Chapter

Trained Immunity-Based Vaccines: A Ready-to-Act Strategy to Tackle Viral Outbreaks

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Abstract

Viral outbreaks have become significant threats to global human public health. New emerging viruses, pathogen mutations, and even the progressive loss of efficacy in some existing vaccines are behind this problem, which is amplified by the rapid virus spread given the ease of current mobility. Taking into account that these outbreaks arise in the absence of conventional effective vaccines, alternative approaches based on trained (innate) immunity are being considered. This immunity is dependent on a functional reprogramming of innate immune cells, leading to an enhanced nonspecific response towards different pathogens, including viruses. Trained immunity-based vaccines (TIbVs), defined as vaccine formulations containing trained immunity inducers, could be used during viral outbreaks to confer non-specific protection but also to enhance adaptive specific immune responses. In this chapter, we aim to illustrate how TIbVs could tackle the above-mentioned situations derived from viral outbreaks, reviewing the potential of available TIbVs in such urgent situations with a special mention to COVID-19.

Keywords: trained immunity-based vaccines, innate immune training, pandemic, viral outbreak, BCG, MV130

1. Introduction

Pandemics and epidemics of infectious origin are large-scale outbreaks that can greatly increase morbidity and mortality globally or over a wide geographic area, respectively [1]. Pandemics have occurred throughout history and appear to be increasing in frequency in the last centuries. Noteworthy examples include the Black Death at the end of the Middle Ages, Spanish flu in 1918, the 2014 West Africa Ebola epidemic or the current COVID-19 pandemic. The direct impact of pandemics on health can be dramatic. These large outbreaks can disproportionally affect younger or active workers, but vulnerable populations such as the elderly are at a particular high-risk. Pandemics can cause acute, short-term as well as longer-term damage to economic growth due to public health efforts, health system expenditures, and aid to affected sectors. Evidence suggests that epidemics and pandemics can have significant social and political consequences too, by debilitating institutions, amplifying political tensions, stigmatizing minority populations, or encouraging sharp differences between social classes [2].

Outbreaks by respiratory ribonucleic acid (RNA) viruses such as influenza or coronaviruses entail the principal threat due to their ease of spreading among humans, their potential severity and recurrence. However, other RNA viruses such as flaviviruses (Zika) or filoviruses (Ebola) must be taken into consideration due to a great overall burden of morbidity and mortality [3]. Antiviral drugs can help mitigate a viral outbreak by reducing the disease in infected patients or their infectiousness. While these drugs can be very successful against some viruses (*e.g.* hepatitis C virus [HCV]) [4], they are not universally effective as exemplified in the current SARS-CoV-2 pandemic [5]. Nowadays, having effective vaccines may be the only tool to reduce susceptibility to infection and thus, prevent the rate of virus spread [2].

Vaccination has dramatically decreased the burden of infectious diseases. Vaccines have saved hundreds of millions of lives over the years [6]. It has been estimated that approximately 103 million cases of childhood diseases were prevented in the United States through vaccination between 1924 and 2010 [7]. The eradication of smallpox in 1980 through vaccination is considered one of the crown accomplishments of medicine. Despite these achievements, effective vaccines have been developed against just over 30 pathogens among bacteria and viruses. There are many pathogens, including viruses such as human immunodeficiency virus (HIV) or respiratory syncytial virus (RSV), for which all efforts for vaccine development have failed so far. In addition, current available vaccines for worldwide important viral diseases like influenza are suboptimal, especially in the elderly, resulting in vulnerability among billions of at-risk populations [6]. On the other hand, having a new effective and safe vaccine in time to control highly contagious emerging viruses that cause epidemic or pandemic threats is an almost impossible task considering the timeframes for vaccine development. This includes preclinical and clinical research, its approval by the regulatory authorities, as well as its production and distribution [3].

Altogether, it has been postulated that one possibility of filling the gap between the appearance of a viral outbreak by an emerging pathogen and the availability of a specific vaccine is to take advantage of the heterologous protection of some existing vaccines, in order to increase the non-specific resistance of the host through trained immunity [8, 9].

2. Specific anti-infectious vaccines

Conventional (specific) anti-infectious vaccines are biological preparations containing live-attenuated or dead microorganisms, their antigens or nucleic acids encoding for them, designed for specific pathogens. The purpose of vaccination is to induce a long lasting adaptive immune response against key antigens able to confer host resistance for future encounters with the corresponding pathogen. Either the production of antibodies, generation of T helper/effector cells, or both, may play a critical role in such a resistance, which greatly depends on the type of pathogen, the route of entrance and the host-pathogen relationship (*e.g.,* extracellular and/or intracellular) [10]. Successful vaccines are highly effective not only in inducing long-lasting immunity against disease-causing pathogens, but also in providing herd immunity to the community that substantially restricts the spread of infection [6].

Most of the vaccines available today have been developed empirically and used successfully long before their mechanism of action on the immune system was understood. Early protection is associated to induction of antigen-specific antibodies, being their quality (avidity, specificity, or neutralizing capacity) key factors for

their efficacy. Long-term protection relies on the persistence of vaccine antibodies and availability of immune memory cells capable of rapid and effective reactivation with subsequent microbial exposure. On the other hand, T cells have a critical role in the induction of high affinity antibodies and immune memory. Furthermore, T cells have a direct role in protection conferred by some vaccines, including the tuberculosis Bacille Calmette-Guérin (BCG) vaccine [11].

Vaccines using whole pathogens have been classically classified as either live attenuated or inactivated (killed). Subunit vaccines contain just selected antigens (*e.g.,* proteins, polysaccharides). Recently, due to a growing availability of bioinformatics and sequencing tools, there has been an increase interest on so-called "rational" vaccine design approaches for subunit vaccines, such as the reverse vaccinology [12]. In this regard, modern vaccines include recombinant proteins or nucleic acids [13]. Rather than administering the antigen itself, DNA and mRNA vaccines targeting dendritic cells (DCs) encode the antigen of interest that will be produced by the vaccinated host, representing a new era in vaccinology [14]. In fact, the first RNA vaccine licensed for humans in Western countries has been recently developed for SARS-CoV-2.

