examples of vaccines – one of a well-understood and successful case, and the other of a vaccine in the development stages – to appreciate some of the questions described in the introduction.

Poliomyelitis, or polio, was one of the most feared infections in the 20th century and has caused paralysis and deaths throughout most of human history. The development of a poliovirus vaccine, followed by its widespread usage in the 1950s, resulted in a 99.9% drop in polio cases[2] – a remarkable illustration of how targeted vaccination programmes with sufficient funding can tackle a global health challenge. Two major vaccines have been in circulation throughout the history of polio – inactivated poliovirus vaccine (IPV) and oral poliovirus vaccine (OPV). There are two conceptual differences between them. First, IPV consists of an inactivated virus (cultured viruses that can no longer cause disease), while OPV consists of a live attenuated (or weakened) virus. IPV is administered intramuscularly/intradermally, whereas OPV, as the name suggests, is given orally. Among other consequences, OPV induces a significantly higher amount of intestinal immunity, possibly reducing foecal–oral transmission[5]. Is the route of vaccine delivery contributing to the difference in the type of immunity induced, or can this be attributed purely to differences in the level of vaccine attenuation? Or, are there other factors which influence the success of these vaccines? Furthermore, the differences between IPV and OPV are not solely in immunogenicity but also in the required number of doses and degree of reversal to infective virus state – all of which play a crucial role in deciding the vaccine administration strategy in countries like India[6]. These questions have not been examined, albeit being extremely crucial in our understanding of poliovirus pathogenesis and vaccine development.

In 2020, countries worldwide saw the outbreak of COVID-19 – an infectious disease caused by SARS-CoV-2 and spread commonly by oral discharge from an infected person's breath, cough and sneeze. Throughout 2020, biotechnology companies and researchers were engaged in developing a vaccine against SARS-CoV-2, with a multitude of vaccine candidates reaching clinical trials in a short time span. Despite many successful candidates, multiple questions have emerged among the scientific community regarding our understanding of certain aspects. Most of the vaccine candidates are based on a systemic route of immunization. However, studies have established that a single dose of an adenovirus-based COVID-19 vaccine administered through the nasal route causes a strong immune response in the respiratory mucosa in mice, showing no signs of further infection[7,8]. Is this mucosal response stronger than existing vaccines against COVID-19 in providing sterilizing immunity? Extensions of these studies have also resulted in the development of a vaccine candidate called BBV154; an intranasal, single-dose vaccine currently in phase 1 of clinical trials. In what way are these better than systemic vaccine delivery? What are the differences in the nature and quantum of systemic immune responses[9]? Can such results be extrapolated to other infectious diseases? One can identify such concerns in almost all vaccines. The common factor in all of these is that our understanding of host–pathogen interactions from the perspective of life-history traits is not adequate.

What are life-history traits and how can they help?

Life-history traits refer to the features corresponding to various events in the journey of an organism from birth to death[10]; here, it refers to the factors involved throughout

pathogenesis. These include – but are not limited to – the modes of transmission, portals of entry (or route of infection), the severity of infection (whether the pathogen infects tissues locally or systemically), and the type of immune response triggered, e.g. mucosal or systemic response. These factors are expected to influence host and pathogen behaviour to varying degrees, thus altering the outcome of infection and influencing vaccine development. Life-history traits can be explored more deeply in light of the pathogenesis cycle (Figure 2). Every 'trait' in the initial stages of the cycle, such as the portal of entry or mode of transmission, can alter the subsequent steps of infection. For example, transmission through food (via the mouth) allows the pathogen to access the alimentary canal. On the other hand, a pathogen transmitted through air droplets can enter through the nose, access and then affect the upper and lower respiratory tract. Traits like the systemicity of the infection and of immune responses are essential to understanding the spread of the pathogen within a host and the type of immune response elicited. Let us consider the portal of pathogen entry. Studies on different routes of *Brucella melitensis* infection have shown that pathogenesis in each case is associated with a varied subset of virulence gene expression, and a varied lymphoid subpopulation contributing to protective immunity[11]. A comparative study comprising data from multiple human pathogens also shows that the route of pathogen entry can influence the extent of damage on host tissues and the required infectious dose required to do so[12]. Such studies, spanning various host and pathogen species, show that the portal of pathogen entry affects the virulence factors associated with infection, the correlates of immunogenic protection and the extent of damage caused. Interestingly, the route of entry also affects downstream life-history traits – the type and extent of immune response triggered in the host and the factors that assist in immunological memory[11,13].

