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Perspective

Immunoengineered adjuvants for universal vaccines against respiratory viruses

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Over the past 100 years, several pandemics have wreaked havoc across the world. Respiratory viruses, such as Influenza viruses and, more recently, Coronaviruses, have been the major causative pathogens of these pandemics. During 1918-1919, the 1918 influenza pandemic was speculated to cause 50-100 million deaths worldwide. Thereafter, pandemics caused by descendants of the 1918 virus occurred repeatedly in 1957, 1968, and 2009 [1]. Each took a heavy toll on human life, as well as significant societal costs. As the most recent and ongoing pandemic, a newly emerged coronavirus, SARS-CoV-2, have caused more than 95 million confirmed cases and 2 million deaths globally by Jan 18, 2021. Influenza viruses and coronaviruses will likely continue to be on the top of the suspect list for the next pandemic, owing to their high transmission efficiency, high morbidity and mortality, and lack of effective therapeutics. Vaccines are deemed the most cost-effective means to eliminate the current pandemic and prevent the next one. However, current influenza and coronavirus vaccines are imperfect. A number of obstacles are still blocking the path toward an “ideal” vaccine, which may be defined as one able to elicit fast and long-lasting protective immune responses against a broad spectrum of viral strains among all populations. Two key components in a vaccine formulation, optimized antigens and adjuvants, are crucial for meeting this goal. In this perspective, we will focus on recent advancements in the field of vaccine adjuvant development, and introduce how the evolutionary path from empiricism to immunoengineering can accelerate the development of adjuvants to improve vaccine performance.

1. Essential roles of adjuvants in pandemic vaccines

Along their long history of clinical use, influenza vaccines have contributed significantly to the reduction of influenza infection-caused morbidity and mortality over the past century. Since development and data collection for current safety and efficacy trials of SARS-CoV-2 vaccines are ongoing, we will limit the scope of the current work to current influenza vaccines and the potential use of existing and future adjuvants, commenting on SARS-CoV-2 when appropriate.

An adjuvant is an additive used in vaccine formulation to optimize antigen distribution and thus classified as a delivery system, or stimulate immune cells and thus classified as an immunostimulatory component. No clear border exits between these two classes in modern vaccine adjuvants, some of which comprise a group of additives serving in both roles (Fig. 1). Aluminum salt adjuvant (Alum) has been widely used in numerous licensed vaccines, including seasonal and pandemic influenza vaccines, as well as inactivated SARS-CoV-2 virus vaccines developed recently in China [2]. In the last two decades, a handful of new adjuvants have been licensed, or are in phase III clinical trials, such as squalene-based emulsion (e.g., MF59 and AS03), Toll-like receptor agonist (e.g., MPL), Saponin-based adjuvants (e.g., QS21, Matrix-M), and their combinations (e.g., AS04). As summarized in Fig. 1, these existing adjuvants may contribute to overcoming some, if not all, obstacles before an “ideal” pandemic vaccine could be achieved. First, adjuvants could enhance the immunogenicity of antigens. As a result, fewer antigens and fewer boosts are required to confer sufficient protection. This is particularly meaningful at the early stage of a pandemic when the vaccine supply is limited. AS03 and MF59 were incorporated in pandemic influenza vaccines in 2009-2010. Alum and Matrix M adjuvants were similarly used for inactivated SARS-CoV-2 vaccines or an S-protein subunit vaccine, respectively [2,3]. Next, adjuvants, may improve the efficacy of vaccines for newborn and elder (e.g., MF59) populations which are most vulnerable to pandemic, but typically suffered from low vaccine efficacy owing to either immature immune system or immunosenescence. Finally, adjuvants could modulate the type of immune responses towards protection and away from vaccine-induced disease enhancement. This is particularly attractive in the development of coronavirus vaccines in which a Th2 response-associated disease enhancement is always a concern. Whether a Th1-biased or a Th1-Th2 balanced adjuvant is beneficial for improving the safety of SARS-CoV-2 vaccination merits further study.