As commented before, a vaccine response is linked to the induction of T and B cell specific responses to the antigens contained in the vaccine. This requires lymphocyte activation, proliferation and differentiation on specialized lymphoid tissues (*e.g* lymph nodes), where antigen presenting cells, like DCs for T cells or follicular dendritic cells (FDCs) for B cells, are present. Mature DCs are recruited into the T cell areas of lymph nodes from the periphery, *e.g.,* at the site of injection of the vaccine. DCs express pattern recognition receptors (PRR) that recognize evolutionary conserved pathogen-associated molecular patterns (PAMPs) that are not contained in self-antigens and are identified as "danger signals" [15]. When immature DCs are exposed to the vaccine-derived antigens at the site of vaccination, they uptake them and become activated [16]. This activation will lead to their maturation with the expression of homing receptors at their surface, triggering DC migration to the draining lymph node through afferent lymphatic vessels, where the activation of T and B lymphocytes will occur. Mature DCs process the up-taken antigens and present them to naïve T cells associated to molecules of the major histocompatibility complex (MHC) within the T cell areas of lymph nodes. On the other hand, unprocessed native antigens, either free or complexed with antibodies or complement, access the B cell areas of lymph nodes (lymphoid follicles) where they are captured by FDCs and displayed from their cell surface to the B cells. Antigen-specific B cells will rapidly proliferate forming a germinal center and differentiate into plasma cells producing low-affinity immunoglobulin (Ig) M antibodies. The B cells will then receive additional signals from activated T cells, undergoing isotype antibody switch from IgM to IgG or IgA and affinity maturation of the antibodies produced.

For a vaccine to be immunogenic enough, DC activation, that can be achieved by adjuvants, is essential. Live attenuated and inactivated whole-cell vaccines are considered "self-adjuvanted" as they naturally present sufficient PAMPs to activate innate immune cells, including DCs; thus, promoting a robust antigen-specific immune response. In contrast, subunit vaccines generally require different types of adjuvants to enhance and/or drive the immune response in the desired direction [15, 17].

2.1 Difficulties for novel specific vaccines in a viral outbreak situation

Viral outbreaks appear when there is a sufficient number of susceptible individuals within a nearby population. Although susceptibility is a balance between host factors (high/low resistance) and pathogens (high/low virulence), in many cases it reflects a

lack of prior contact with a given pathogen. In general, this is related to the emergence of new viruses or the lack of effective vaccines against known viruses. As pointed above, the development of effective vaccines is not an easy task against certain viruses. We are still lacking vaccines for some of the most lethal viral infections, including HIV and MERS-CoV, among others. These pathogens are difficult to tackle, as we do not fully understand their mechanisms to evade the immune system or how to elicit protective immunity against them [13]. However, encouraging progress is being made against these pathogens and there are currently several "pipeline vaccines" in development, such as RSV, universal influenza vaccine, and SARS-CoV-2 [18–20]. Apart of SARS-CoV-2 for obvious reasons in the current pandemic, there is an urgency to have a universal influenza vaccine that provides a broad and durable protection from influenza virus infection. Yet, the high level of antigenic diversity and variability, and antigenic drift in the surface antigens, enable these viruses to escape antibody-mediating neutralization [21]. On the other hand, there is a number of vaccines currently licensed, including the influenza A virus vaccine, that provide incomplete protection, especially in high-risk groups [22]. Mumps outbreaks observed in Ireland, United Kingdom and United States in vaccinated subjects with Measles Mumps Rubella (MMR) vaccine is another example [23]. Different factors have been postulated to contribute to mumps outbreak, including waning immunity and primary and secondary vaccine failure. Yet, their actual contribution is not fully understood [23].

Vaccine efficacy must consider different target populations as well. Adaptive immune response to vaccines may be limited in newborn and the elderly. Early in life, immune responses are dampened compared to adults [24, 25]. Neonates have underdeveloped germinal centers in lymph nodes and the spleen, and low expression of B cell receptors which in turn results in low levels of primary IgG responses to infections and vaccines [26]. As we age, our immune system undergoes age-related changes that lead to progressive deterioration of the innate and the adaptive immune responses, this is termed immunosenescence. The most common features of immunosenescence are short-lived memory responses, impaired response to new antigens, increased predisposition to autoimmune diseases and low-grade systemic inflammation (*inflammaging*) [27, 28]. Immunosenesence results in increased susceptibility to infections and deficient response to vaccination causing high hospitalization and mortality rates. For example, influenza vaccine efficiency has been reported to be 17–53% in the elderly, compared with the 70–90% efficacy in young adults [29]; and vaccination with Varicella zoster virus (VZV), also an important pathogen in elderly people, only partially prevents reactivation of herpes zoster [27].

If the difficulties listed above are outlined for existing or developing vaccines, quickly obtaining an effective vaccine to urgently control a new virus outbreak is almost an impossible task in the short-term as pointed above. This is well exemplified by the SARS-CoV-2 vaccine race pushed by the devastating COVID-19, with more than 100 vaccine candidates in the running. It is considered that no less than 1 year will last the time until the first licensed vaccine can provide protection in the best scenario [30]. This, in spite of greatly shortening the usual clinical development time and regulatory obstacles for a new vaccine and, therefore, without knowing its true performance and/or safety in the medium term compared to other authorized vaccines [31].

3. Trained immunity and infections

It has become evident from epidemiological, clinical and experimental data that some conventional whole-cell vaccines, like BCG and others, also provide resistance to infectious diseases not related with the specific pathogen targeted by the vaccine [32–34]. Much of these non-specific "heterologous" effects appear to depend on

the activation of innate immune cells by the PAMPs contained naturally in these vaccines [10], although other mechanisms such as cross-reactive epitopes between different pathogens could also account for this protection in some cases [35].