One can then connect the route of pathogen entry to the mode of vaccine administration – the path through which a laboratory-altered 'pathogen' enters into a host. As one expects, vaccines delivered through the nasal tract versus through IV administration will not have access to the same organs and will possibly trigger different immune response cascades; therefore, they will vary in their efficacy. Numerous vaccines have been administered using various routes, such as polio and SARS-CoV-2 vaccines described previously. The Bacillus Calmette-Guérin (BCG) vaccine against tuberculosis is another example – it has been proven to be more effective when delivered intravenously by inducing more antigen-responsive CD4+ and CD8+ T cells at various locations in macaques[14]. On the other hand, vaccines against measles prove to induce better response when delivered through the respiratory tract than the subcutaneous route[15]. One hypothesis in the field suggests that matching the route of vaccination to the route of infection is advantageous[16,17], as seen in the cases of measles, poliomyelitis and COVID-19. However, as depicted by the contrast in the case of BCG, our understanding of this phenomenon is incomplete.

Furthermore, many well-characterized diseases result in either local or systemic infections (yet another life-history trait) depending on the individual and environmental factors. For example, SARS-CoV-2 infections begin locally at the alveolar spaces and later transition into systemic infections due to the 'cytokine storm' syndrome. This transition, however, occurs only in a minority of individuals as the onset of the cytokine storm is known to depend on the early defence mechanisms and immune response time of an individual[18,19]. In such a scenario, one must ensure that a vaccine can address all possible cases. Similarly, one must ensure that a systemically delivered vaccine which circulates and reaches multiple locations through the bloodstream can effectively address local infections. In the case of tuberculosis, the lungs are the primary infection site, and local immunity in this region can be induced by delivering appropriate signals to the lung innate immune system. However, it is observed that parenteral immunization results only in delayed initiation of immune responses to *Mycobacterium tuberculosis*, possibly because the right innate signals are not induced[20]. The scientific community has made great leaps in addressing life-history traits. However, by connecting the above dots, we identify multiple gaps in our understanding – especially in characterizing life-history traits, taking these observations forward to human systems and implementing them during vaccine development.

The path from life-history traits to vaccine development

Table 1 describes the current protocols for vaccine design and development. These have multiple branches based on the pathogen of interest and the extent of our understanding of the pathogen/infection/disease[4]. We believe that throughout the stages of vaccine development, one must be mindful of the pathogenicity cycle and life history of the pathogen in a host. These considerations may prove crucial in designing effective vaccines and predicting vaccine behaviour in humans. Some such identified considerations are enumerated here.

Vaccine type governs some but not all aspects of immune response

The immune response generated by a vaccine is well-known to depend on the chosen antigen and how and where it interacts with the immune system. Instead, we consider a closely related concept – 'vaccine type', or the platform used to present the chosen antigen. Vaccine types include, but are not limited to, live attenuated, inactivated whole pathogenic, subunit protein, toxoid, genetic, recombinant and viral vector vaccines; each has its advantages and limitations[21]. Notably, each vaccine type is understood to elicit different types of immune responses (Table 2).