Above are three obstacles that could be overcome at least in part by existing vaccine adjuvants. Other “high hanging fruits” remain to be confronted by the next-generation vaccine adjuvants, including how to accelerate the induction and extend the longevity of protective immune

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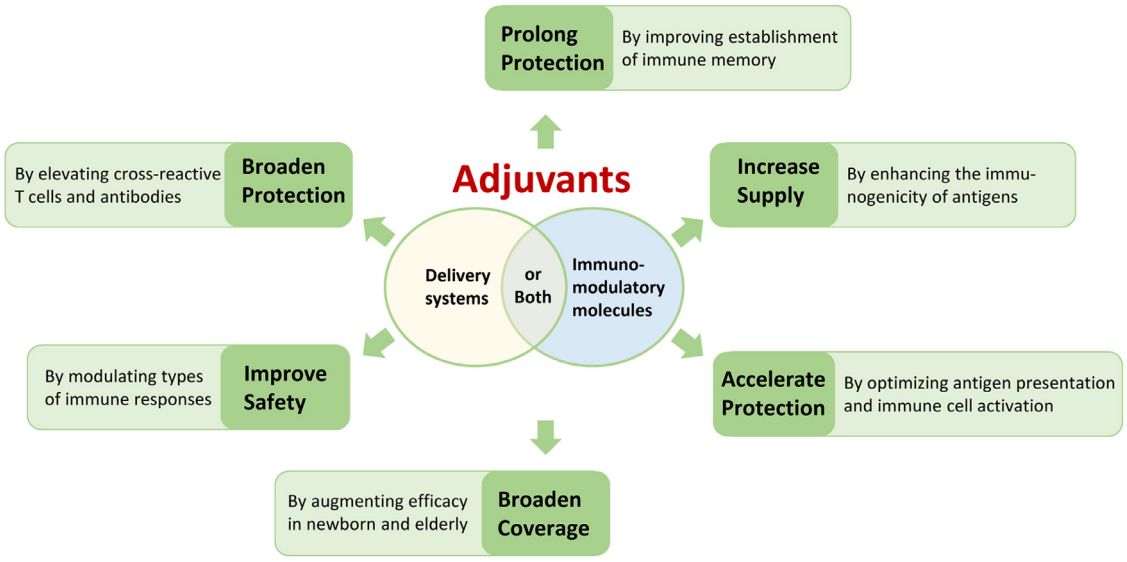

Fig. 1. Essential roles of adjuvants. Adjuvants are defined as a vaccine component to improve the distribution of antigens (delivery system) and/or stimulate the immune system (immunomodulatory molecule). Major problems of current pandemic vaccines could be confronted by existing or next-generation adjuvants.

responses and, pernhaps most important, how to broaden immune protection against mutated viruses and emerging viruses from other species or subtypes.

2. Broaden protection by immunoengineering approaches

An “ideal” pandemic vaccine should not only protect against the original viral strain, but also against mutated viruses that evolve as the pandemic continues. More ideally, this vaccine should be able to prevent the next pandemic caused by novel viruses formed through reassortment with zoonotic viruses. In vaccinating against influenza, immune escape as a result of antigen shift or drift has long been a problem. Licensed influenza vaccines, including whole inactivated virus, split virus, virosomes, and subunit vaccines, mainly induce humoral responses against envelope proteins (Hemagglutinin, HA, and Neuraminidase, NA) on the viral surface, while antiviral cellular immune responses (CD4+ and CD8+ T cells) are very low, even in the presence of existing vaccine adjuvants. Two genera of influenza viruses infect humans, Type A and B. Within Influenza A, numerous subtypes occur as a result of reassortment among 18 kinds of HA and 11 NA. Each subtype is a large group of strains derived from antigen drift. A large number of these subtypes and strains can infect humans. Some of them are highly pathogenic (e.g., H5N1, H7N9), or highly transmissible (e.g., 2009 H1N1), or even both (e.g., 1918 H1N1). Unfortunately, the humoral immune responses induced by current influenza vaccines are highly susceptible to mutations. Thus, vaccine-induced protection is constrained to a very small group of strains that are genetically similar to the vaccine strain. Some flu seasons have seen mismatch between circulating viral strains and the vaccine by just a few amino acids. This has resulted in a drop of efficacy of vaccination from an average of 60% to as low as 10% [4]. In some years (e.g., 2009), such mismatch has led to the outbreak of a pandemic. Where genomic surveillance is practiced, SARS-CoV-2 is being monitored for such mismatch and its impact on current vaccine production. Emerging mutants capable of escaping from immune response induced by current SARS-CoV-2 vaccines is always a concern, especially in the light of several recent cases showing that a second infection by mutated viruses did occur in some patients [5]. Moreover, many viruses from various species within the genera of betacoronavirus could infect humans, including SARS, MERS, and OC43. Such potential risks call for the development of a cross-protetive vaccine or, better, a universal vaccine against multiple subtypes or species.