Immunological memory, understood as the ability to "remember" past encounters with pathogens, has been classically attributed to the adaptive branch of the immune system exclusively, by virtue of the antigen-driven clonal expansion of T and B lymphocytes and exemplified by the mechanism of conventional specific vaccines pointed above. However, the notion that innate immunity was unable to induce immunological memory has been challenged in recent years, particularly from studies in organisms that lack adaptive immunity, such as plants or invertebrates, as well as early studies in mice lacking the adaptive immune system [8, 36]. Altogether, the term 'trained immunity' was coined to define an innate immune memory that lead the innate immune system to an enhanced response to secondary challenges [37]. Importantly, trained immunity seems to be underlying the heterologous effects of an increasing number of vaccines [38–40].

3.1 The concept

What is trained immunity? - Trained immunity is defined as the memory of the innate immune system, where an encounter with a first stimulus (*e.g.* a microbial insult) results in a subsequent long-term adaptation and enhanced non-specific response by innate immune cells against a secondary challenge (the same or unrelated), thus providing non-specific, broad-spectrum, long-term protection in case of infection [8, 9, 37, 41].

Which cells can be trained? - Trained immunity properties have been defined for distinct cell subsets of the innate immune system [9, 42], including natural killer (NK) cells and innate lymphoid cells [43]. Of note, training of myeloid cells [42], particularly monocytes and macrophages [44, 45], and more recently DCs [46, 47] and hematopoietic stem cells [48], have been extensively studied. Finally, the acquisition of this immunological memory has also been demonstrated to a lesser extent for non-immune cells [49].

How to get trained? - A wide variety of stimuli can train innate immune cells, particularly when considering monocytes and macrophages [9, 50]. Among infectious agents, live microorganisms such as the tuberculosis vaccine BCG [51], *Candida spp* [52] or viruses [53, 54]; bacterial components, such as flagellin, lipopolysaccharide, muramyl dipeptide [55], fungal components as β-glucan [52] or even helminth products [56]. In general, microbial ligands engaging some PRR, like C-type lectin receptors (CLRs), nucleotide-binding oligomerization domain-like receptors (NLRs) are well established training inducers, whereas those engaging toll-like receptors (TLRs) may have opposite effects depending on the TLR type and concentration [55, 57]. Intriguingly, not only infectious agents but also endogenous inducers and metabolites such as oxidized low-density lipoprotein or mevalonate can induce trained immunity [50].

What hallmarks define trained immunity? - In contrast to adaptive immune responses, epigenetic reprogramming of transcriptional pathways — rather than gene recombination — mediates trained immunity. This training phenomenon comprises three key hallmarks that occur at the intracellular level: increased cytokine production upon rechallenge, changes in the metabolism and epigenetic reprogramming [9, 58, 59], which eventually support increased protection upon infection.

Among those cytokines whose production is augmented after re-exposure in trained cells, proinflammatory molecules such as tumor necrosis factor α (TNF-α), interleukin (IL)-6, IL-1β and interferon γ (IFN-γ) are fairly constant [45, 52, 55, 60, 61]. Modulation of IL-10 varies between studies [45, 52, 56, 62, 63]. A noted

shift from oxidative phosphorylation to aerobic glycolysis (Warburg effect) is the main change in cellular metabolism during the induction trained immunity [64]. Moreover, glutaminolysis, cholesterol synthesis and the tricarboxylic acid cycle are non-redundant pathways required for trained immunity to take place [64, 65]. Epigenetic reprogramming, mainly mediated by histone modifications, is one of the bases for the long-lasting effect of trained immunity [8, 66–68]. Immune pathway activation and changes in metabolism serve as basis for epigenetic rewiring [65]. As a result, epigenetic modifications have been found at the level of important promoters for the training process, which makes chromatin more accessible and conditions gene expression patterns of trained cells upon stimulation with a secondary challenge [69].

As a result of the whole process, enhanced, broad-spectrum, non-specific protection mediated by innate immune cells is found upon infection. This cross-protection has been observed for a wide range of human pathogens including fungi [51, 52], parasites [70, 71] and different bacterial infections [72–75]. Importantly, induction of trained immunity has been proved to be effective against viral infections including yellow fever [76], influenza A virus [77] and others [78, 79]. In this line, the induction of this phenomenon has been also proposed as a tool for reducing susceptibility to emergent SARS-CoV-2 infection, as will be described at the end of the chapter [78, 80].

How long does trained immunity last? – Trained immunity phenotypes have been observed for months and up to one year after the training insult. This was initially controversial, as trained immunity properties had been attributed to short-lived myeloid cells such as monocytes or DCs [38]. In this regard, several studies have shown that modulation of bone marrow progenitors is also an integral component of trained immunity, supporting the long-lasting effect of this phenomenon [9, 81]. In this way, trained immunity inducers [82–85] would be able to reprogram and induce expansion of hematopoietic progenitors with a particular bias to the myeloid lineage. Thus, bone marrow-derived mature cells would be also trained [86], showing improved clearance of infection [83].

Complementary to progenitor reprogramming, peripheral trained immunity induction would take place in tissue-resident cells [9]. This is especially relevant at the mucosal level, where cells encounter most of the infectious training inducers. Alveolar macrophage (AM) memory was demonstrated following viral infection [87, 88]. Training of these long-living cells led to increase antimicrobial properties, independently of systemic immunity [87, 89]. This local training of AM was further reproduced following respiratory mucosal administration of tuberculosis vaccine, being crucial for *Mycobacterium tuberculosis* clearance [90]. On the other hand, training of NK cells lead to long-lived, self-renewing, stable expanded cells with memory-like properties, both in an antigen-dependent or independent manner [91–93]. Finally, it was also reported that self-renewing long-living skin epithelial stem cells exhibited local trained immunity, providing faster wound healing in primed mice than in naïve mice [94, 95].