Table 1. Overview of the steps involved in vaccine development

(1)	Designing a vaccine	Major characteristics of the vaccine are determined during this stage: the type of vaccine, correlates of immunogenic memory, delivery vehicle and the need for adjuvants. This extensive process involves a broad range of interdisciplinary researchers studying the host–pathogen interaction at multiple levels.
	(1.1) Identifying the 'vaccine target'	A potential 'vaccine target', target antigen or immunogen is identified using existing knowledge on the infection cycle of the pathogen and its interaction with the immune system. This vaccine target must mimic most features of the pathogen in order to induce similar immune responses, but lack certain other features to prevent a full-blown infection. Targeting conserved regions/features can help develop a universal vaccine which allows protection from multiple strains of the pathogen, and from mutation or antigenic drift that may arise in structural elements. Insights from structural biology and bioinformatics help in identifying potential vaccine targets.
	(1.2) Identifying antigen display and delivery platforms	Two crucial parameters of the vaccine are the type of vaccine and the mode of administration. These are based on an in-depth understanding of pathogenesis, and studies on the advantages and limitations of each option. They are determined prior to production and testing processes, since they influence the behaviour of the vaccine candidate. In case of protein vaccine, the choice of the best adjuvant combination is identified at this stage.
	(1.3) Optimization of production	Following this, the formulation and potential upstream and downstream production processes are optimized. The 'product' can be taken forward once sufficient expression levels of the target antigen are achieved. Biosafety issues from potential toxins and undesired antigens are monitored while manufacturing and testing the vaccine candidate.
(2)	Preclinical trials	The vaccine candidate, now equipped with appropriate delivery and antigen display methods, needs to be characterized. Specifically, it needs to be tested for its safety, immunogenicity and protective efficacy in a biological system. This is achieved in small animal models and non-human primates. This step also helps establish if an adjuvant is required. Utilizing this data, one gets a preliminary idea of the dose of the vaccine candidate and the corresponding toxicity in humans. These are expanded upon to determine the parameters to be used for clinical trials.
(3)	Clinical trials	This step reveals whether the vaccine candidate is viable and functional in healthy human volunteers – for both confirmation and fine-tuning. Most clinical trials are performed in three stages, each one dedicated to explore a different aspect of vaccine behaviour, efficacy and clinical end-point. In phase I, a small number of volunteers receive the vaccine candidate to test for safety. In phase II, an increased number of volunteers with characteristics similar to the target population receive the vaccine candidate to test for immunogenicity and efficacy. Phase III, which is a scaled-up version of the phase II, involves a much larger number of volunteers to assess a broader-scale impact of the vaccine candidate.
(4)	Licensure and distribution	Success in clinical trials is followed by technology transfer and mass production of the vaccine by a combined effort of pharmaceutical companies and Government/public funding agencies. Effective distribution of the vaccine is necessary to ensure that a significant proportion of the population is protected against an infectious disease.
(5)	Post-immunization surveillance	Following immunization of widespread individuals, the effectiveness of the vaccine is continuously monitored in the large population during the subsequent years. The long-term responses, efficacy and side-effects of the vaccines are the factors that are monitored.

Live attenuated vaccines (LAVs) consist of a laboratory-generated 'version' of the pathogen that can replicate sufficiently enough to evoke most (and sometimes all) immune responses attributed to natural infection and disease. In other words, LAVs can elicit the strongest immune responses without causing a strong disease[22]. A direct proof of this comes from the high success of the smallpox vaccine by maintaining serum antibody titres even 75 years after vaccination. LAVs have also been shown to activate the innate immune system through their action on Toll-like receptors[23]; the yellow fever vaccine YF-17D and BCG are common examples of approved vaccines that function via Toll-like receptor activation[22]. On the other hand, subunit vaccines have a limited capacity to activate immune responses. For such vaccines, the specificity of the induced immune response is controlled by the subunit itself, but its potency or strength is generally attributed to adjuvants[24]. In fact, these vaccine types do not activate the innate immune responses when delivered without adjuvants, possibly due to the inability to activate co-stimulatory receptor interaction on antigen-presenting cells. Adjuvants, therefore, play a crucial role in generating sufficient levels of immune response, especially when using subunit vaccines and also help limit adverse reactions (for a recent review on the selection of adjuvants, see Pulendran *et al.*[25]). Furthermore, unlike LAVs, it is difficult to get a high degree of overlap in B cell and T cell epitopes using a single protein antigen.

In recent years, vaccinology has seen an increased value in inducing innate immune responses[26,27]. This is due to our new understanding of the 'trained immunity' set up by vaccine-induced reprogramming of macrophages against a wide variety of diseases[28]. In addition to being upstream to T cell responses, the type and strength of innate immunity are now also understood to modulate the vaccination outcome[29].