Eliciting broadly neutralizing antibodies or cross-protective T cell responses are two long sought-after strategies. A potent adjuvant system, termed GLA-SE, is a combination of squalene emulsion SE and TLR4 agonist GLA. It could broaden the protective spectrum of a subunit pandemic H5N1 vaccine to other H5N1 viral strains, most likely by elevating cross-reactive antibody titers [6]. A further improvement in inducing heterosubtypic protective antibodies requires a deep understanding of broadly neutralizing epitopes (e.g., HA stem) and advancement in antigen design to adequately expose these epitopes. Additionally, a potent adjuvant is still required since the immunogenicity of these antigens is always poor. A recent study showed that vaccination with a smart recombinant HA stem based on H1 subtype in the presence of Matrix M adjuvant conferred protection against H5N1 viruses [7]. Nevertheless, viruses carrying mutations may occur under the selective pressure of vaccine-induced anti-stem immunity [8], suggesting that the antigen design of a universal influenza vaccine is still challenging, even when it is based on extensive understanding of influenza viruses gained over the past 100 years. Whether or not designing a universal vaccine for a newly emerged virus requires such a long time fascinates open questions for future research.

Accumulated evidence demonstrated that T cell responses, which always target conserved epitopes in internal viral proteins, could mediate cross-protection, especially when these T cells bear a tissue-resident memory phenotype [9]. These tissue-resident memory T cells (TRM) could be rapidly recalled at the onset of disease, enabling more efficient control of viral infection and spread. Therefore, eliciting TRM beneath respiratory mucosa has recently gained increasing interest. Unlike B cell epitopes, which are always conformational, T cell epitopes are linear. Theoretically, mucosal immunization with a conventional inactivated virus or subunit vaccine could induce such cross-protective T cell response when an appropriate adjuvant is included. However, a highly efficient adjuvant is still a bottleneck, which can be ascribed to the complexity raised by multiple layers of targeted delivery and precise regulation of multiple cell types in mucosal immunization. Key steps and associated issues towards an effective induction of respiratory TRM are summarized in Fig. 2, including breaching anatomical and physiological barriers, increasing antigen uptake and activation of dendritic cells (DC), optimizing antigen presentation in DC, and enhancing the establishment of local residence of memory T cells. Besides efficacy, safety should be taken into account since vaccines are given to healthy populations. Side effects could be avoided by precise control of the type, location, and