3.2 Trained immunity on ongoing immune responses

3.2.1 Effect on adaptive immunity

Non-specific effects of vaccines have been extensively studied and reported over the last decades. Although trained innate cells could partially account for these effects, involvement of adaptive immunity has also been suggested [96]. An adaptive immune mechanism of non-specific effects could be heterologous immunity; vaccine antigens can give rise to T cell cross-reactivity against other antigens that may confer some protection against unrelated pathogens [96, 97].

However, innate immune cells constitute the bridge between the intrusion of microbial threats and the activation of adaptive immunity. As said before, following sensing of pathogens by PRRs, activated innate immune cells secrete different factors and act as antigen-presenting cells (APCs) to initiate activation of adaptive immunity [98]. Thus, it would not be unexpected that trained innate immune cells, within their acquired enhanced properties, would be able to induce stronger adaptive immune responses [39]. In this regard, BCG vaccine, a well-known trained immunity inducer, has shown to enhance the antibody titer and alter heterologous T cell responses against a wide range of vaccines and unrelated infections [99–101]. In different experimental models, BCG-mediated protection against viral and *Plasmodium* infections was abrogated in the absence of T cells. In these models, BCG vaccination has been mainly associated with modulation of $CD4^+$ T helper (Th) 1 responses. Similar observations have been found in different clinical studies [99]. Of note, BCG vaccinated human volunteers displayed a long-lasting heterologous Th1 and Th17 response upon stimulation with unrelated pathogens and TLR-ligands [38]. To some extent, similar observations have been found in other vaccines such as diphtheria-tetanus-pertussis (DTP) or measles vaccine [99].

As said before, trained immunity properties have been recently described also for DCs. As being the most professional APCs, they emerge as crucial bridge for potentiating adaptive immune responses. In this sense, DCs with high immunostimulatory properties that enhance adaptive immune responses via IL-1β release had been described [102]. More recently, programmed memory DCs have shown to increase Th1/Th17 immunity and confer protection during cryptococcosis [46]. Finally, different polybacterial preparations of whole-cell inactivated bacteria, have shown to prime DCs and induce enhanced Th1, Th17 and IL-10 T cell responses against related and unrelated stimuli [103, 104]. This capability of modulating heterologous T cell responses by APCs have been also described to suppress pathogenic T cell immunity in experimental models of autoimmune encephalomyelitis [56].

3.2.2 Effector functions on trained innate immune cells

As noted above, a hallmark of trained innate immune cells is the enhancement of some effector functions leading to increased non-specific resistance against a variety of pathogens. In this regard, β-glucan-trained monocytes show enhanced candidacidal activity and efficiently inhibit the *C. albicans* outgrowth [52]. Production of reactive oxygen species (ROS) has shown to be also affected by the induction of training. Thus, BCG-trained monocytes [45], β-glucan-trained macrophages [105] or β-glucan-trained neutrophils [106] produced increased amount of ROS following different challenges. Finally, increased phagocytosis and production of microbicidal molecules have been observed in β-glucan-trained macrophages [70, 105]. Mechanisms underlying this enhanced effector function could be an intrinsic cell reprogramming as consequence of the training, as well as be supported increased expression of different PRRs and surface molecules [45, 60, 87]. Altogether, these enhanced effector responses could improve pathogen clearance by increasing host resistance.

On the other hand, a substantial part of the adaptive immune response is directed at recruiting other effector cells from the innate immune system to eventually resolve an infection. Both T helper and B responding cells release cytokines, antibodies, and other mediators that activate monocytes, macrophages, NK cells or neutrophils to clear extracellular and intracellular pathogens [107]. Multiple studies have demonstrated the importance of IFN-γ-mediated priming in the activation of macrophages [108, 109], produced by $CD4^+$ Th1 and $CD8^+$ T cells [107]. In this sense, it has been previously demonstrated that adaptive T cells render innate macrophage memory via IFN-γ-dependent priming [87, 89]. Furthermore, a

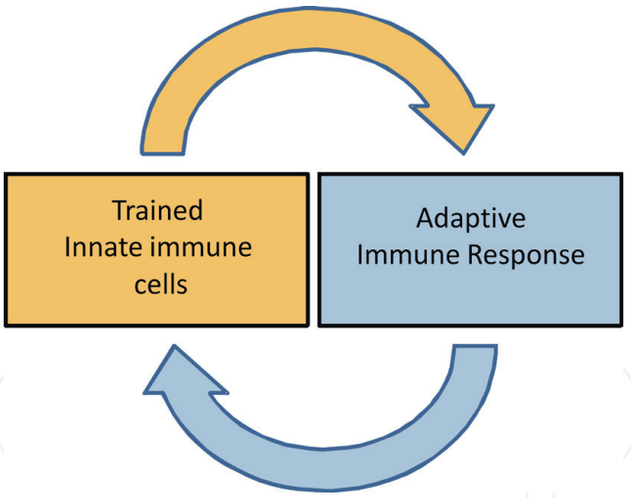

Figure 1.
Effect of trained immunity on ongoing immune responses. Induction of trained immunity allows trained cells to enhance adaptive immune responses and vice versa, final effector functions of trained cells can be further potentiated by enhanced adaptive responses.

deep crosstalk between Th17 and neutrophils have been widely demonstrated, via production of IL-17 and other related cytokines [110].

Taken into account the potential role of trained innate cells in both the induction of adaptive and effector responses, a notable amplification loop in the global immune response could be considered (**Figure 1**).