Table 2. Comparison of immune responses elicited by different vaccine types

Vaccine type	Examples		Route of administration	Immune responses
	Disease	Vaccine/ manufacturer		
Live attenuated	Smallpox	Smallpox (Vaccinia) vaccine, Live (ACAM2000)	Percutaneous injection	Non-specific innate immune response inhibiting viral replication. Adaptive immune response: T helper cells, T cells, B cells which act both directly and by the production of cytokines. Humoral immune response. Memory T and B cells[74].
	Yellow fever	Yellow fever (YF) 17D	Subcutaneous/ intramuscular injection	Innate immune response, majorly in the form of dendritic cells. Adaptive immune response: T cell responses, with CD8+ T cells clearing virus, and B cells that can either be cytotoxic or produce cytokines. Humoral immune response: High titres that persist for long durations (~40 years). Memory T cells, majorly in the form of CD8+ memory T cells[75].
	Tuberculosis	Bacillus Calmette-Guérin (BCG)	Intradermal injection	Epidermal macrophages and dendritic cells activation at the site of injection, resulting in innate immunity and production of pro-inflammatory cytokines. Adaptive and cell-mediated immune response: Extensive T cell recruitment, proliferation and maturation into different types and B cells. Minimal humoral immune response, as their contribution is minimal due to the intracellular nature of the infection. Systemic immune response, with relatively low but crucial mucosal response in the lungs. Antigen-specific memory T cells and memory B cells[76].
	Influenza	Live attenuated influenza vaccine [LAIV] (the nasal spray flu vaccine)	Intranasal	Mimics natural infection, starting with the replication of the LAV in the upper airways. Mucosal immune response, in the form of nasal IgA antibodies. Adaptive and cell-mediated immune response: T cells and B cells that act at the site of infection (specific immune response). Humoral immune response, with both local and systemic production of antibodies. Memory B cells[77,78].
	Other examples: MMR (Priorix; M-M-RVAXPRO, MSD or Priorix-Tetra; ProQuad, MSD), varicella (from Oka strain of varicella-zoster virus), chickenpox (from varicella-zoster virus), rotavirus (Rotarix), typhoid (Ty21a), rabies (RabAvert), poliomyelitis (OPV), COVID-19 (Codagenix), dengue (Dengvaxia:CYD-TDV), Japanese encephalitis (SA-14-14-2)			
Inactivated whole pathogenic	Poliomyelitis	IPV	Subcutaneous or Intramuscular injection	Induces strong systemic immunity and humoral responses. Mucosal immunity generated in the intestine is low (as replication and faecal shedding of the virus from the intestine can still occur), however, pharyngeal shedding of the virus is markedly reduced. Less development of secretory IgA antibody and little/no protection against subsequent infection[79,80].
	Hepatitis A	Havrix	Intramuscular injection	Adaptive and cell-mediated immune responses: T and B cells are produced effectively. Humoral immune response with high antibody levels. Memory T and B cells persist with high antibody responses in individuals even after ~20 years[81].

(*Contd*)

Table 2. (*Contd*)