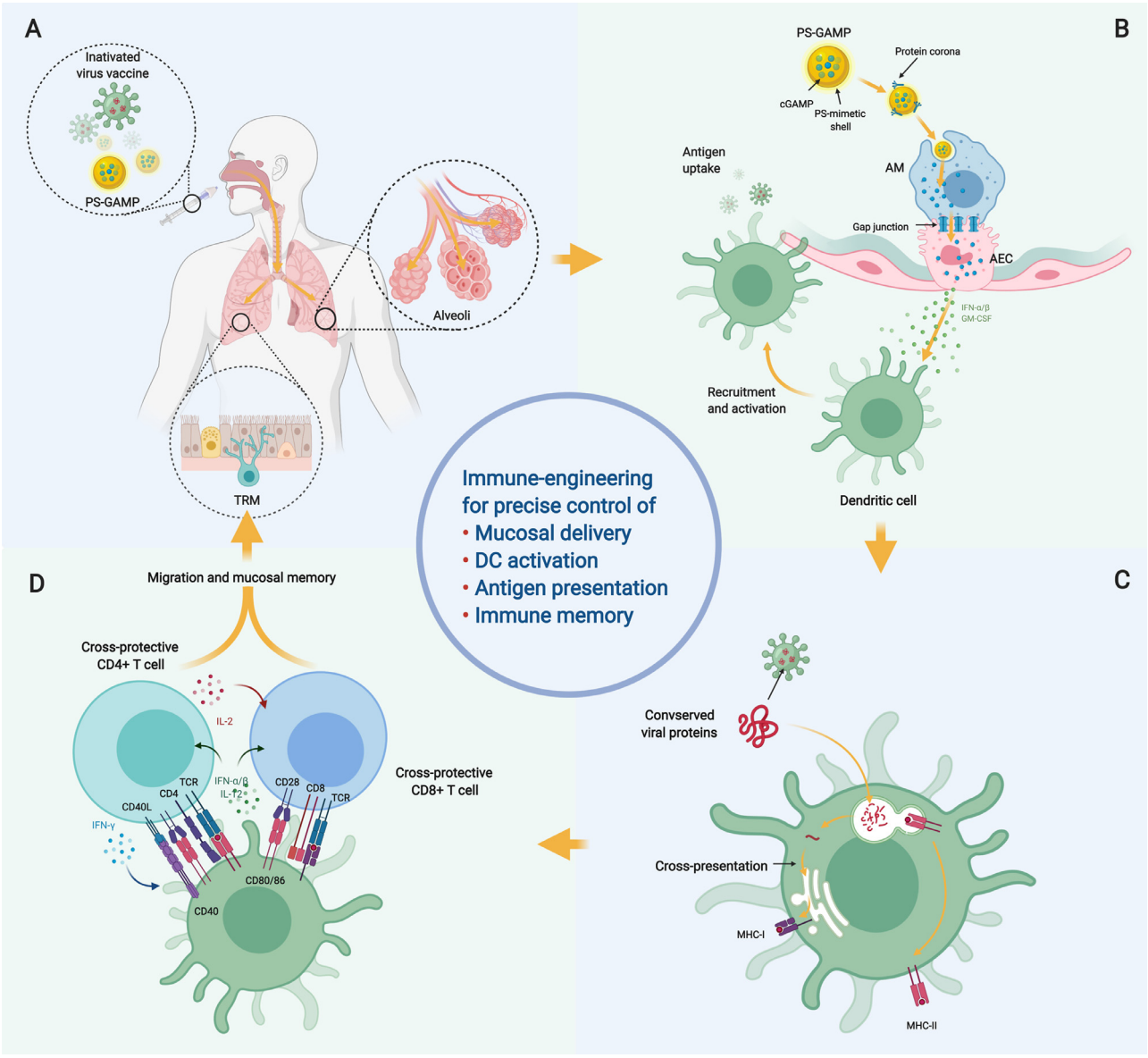

Fig. 2. Key steps of inducing a cross-protective T cell response in respiratory mucosa. Vaccination of inactivated influenza vaccine and PS-GAMP were illustrated as an example showing key steps of inducing a cross-protective T cell response. (A) Mucosal delivery of vaccines and adjuvants into respiratory tracts, especially deep lung, faces many anatomical and physiological barriers. Both inoculation devices and adjuvants (e.g., PS-GAMP) should be tailored accordingly. (B) Recruitment and activation of dendritic cells are the next key steps for effective antigen uptake. PS-GAMP indirectly activates DC in a way that harnesses a gap junction-mediated transfer of cGAMP between AM and AEC. (C) Epitopes within conserved internal viral proteins are subsequently presented by MHC I and MHC II. A cascade of intracellular processes, herein termed as cross-presentation, is required for antigen presentation through MHC I. (D) Co-stimulatory signals and cytokines are required for optimal activation of cross-protective T cells. Local environment educated by mucosal vaccination finally promotes the establishment of long-lasting memory and tissue residence. Opportunities for immunoengineering approaches are summarized in the middle. This figure was created by BioRender.

duration of innate immunity activated by adjuvants. Transient and localized, rather than systemic and long-lasting, activation is preferred. Given the complexity of this process, developing an “ideal” adjuvant cannot be satisfactorily addressed by traditional empirical strategies.

We recently developed a new mucosal adjuvant capable of improving the protective spectrum of inactivated influenza vaccines in mice and ferrets [10]. The adjuvant, termed PS-GAMP, delivered natural STING agonist cGAMP into respiratory mucosa via rationally designed pulmonary surfactant (PS) mimetic nanoparticles. When codelivered intranasally with inactivated H1N1 vaccines, PS-GAMP significantly augmented vaccine efficacy in inducing both systemic and mucosal immune responses, including IgA and TRM, and protected animals from lethal challenges by heterologous H1N1, H3N2, H5N1, and H7N9 viruses. Distinct from the traditionally empirical development of vaccine adjuvants, an immunoengineering approach was involved in the design of this novel adjuvant system.