4. Trained immunity-based vaccines

Based on trained immunity pillars, a next generation of anti-infectious vaccines has been postulated, coined as 'Trained Immunity-based Vaccines' (TIbVs). TIbVs would be conceived to confer a broad protection far beyond the antigens they contain. By proper targeting of innate immune cells to promote trained immunity, a TIbV may confer non-specific resistance to unrelated pathogens while trained immunity memory is still present, in addition to the specific response given by intrinsic antigens [39].

A *bona fide* TIbV would consist of two main components: the trained immunity inducer(s) and the specific antigen(s). The antigen(s) mission is to generate an adaptive (specific) immune response as any conventional vaccine. The trained immunity inducers aim to promote the training of innate immune cells. This innate immune training would confer non-specific resistance against unrelated pathogens for a window of time (months) plus an enhanced adaptive immune response to the antigens present in the vaccine itself or from other sources (*e.g.,* coming from eventual infections or bystander pathogens) [39].

Two additional concepts arise under the TIbV umbrella: i) trained immunity-based immunostimulants (TIbIs) and ii) trained-immunity-based adjuvants (TIbAs). The former (TIbIs) would induce the training of innate immune cells, so they would be ready-to-act against upcoming infections conferring broad non-specific protection while trained immunity is present, still enhancing adaptive immune responses following any eventual natural infection. The latter (TIbAs) would enhance adaptive responses against specific antigens incorporated either to the trained inducers as in *bona fide* TIbVs, or in a separated but combined vaccine [39] (**Figure 2**).

Following the above features, the TIbV concept can be applied to existing anti-infectious vaccines composed of microorganisms that show heterologous protection ascribed to trained immunity.

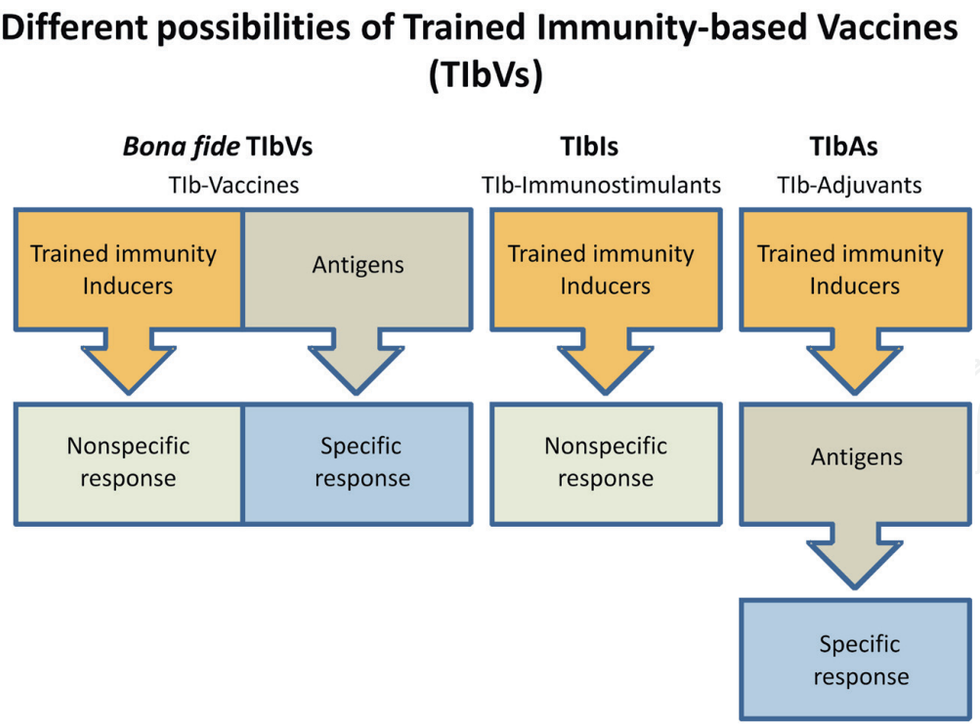

Figure 2.
Different possibilities of trained immunity-based vaccines (TIbVs). Under the umbrella of trained immunity-based vaccines (TIbVs) different possibilities exist depending on their design and purpose. Bona fide TIbVs are those containing both trained immunity inducers and antigens in the same vaccine as occurs in conventional vaccines with trained immunity inducing properties. These vaccines show heterologous protection in addition to the specific response to the target antigen. TIbIs are intended just to confer non-specific protection by means of trained immunity induction beyond the intrinsic antigens they may contain. TIbAs are intended to enhance the specific response of other vaccines that are administered later, once trained immunity has been induced, or specific antigens combined in the same vaccine as any other adjuvant.

4.1 Available vaccines with heterologous protection against viral infections

During the last decades, robust epidemiological data has demonstrated the role of certain vaccines leading to protection against heterologous infection with a high impact on overall mortality in children [111–113]. This protection could not only be explained by protection achieved by the target disease. Studies on MMR vaccination in high-income settings have also evidenced a reduction in non-target infections, particularly in respiratory infections [114]. A limitation for most of these epidemiological studies is that they do not identify the agent (viral, bacterium or parasite) responsible for the infection. These heterologous effects of certain vaccines conferring non-specific protection for a quite long time are believed to be largely due to non-specific stimulation of the innate immune system. It is not yet clear whether this is a direct reflection of trained immunity induction (*i.e.,* acting as TIbVs) in every case. The fact that most of these vaccines use live-attenuated microorganisms, *i.e.,* self-replicating agents, may suggest that a continuous stimulation of innate immune cells is necessary to obtain protection and/or to achieve a proper trained immunity for this purpose.