Vaccine type	Examples: Disease	Examples: Vaccine/ manufacturer	Route of administration	Immune responses
	Rabies	Human diploid cell culture-produced and purified chick embryo cell culture-produced	Intramuscular injection	Adaptive and cell-mediated immune responses: T cell-dependent immune responses through maturation of CD4+ T cells, but also a strong rabies-specific B-cell response. Humoral immune response, with high IgA and IgG levels. Memory B cells. NK cells and CD8+ T cells-mediated cytotoxicity upon revaccination[82].
	Other examples: Cholera (Dukoral, Shanchol, Euvichol-Plus), COVID-19 (Sinovac, Covaxin, Valneva), tick-borne encephalitis (TicoVac), diphtheria, tetanus and pertussis (DTap and Tdap)			
Subunit				Adaptive immune response: B cell responses are
Capsular polysacchride subunit	Pneumococcal	PPSV23	Subcutaneous or intramuscular injection	generated in a T cell-independent manner due to capsular polysaccharides antigens. Humoral immune response is generated, specifically against the serotypes included in the vaccine. No memory cells are generated as the response is T cell-independent (although antibodies can persist for ~5–10 years). Conjugation of the polysaccharide antigen with a carrier protein is essential[83,84].
Protein subunit	COVID-19	Novavax	Intramuscular injection	Adaptive immune response: CD4+ and CD8+ T cells, and B cells are produced. Humoral immune responses, with antibody response directed towards the spike protein of SARS-CoV-2. Memory CD4+ T cells, B cells and antibodies could be detected after six months of vaccination, but memory CD8+ T cells were detected only in 10–50% of volunteers[85–88].
	Other examples: Influenza (Fluvirin, Agriflu, Flucelvax), Haemophilus influenzae type b, meningococcal meningitis			
Toxoid	Diphtheria and tetanus	Td	Intramuscular injection	Adaptive immune response: Limited T and B cell proliferation. Humoral immune response is generated in terms of tetanus- and diphtheria-specific antibodies that persist for ~65 and 20 years respectively. Memory T and B cells, of which the latter are crucial for long-term secondary immune response[89,90].
Recombinant	Hepatitis B	HB-Vax, HBVAXPRO, INN-hepatitis B	Intramuscular injection	Adaptive immune response: T and B cells. Humoral immune response: Significant antibody levels are generated in the form of hepatitis B surface antibodies; these persist for more than three years. Memory T cells (which persist for more than 10 years)[91–93].
	HPV	9vHPV (9-valent HPV), 4vHPV and 2vHPV	Intramuscular injection	Adaptive immune response: T cell (mainly characterized for CD4+ T cells) and B cell responses. Humoral immune response: High levels of type-specific neutralizing antibodies, which last for a long duration. Memory B cells[94,95].
	Other examples: Influenza (Flublok), serogroup B meningococcal			
Genetic mRNA vaccine	COVID-19	Comirnaty and Spikevax	Intramuscular injection	Cellular immune response: Extensive B and T cell response. Humoral immune response: High titres of neutralizing IgG with increased ability to tackle SARS-CoV-2 variants which persist for at least six months. Memory T cells that persist at least six months after vaccination and memory B cells[85,88,96–98].

(*Contd*)

Table 2. (*Contd*)

Vaccine type	Examples: Disease	Examples: Vaccine/ manufacturer	Route of administration	Immune responses
DNA vaccine	COVID-19	ZyCoV-D	Intradermal (needle-free)	Cellular immune response observed and documented mainly for T cells. Humoral immune responses are generated in the form of specific neutralizing IgG against the spike glycoprotein of SARS-CoV-2, maintained for more than three months in mice. Memory B cells[99,100].
	Other examples: DNA vaccine clinical trials are underway for multiple diseases such as malaria, AIDS, influenza, Ebola and herpesvirus. mRNA vaccines for multiple diseases such as rabies, influenza, Zika virus, cytomegalovirus and Chikungunya virus are underway, but not yet approved.			
Viral vector	COVID-19	Covishield/ Vaxzevria (AstraZeneca)	Intramuscular injection	Innate immune response: Activation of innate immune cells and production of proinflammatory cytokines and chemokines. Adaptive immune response: T and B cells. Humoral immune response: Neutralizing antibody production. Memory T and B cells[88,101,102].
	Other examples: COVID-19 (Jannsen, Sputnik V, Convidecia), Ebola (rVSV-ZEBOV, Zabdeno/Mvabea).			

The efficacy of a vaccine is tightly linked to the immunogenic activity of the chosen antigen. Therefore, LAVs are at the top of this list, followed by an inactivated pathogen, subunit vaccines and finally, toxoid vaccines, all of which require booster shots at regular intervals. However, this must also be considered in light of a possible full-blown infection or disease caused by the vaccine, especially in immunodeficient individuals. For example, LAVs run the risk of initiating fatal reactions due to uncontrolled pathogen replication, reversal to natural pathogen forms, or dissemination of the live attenuated pathogen to other sites (such as during BCGosis[30]).