Immunoengineering is a newly emerged concept in which an advanced understanding of the immune system is enrolled to guide the development of engineering solutions. On the other hand, engineering innovation provides feedback to shed new light on the immune mechanism for a further improved design. This two-way feedback system resulted in the accelerated development of PS-GAMP. Taking the aforementioned understanding of mucosal immune response induction as the initial guideline, a biomimetic strategy was used to facilitate mucosal delivery, but avoid adverse effects. We found that a particle mimicking pulmonary surfactants was highly effective in reaching the deep lung as compared to many nanoparticles with different physicochemical properties (Fig. 2A) [10]. Targeted delivery of immunostimulatory

molecules to dendritic cells is the most straightforward strategy of adjuvant design. However, our investigation involving fluorescent nanoparticles and probes indicated that delivering PS particles into dendritic cells was much more difficult than delivering such particles to alveolar macrophages (AM). The protein corona of surfactant protein (SP)-A and -D on PS nanoparticles favored an engulfment of these particles by AM, but not other cell types. Furthermore, redirecting PS particles to dendritic cells might further increase the complexity of adjuvant design since a surface ligand that is highly selective for DC, but not AM, must be added. Additional machinery that rejects AM engulfment without significant impact on DC may be required as well. Fortunately, a rational design guided by recent immunological findings may simplify adjuvant development. Gap junctions between AM and alveolar epithelial cells (AEC) enabling an intercellular transfer of small molecules were recently found [11]. A potent immunostimulatory, Cyclic guanosine monophosphate–adenosine monophosphate (cGAMP), is such a small molecule ready to be transferred through gap junctions [12]. Moreover, activation of AEC in natural influenza infection could recruit and activate DC for a better T cell response [13]. These findings point out a new approach to stimulate DC in an indirect, but equally effective, way without increasing the complexity of nanoparticles. As a result, a cGAMP-encapsulated PS-mimetic strategy was established. PS-GAMP enhances the antigen uptake and activation of DCs in an indirect way that uses AEC as an interpreter (Fig. 2B). PS-GAMP effectively strengthens the capability of inactivated influenza vaccines in inducing T cell responses against conserved internal viral proteins, leaving a long-lived TRM for heterosubtypic protection [10].

3. Implications for next-generation vaccine adjuvants

PS-GAMP may not be the ultimate adjuvant for future pandemic vaccines, but this initial advancement in providing conventional influenza vaccines with the capability of inducing heterosubtypic immunity reveals the potential of immunoengineering approaches for a future breakthrough in adjuvant development.

Based on this very early starting point, we make some observations. First, novel delivery techniques are urgently needed if memory responses in the lower respiratory tract are further demonstrated to be critical for influenza or coronavirus infection in humans. Unlike small animals that have a relatively small-sized respiratory system, an inhaler or nebulizer enabling deep lung delivery is required for large animal studies and human vaccination (Fig. 2A). However, no such technique has been used for mucosal vaccination to date. Second, for a protein-based vaccine aiming for high CD8+ T cell responses, a cascade of processes enabling an effective cross-presentation is required (Fig. 2C). Further improvement enabling more precise control of antigen processing inside DC would be a future direction. Manipulating the fate of antigens has always been neglected in vaccine research for infectious diseases, but it is thriving in cancer vaccine research. One recent study described a proton-driven transformable nanovaccine that mechanically disrupted the endosomal membrane and delivered the antigenic peptide into the cytoplasm by undergoing a dramatic morphological change from nanospheres to nanosheets [14]. These novel immunoengineering approaches for cancer vaccines may inspire the development of pandemic vaccines. Third, multiple stimulatory signals are required for an optimized expansion of T cells and the establishment of memory and tissue residence (Fig. 2D). Our recent study indicated that a spiky nanostructure could be a new kind of stimulus for DC and substantially augment the efficacy of influenza vaccines in inducing both humoral and cellular immune responses [15]. Incorporating nanostructures into an adjuvant design in addition to conventional immunostimulatory components (e.g., TLR and STING agonists) to further improve adjuvanticity merits further study.

In spite of a long history of influenza vaccination and the avent of SARS-CoV-2 vaccines, the virus's propensity to mutate calls for the development of a universal vaccine. Recent advancements in adjuvant development demonstrate the essential role of a potent adjuvant in broadening the protective spectrum of first-generation vaccines. In the future, a universal vaccine would be achieved by a combination of a more transformative immunogen exposing broadly neutralizing epitopes and/or a panel of conserved T cell epitopes and an ideal adjuvant that precisely modulates the delivery, DC activation, antigen presentation and immune memory. Improved coordination among multiple scientific disciplines, including immunology and biomedical engineering, is urgently needed in the hunt for such adjuvants.

Declaration of Competing Interest

The authors declare that they have no conflict of interest

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