4.2 Live attenuated vaccines

4.2.1 BCG

The BCG-Denmark strain was tested in randomized-controlled trials (RCT) in infants who normally did not receive the BCG vaccine at birth. These studies carried out in Guinea-Bissau demonstrated that vaccination at birth was associated

with lower neonatal mortality, especially due to neonatal sepsis, respiratory infections, and fever [111, 115]. In these lines, a meta-analysis commissioned by the WHO concluded that BCG administration during the first month of life reduces all-cause mortality by 30% [116]. In these studies authors did not discriminate the etiology of infection (bacterial vs. virus); therefore, a reduction in viral infections may explain, to some extent, this result. However, in two studied carried out in India in neonates with BCG-Russian strain no such effect was observed [117]; suggesting that different immunological effect of diverse BCG strains may account for these discrepancies. A study carried out in infants to assess the impact of BCG vaccination on the incidence of RSV infection suggested a possible protective role for BCG vaccination against acute lower respiratory tract infection [118]. Other clinical studies have provided evidence suggesting a protective role for BCG on secondary viral infections [79]. In this regard, the impact of BCG vaccination on viral infection in human healthy volunteers has been assessed using the live attenuated yellow fever vaccine (YFV) as a model of viral human infection [76]. BCG vaccination induced epigenetic reprogramming in human monocytes, and these modifications correlated with IL-1β upregulation and the reduction of viremia, all these features being the hallmarks of trained immunity [76].

Similar protective effect of BCG was observed in several studies in elderly people regarding respiratory tract infections. BCG vaccination in subjects of 60–75 years old once a month for three consecutive months resulted in reduction of acute upper respiratory tract infection, concomitant to significant increase in IFN-γ and IL-10 compared with those receiving placebo [119]. A recent randomized trial of BCG vaccination was carried out in elderly patients (age ≥ 65 years) returning home from hospital admission, these subjects are at high risk to develop infections. The BCG vaccination increased the time to first infection (primary outcome) and decreased the incidence of a new infection [120]. Besides, results demonstrated that BCG vaccination resulted in lower number of infections of all causes, especially respiratory tract infections of probable viral origin, although no discrimination was made between respiratory tract infections caused by bacteria or viruses.

BCG has also been shown to enhance the response to vaccines directed against viral infections [79]. A clinical study in healthy volunteers demonstrated that BCG administration prior to influenza vaccination increases antibody titers against the 2009 pandemic influenza A (H1N1) vaccine strain, concomitantly with an enhanced IFN-γ production to influenza antigens compared with the control group [121].

4.2.2 Influenza vaccine

The cold-adapted, live attenuated influenza vaccine (CAIV) has been shown to provide non-specific cross-protection against RSV in an experimental model of infection [122].

In a randomized pilot study conducted in healthy volunteers receiving a trivalent influenza vaccine, cytokine responses against unrelated pathogens were observed [121]. During the 2003–2004 influenza A (H3N2) outbreak, an open-labeled, nonrandomized vaccine trial was carried out in children 5 to 18 years old. Subjects received either trivalent live attenuated or inactivated influenza vaccine. Live attenuated influenza vaccine but not trivalent inactivated vaccine was effective in children administered during influenza outbreak, despite the dominant circulating influenza virus was antigenically different from the vaccine strain [123].

4.2.3 Measles vaccine

Measles vaccine (MV) is among the live vaccines that have been shown to have beneficial effects reducing all-cause mortality [124]. Randomized clinical trials and observational studies from low-income countries have concluded that measles vaccination is associated with decreased overall mortality and morbidity [100]. However, a systematic review carried out by Higgins and colleagues has pointed out that most of these studies were considered at high risk of bias [116]. Nevertheless, MV seems to induce a transient suppressive effect on both the lymphoproliferative and innate response evaluated in peripheral blood mononuclear cells (PBMCs) from children, with slight increase in innate immune response, measured by non-specific cytokine production [100]. It has been reported that following measles vaccination, the *ex vivo* production of both innate (IL-6 and TNF-α) and adaptive (IFN-γ and IL-2) cytokines decreases for 2 weeks, but levels of IL-2, IL-6 and IFN-γ are increased at day 30 post vaccination compared with baseline [125]. Differences in males and females have been reported, where girls seem to receive stronger beneficial effects. In this regard, a study of MV-specific innate responses following MMR vaccination found higher TNFα, IL-6 and IFN-α secretion, cytokines associated to trained immunity, in adolescent girls than boys [126].

4.2.4 Oral polio vaccine

There are currently only three countries where polio remains endemic. Thus, polio-free, high income countries are introducing the use of the inactivated polio vaccine (IPV). However, there are still many countries that use the live-attenuated oral polio vaccine (OPV). Despite current WHO policy to replace OPV by IPV, there is epidemiological evidence that supports that replacing OPV by IPV might have an impact on overall mortality [96], since OPV has shown strong non-specific beneficial effects even in settings where the incidence of the targeted infection is low. In this regard, campaigns to eliminate polio in West Africa have been associated with lower child mortality rates [127].

4.3 Inactivated vaccines

As pointed above, most of the vaccines described so far showing non-specific heterologous effects contain live-attenuated microorganisms. Nevertheless, fully inactivated bacterial vaccines have also been described conferring protection against viral infections, and some of them for a fairly long period of time. Interestingly, these vaccines are mucosal preparations that are administered daily for long periods of time (weeks/months) rather than single, or seldom, doses used in live attenuated vaccines. Thus, it seems that the much longer administration of these inactivated mucosal vaccines resembles the effect achieved by live vaccines on heterologous protection associated to trained immunity (**Figure 3**).

4.3.1 Polybacterial whole-cell vaccines

These vaccines are used for the prevention of recurrent infections in susceptible subjects, mainly associated to the respiratory and urogenital tracts [128–134]. Since they target infections occurring in these tracts, their administration is generally through mucosal tissues to obtain a better mucosal response [135, 136].