Albeit significant differences in the potency of immune responses generated, their systemicity is not limited by vaccine type. The general aim for current vaccines is to achieve systemic immunity – that is, antigen-specific immune responses throughout the body, along with mucosal immunity – tolerance against the pathogen at the mucosal linings that generally surround the portals of entry to various internal organs. Live attenuated influenza vaccines[31], as well as subunit vaccines against influenza (with adjuvants)[32] can induce mucosal immunity. The issue of systemicity, instead, seems to be associated with another aspect of vaccination, as discussed below[33].

Immune responses can be fine-tuned using varied portals for vaccine administration

One of the crucial aspects determined during vaccine design is the portal through which it heads to be administered, a factor which is a life-history trait. Vaccines currently in use commonly utilize the oral, edible, intranasal, subcutaneous, intramuscular, intravenous and intradermal routes for their administration[34]; the last four can also be injected at various sites across the human body. Each of these routes is associated with varying downstream effects. Oral and edible methods of administration are used in cases where the gastrointestinal tract needs to be accessed. These are hypothesized to have myriad advantages[35], but are constrained by difficulties of antigen survival in the harsh environment of the gut[36]. Some examples of licensed oral vaccines are the OPV and the live oral typhoid vaccine, Ty21a. Edible vaccines are genetically modified plant and animal-based agents that trigger an immune response in the host[37]. Edible vaccines overcome the safety-related issues of traditional vaccines but are currently only in the developmental stages[38].

A vaccine can also be injected into various skin layers of a host. Intradermal delivery (IDD) involves injecting a vaccine at the topmost layer of the skin, i.e. the dermis. It is currently only used for the delivery of the BCG vaccine against tuberculosis. Research continues into the possibility of this method contributing to higher safety and better vaccine efficacy with lower dosage[39]. Subcutaneous administration consists of injecting the vaccine into the subcutaneous layer above the muscle and below the skin. This method is used for vaccines against measles and yellow fever. However, subcutaneous injection routes are becoming obsolete due to local adverse side effects of these vaccines[40]. Intramuscular delivery involves injecting the vaccine into the muscle mass, deep within the skin layers and remains a preferred route of administration due to its convenience. Most vaccines use this method; a common example is the DTaP/Tdap (DPT) vaccine against diphtheria, pertussis,

tetanus and many COVID-19 vaccines. This form of administration has been shown to reduce local adverse events[40]. Intranasal administration, a developing method, involves insufflating a vaccine through the nasal cavity. This results in the induction of mucosal and systemic immunity while reducing the discomfort caused by vaccine injection[41]. The live attenuated influenza vaccine against influenza virus is used in various countries. This method is also being developed against COVID-19 (ref. 9).

The recurring theme from all the above statements is that vaccine administration strategies are chosen to accomplish a particular end-point. For example, oral and intranasal administration helps target a particular tissue – the gastrointestinal tract and the mucosal lining respectively. Immunological considerations also abound during the determination of the vaccine administration route. Vaccination routes show varied preferences for cellular and humoral immunity activation[42]. Recent evidence suggests that distinct lymph-node clusters can be targeted using intramuscular versus subcutaneous delivery routes[43]. Particular antigen-presenting cell clusters can also be activated by modulating the anatomical site of vaccine injection[44]. In some cases, the extent of immunological response varies with the route of administration[45] or even the depth of vaccine injection[46]. Taken together, multiple lines of evidence suggest that vaccine entry routes contribute to an altered safety, immunogenicity and efficacy profile of a vaccine[46]. However, this observation cannot be generalized. In various instances, the extent of adaptive and innate immunity[43] and systemic and humoral immunity induced[47] has been shown to be similar across immunization routes. Therefore, it remains essential that each vaccine is adequately tested with varied administration routes to ensure that the best possible strategy is implemented.

Do vaccines combat infection or disease?