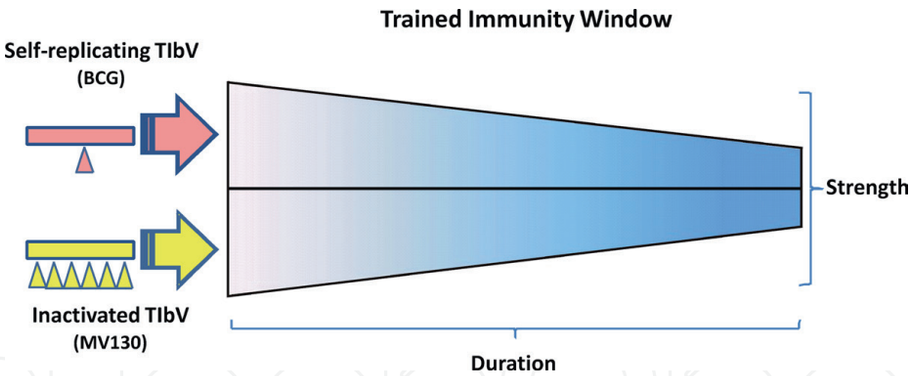

Figure 3.
Trained immunity window by self-replicating and inactivated TIbVs. Trained immunity-based vaccines (TIbVs) containing live-attenuated self-replicating microorganisms (e.g. BCG) may require fewer administrations to induce an adequate trained immunity window of sufficient intensity, quality and/or duration than vaccines with dead microorganisms. Fully-inactivated TIbVs can be enhanced to induce trained immunity with a multiple dose schedule (e.g. MV130).

MV130 is a sublingual vaccine used to prevent recurrent respiratory tract infections [128, 129] containing inactivated whole-cell bacteria that are common pathogens in the airways. Its ability immunomodulating DCs has been addressed experimentally *in vitro* and *in vivo*. MV130 triggers the release of cytokines ascribed to trained immunity in different setting, including TNF-α, IL-1β and IL-6 [103, 137, 138]. Sublingual immunization of mice with MV130 induces a systemic Th1/Th17 and IL-10 enhanced responses against unrelated antigens [103]. Similar enhancement was shown in patients treated with MV130 where an increased T cell response to flu antigens were described [128]. MV130 was successfully used in infants with recurrent wheezing, a condition triggered in most cases by viral infections. It is noteworthy that the protective effect was also shown 6 months after discontinuation of treatment, which points to a long-lasting effect that fits with the memory ascribed to trained immunity (Nieto et al., under review). In this regard, MV130 has been shown to induce trained immunity and to confer protection against experimental virus infections (Brandi et al., under review). Recent studies have assessed the clinical benefit of MV130 as a TIbV in the context of recurrent respiratory infections in vulnerable populations such as patients with different primary and secondary immunodeficiencies showing a reduced rate of respiratory infections [130, 139] (Ochoa-Grullón et al., in press).

4.3.2 Polybacterial lysates

Although not considered vaccines but immunostimulants, these bacterial preparations are, like MV130, used for the prevention of recurrent respiratory infections. OM-85, one of the best studied, is composed of chemically treated bacterial lysates for oral administration, acting through the gastro-intestinal mucosa. OM-85 has been shown to be effective in experimental viral infections [140] and in children with recurrent wheezing [141], a condition triggered by viruses as noted above. OM-85 stimulates the release of proinflammatory cytokines such as IL-1β, TNF-α and IL-6 by macrophages [142], typical of trained immunity induction, as well as Th1 cytokines including IFN-γ [143]. It is not known, however, the role of trained immunity in their mechanism of protection. A recent study conducted in infants, the observed protection against respiratory infections under OM-85 treatment stopped when treatment was discontinued [144], which may point against the memory ascribed to trained immunity.

4.4 The potential clinical applications of TIbVs in the context of viral outbreaks

The non-specific mechanism of TIbVs against widely differing pathogens associated to the induction of trained immunity can be exploited clinically. This makes TIbVs as a ready-to-act tool to tackle disease outbreaks from different angles where conventional specific vaccines have proven their limitations:

Newly emerging disease outbreaks, with no conventional vaccines available. Even in the presence of therapeutic options, vaccines are the best tool to prevent infections. However, even with worldwide efforts, getting a vaccine to the public takes time. In addition, side effects, dosing issues, and manufacturing problems can all cause delays [3]. Herein, using available TIbVs could mitigate the devastating consequences of emergent outbreaks by means of non-specific protection, until a suitable specific vaccine is available.

Newly emerging disease outbreaks, first coming vaccines with partial efficacy. Even if a vaccine gets available to the market, conventional strategies might raise some issues. The unpredictable identity of largely unknown emerging pathogens, the lack of appropriate experimental animal models, and the time for faster developing may give raise to an upcoming vaccine with no full efficacy [3]. On the other hand, limitations of current vaccines, such as mumps, also include a low efficacy resulting from an unacceptable drop in the immune response over time, requiring re-immunization [145]. In these contexts, the administration of a TIbV prior to the specific vaccine may enhance the response to the latter (111).

Re-emerging disease outbreaks, pathogens with high mutation rates and loss of vaccine efficacy. Mutations are the building blocks of evolution in any organism. Viruses are among the fastest evolving entities, especially RNA viruses such as influenza. Implications in conventional vaccine design are numerous, as a high mutation rate makes it hard to design a vaccine that is universally effective across many years. As a result, this makes a vaccine effective for shorter and raises the need for yearly vaccination programs [22, 146]. Since the underlying mechanism of TIbVs extend well beyond their nominal antigens and have a broad-spectrum of protection, TIbVs could overcome the troublesome of highly specific vaccines that promote antigen variant switching [147].

Disease outbreaks in vulnerable populations. During infectious disease outbreaks, vulnerable populations are usually disproportionately affected due to an interplay of immunological, epidemiological, and medical factors, which leads to sub-optimal or even under-vaccination [148]. This is well exemplified in the elderly population, where successful vaccination against important infectious pathogens which cause high morbidity and mortality represents a growing public health priority. Age-related immunosenescence and 'inflammaging' have been postulated as underlying mechanisms responsible for decreased response and high mortality, including during COVID-19 pandemic or influenza season [80, 149]. Therefore, more potent vaccines are needed. In this regard, the induction of trained immunity by the use of TIbVs is proposed to overcome the immune dysfunction found in these individuals [28]. Thus, elderly has been proposed as one of the groups to benefit from the use of TIbVs, including severe COVID-19 disease, with the aim of potentiating the immunogenicity of their vaccination [80]. Moreover, some types of immunodeficiencies or immunosuppression may benefit from TIbVs. These formulations, by means of tackling both branches of immunity, especially the innate compartment, may be an achievable alternative to reinforce protection or optimize immunogenicity of vaccination in this population [130, 139].

Altogether, harnessing the TIbV concept has been suggested as a crucial step in future vaccine development and implementation, because a wide range of clinical applications may benefit to some extent from their use [150].

4.5 TIbVs in the time of COVID-19

Despite the tremendous financial and scientific effort invested to rapidly obtain a prophylactic vaccine against SARS-CoV-2, only the first one has been licensed in December 2020. Although this means less than a year since the declaration of the pandemic by the WHO, which is an unprecedented achievement, in the meantime, two pandemic waves of COVID-19 and more than 1.5 million deaths have been declared worldwide. Therefore, alternative strategies have been considered to fill the gap until a safe and effective vaccine is available. As noted earlier in this chapter, TIbVs can play an important role for this purpose by increasing host resistance to other pathogens, including viruses.

A bunch of recent studies have been published supporting the role of certain vaccines, including BCG, OPV and measles, as a possible successful strategy to reduce susceptibility and severity to SARS-CoV-2 through trained immunity induction [80, 151, 152]. Thus, clinical trials are currently being conducted to find out the contribution of trained immunity as a preventive tool in the context of COVID-19 pandemics [153]. In a prospective observational trial, 255 MMR vaccinated subjects were followed searching for COVID-19 cases, thirty-six presented COVID-19 but all with a remarkable mild course [154]. Recent studies have also suggested a potential benefit of influenza vaccine on the susceptibility and the outcome of certain infections including SARS-CoV-2. In this sense, a particular attention has been focused on a high-risk population, the elderly. In a study conducted in Italy, influenza vaccination in people aged 65 and over was associated with a reduced spread and a less severe clinical expression of COVID-19 [155].

Finally, in addition to the potential role of TIbV conferring resistance against SARS-CoV-2 infection, they can eventually be used to increase efficacy of specific anti-COVID-19 vaccines, when available, especially in vulnerable population. In this sense, implications of vaccination route and mucosal immunity have also been raised as a key aspect in the development of safe and effective prophylaxis interventions against SARS-CoV-2. Most formulations in development are parentally administered; only a few COVID-19 vaccine candidates are administered by mucosal routes. Still, studies indicate that even if mucosal immunization against coronavirus does not confer sterilizing immunity, the ability to induce anti-SARS-CoV-2 IgA responses in the airways may prevent virus spread to the lung and avoid respiratory distress [156]. In this regard, mucosal TIbVs could enhance the mucosal response of specific COVID-19 vaccines acting as TIbAs by combining them as pointed above in those especially vulnerable subjects.

5. Conclusions and future perspectives

Viral outbreaks can cause epidemics and pandemics if the route of transmission allows for the rapid virus spread. Given the ease of travel and the global exchange of potential transmitting agents, these situations will be increasingly frequent in the future. Preventing the spread of a virus outbreak caused by a highly contagious agent is not easy in the absence of effective therapies or preventive measures. Although the development of effective prophylactic vaccines specific for the threatening virus is the final goal when possible, this requires a minimum time of almost a year in the best possible scenario. Meanwhile, the consequences of the spread of a deadly virus can be devastating, as it is exemplified during the COVID-19 pandemic. This scenario may also take place in the case of re-emerging viruses tackled by partial efficacious vaccines. In such situations, harnessing the heterologous non-specific protection of some existing vaccines with a known safety track record

is an interesting possibility. This protection may be critical for vulnerable subjects and/or for highly exposed individuals, like healthcare workers.

Non-specific protection of some vaccines is thought to be mainly dependent on their effect on the innate immune system. Increasing evidence gathered over the past few years points that innate immune cells show memory-like features when properly activated. This memory termed "trained immunity" has been associated with the non-specific protection of vaccines. The concept of "trained immunity-based vaccine" (TIbV) has been drawn to exploit the potential of trained immunity in designing novel vaccines or to redefine bacterial-derived preparations conferring broad protection against widely differing pathogens. As trained immunity may have implications on the adaptive immune response and *vice-versa*, its potential to provide enhanced immune responses is quite broad whether considering natural infections or following vaccination.

Taken advantage of the current COVID-19 pandemic, a number of clinical trials have been launched with putative TIbVs in order to address protection in highly exposed subjects. The results are eagerly expected as these initiatives may be considered as a proof-of-concept supporting their use in future epidemics/pandemics to fill the gap until a specific vaccine is available. Nevertheless, as trained immunity can be achieved by different inducers, it is unlikely to obtain the same degree of protection, duration, etc. for all of them, which may also depend on the biological behavior and the route of transmission of the threatening pathogen. As in most instances rapidly spreading viruses are airborne and primarily infect the mucosa of the airway tract, induction of trained immunity at the local mucosal level can confer a more adequate protection. This may be an opportunity for mucosal TIbVs as compared to those given parenterally.

Trained immunity may justify heterologous protection of vaccines, help to explain their underlying mechanisms, open avenues for next generation of vaccines, or be proposed to tackle outbreaks by new pathogens as described here. However, this is an emerging field that requires more clinical data before being a reality in the clinical practice; not only to be used against infectious outbreaks, but to fight against recurrent infections in vulnerable subjects for whom no effective vaccines are yet available.

Conflict of interest

JLS is the founder and CEO of Inmunotek SL, Spain, a pharmaceutical company that manufactures bacterial vaccines. LC and PS-L are employees of Inmunotek.

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