Vaccines are traditionally designed to protect an individual from disease, not infection. That is, a pathogen can enter and colonize host niches, but the subsequent steps of host damage are protected against. Therefore, only some stages of the pathogenicity cycle are addressed by the vaccines, which is an immeasurable feat. However, it remains important to study the role of vaccines in intercepting infection and transmission. Developing a vaccine to prevent infection requires understanding the route of transmission, the points of entry into a new host and the correlates of immune protection at each step of the pathogenicity cycle. At later stages, the prevention of infection by vaccines can be tested through 'human challenge trials'. These require individuals to get vaccinated and be deliberately challenged by the pathogen, which can then check if a vaccinated individual is protected against infection. Of course, this procedure is ethical only if a well-established treatment method is in practice. One important example is the subunit vaccine against the hepatitis B virus, which prevents infection. This was based on a recombinant vaccine based on plasma-derived HBsAg developed in the 1980s that prevented hepatitis B in infants of high-risk mothers at 75% efficacy. Incredibly, a recent study established that a combination of the above hepatitis B vaccine with hepatitis B immunoglobin (HBIg)-based passive immunization increased this efficacy to 95% (ref. 48), a feat achieved by careful consideration of the available antigens and their immunological effects. The OPV has also partially addressed transmission of the virus by faecal–oral routes during the later stages of infection.

Current research in this field comes from work on sterilizing immunity, 'an immune status conferring protection from pathogen infection'[49]. Achieving sterilizing immunity would require that early stages of pathogenesis are prevented. This, in turn, requires vaccine-induced activation of immune responses at the portals of entry. As these entry sites are linked to the internal organs by tracts lined with mucosal surfaces, current strategies aim to establish mucosal immunity through vaccines. In addition to neutralizing antibody responses[50], this type of immunity necessitates T-cell mediated immunity[49]. Multiple studies support the notion that capturing natural infection-like scenarios with T cell and B cell responses assists in establishing sterilizing immunity. For example, intranasal inoculation of the influenza virus results in undetectable levels of virus titres following exposure[49]. However, this may not be the only factor, as an intranasal SARS-CoV-2-targeting vaccine that generates mucosal T cell and neutralizing antibody responses does not confer sterilizing immunity[8]; but an adjuvant-assisted recombinant vaccine delivered intramuscularly produces the derived result in mice[51]. Therefore, we do not yet understand the complete mechanism behind sterilizing immunity. Factors such as the location of the neutralizing antibody responses, systemicity of infection and the duration of mucosal response may be the key to unlocking a guide to preventing infection.

Identifying the immunological correlates of protection

The ultimate question of a vaccine development process, i.e. whether a vaccine should be taken forward to clinical trials, is determined by research on the behaviour of vaccine candidates in animal models[52]. It offers a chance to study the protective effect of an immunogen, antigen recognition[53] and long-term effects in a system which contains all the complex interactions of the immune system[54]. The reliability of such models is dependent on whether human infection and disease can be sufficiently recapitulated. In other words, the route of pathogen entry and infection, level of pathogen replication and damage of the target organ, immunopathogenesis, virulence factors and tissue pathology must correlate between the animal model and humans[52,54].

Choosing the 'right' model for each pathogen remains one of the biggest challenges in studying infectious diseases; analysing these models and devising techniques to improve the similarities are currently in progress.

Despite the up-and-coming strategies concerning animal models, the ethical implications they raised pushed vaccine and drug developers to minimize their use by replacing them with *in vitro* and *in silico* techniques. Most notable among these are the *in vitro* techniques that assess immune responses following vaccine administration. For instance, a monocyte-derived dendritic cell and allogeneic T cell culture have enabled research into innate responses associated with the respiratory syncytial virus and its vaccine[55]. Such assays can also functionally characterize a vaccine candidate. This has been performed for the tuberculosis vaccine candidate H4-IC31 by studying the extent of cytokine secretion and immune stimulation[56]. Another step towards such efforts comes from three-dimensional *in vitro* models of the human immune system, explicitly generated to test the efficacy of vaccines[57]. These are 3D tissue culture systems consisting of human capillaries, interstitium, endothelial cells and patient-specific monocytes. After a few days of culturing, the monocytes differentiate into dendritic cells – which are subsequently used to gauge responses to vaccine antigens. Organoid models, stemming from advances in developmental biology, are also gradually being incorporated into infection biology and immunology[58]. These systems commonly develop from induced human pluripotent stem cells into target cell lineages, recapitulating a large extent of *in vivo* organization and responses of tissues such as the immune system[59]. Vaccine testing in animal models is often doubtful regarding its predictive value, these systems provide a convenient alternative. Multiple organoids, like tonsil[59], airway, gut and intestinal organoids[60] are currently used in vaccine design. Improvements in such models, especially in recapitulating a larger percentage of early human physiology, can help address multiple questions introduced in this article.

In silico studies, too, have made extensive contributions to vaccine design[61]. Immuno-informatics, reverse vaccinology[62] and epitope predicting algorithms[63,64] are the most commonly used protocols. For example, a computational algorithm-based approach is being used by Codagenix to identify and alter the SARS-CoV-2 genomic regions, which are susceptible to high translation in humans by a process known as 'codon deoptimization'. This enables them to generate a LAV, which is currently at stage I of clinical trials. Vaccine candidates can also be validated through *in silico* tools; for example, a Universal Immune System Simulator (UISS) platform developed as a cohesive immune framework has been extended to test the numerous vaccine candidates against SARS-CoV-2 (ref. 65). Mathematical modelling of biological systems is also being used in vaccinology. Kinetic details of immune responses following vaccination have greatly benefitted from this approach[66]. Specific infections, epidemiological dynamics and an in-depth purview into various questions can also be tackled by such models[67,68]. With a surge in the use of mathematical modelling in biological systems and those above-mentioned *in silico* systems, modelling of immune responses taking the routes of vaccine entry into account can be a great contribution to vaccine design.

Conclusion

Do we get the time to go through this extensive process of vaccine development during a pandemic, which requires speedy development timelines and emergency authorization? The points mentioned in this article demand an extensive understanding of the pathogenesis cycle of a pathogen, preferably in its natural host, which would require setting up multiple model systems, understanding the immune correlates and completing the usual extensive process of vaccine development. In fact, multiple factors remain unaddressed in this article, such as the epidemiology of the disease, the time course of the infection and the life history of the susceptible population. However, as seen with the COVID-19 vaccines, which have taken a few months to design, manufacture and distribute to large populations, one can speculate that some steps may indeed be bypassed. After all, it would be advantageous for all infections to have quick-developed vaccines, and therefore, questioning the necessity for the concerns raised in this article. However, researchers consider that such an accelerated pace of vaccine development for COVID-19 has been made possible by years of study on the related coronaviruses. Multiple technologies used, like RNA-based vaccines, have benefitted from decades of research – has been at the right stage for immediate use against SARS-CoV-2 (ref. 69). In other words, generations of effort prompted by previous instances of infectious diseases enabled COVID-19 vaccines to be developed and delivered in a matter of months.

An extensive knowledge of the underlying biology, is a prerequisite for developing effective vaccines. For example, a hepatitis A vaccine is known to have exceptional efficiency in terms of protection against the hepatitis A virus (HAV). Specifically, anti-HAV antibodies have been detected in ≥97% of vaccines nearly 20 years following vaccination, with indications that life-long protection against HAV can also be achieved[70]. This has been attributed to multiple studies on immunization, propagation and epidemiology from appropriate animal models and human populations[71] and echoes a similar situation as studies on coronaviruses and COVID-19 vaccines. The vaccines against human papillomavirus (HPV) are also hypothesized to induce long-term protection[72]. This was made possible due to studies on using HPV subunit-based-virus-like particles (VLPs) that helped standardize VLPs and understand their immunogenicity[73]. Similarly, the exceptional success rates of toxoid vaccines against diphtheria and tetanus can be attributed to timely studies on these pathogens – on suitable animal models,

targets of vaccines, correlates of immunogenic protection and responses from human volunteers[71]. All of this demonstrates the necessity of in-depth studies regarding multiple aspects of vaccination. This article deals with our understanding of pathogenesis, immunology and host–pathogen interactions, which would contribute to a better appreciation of pathogen biology.

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