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Laser facilitates vaccination

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Development of novel vaccine deliveries and vaccine adjuvants is of great importance to address the dilemma that the vaccine field faces: to improve vaccine efficacy without compromising safety. Harnessing the specific effects of laser on biological systems, a number of novel concepts have been proposed and proved in recent years to facilitate vaccination in a safer and more efficient way. The key advantage of using laser technology in vaccine delivery and adjuvantation is that all processes are initiated by physical effects with no foreign chemicals administered into the body. Here, we review the recent advances in using laser technology to facilitate vaccine delivery and augment vaccine efficacy as well as the underlying mechanisms.

Keywords: Laser; vaccine delivery; adjuvant.

1. Introduction

Vaccine is the most cost-effective way to control infectious diseases. To date, more than 70 vaccines have been developed to reduce the morbidity and mortality caused by approximately 30 pathogens.[1] The most successful example is smallpox vaccine, which completely eradicated smallpox in humans and saved millions of lives. Despite the enormous success of vaccines, there are still several obstacles to overcome before vaccines can reach their full potential. First, current vaccine manufacturing capacity is far from meeting the global needs, especially in response to a pandemic. Taking influenza vaccine as an example, the global influenza vaccine manufacturing capacity has increased to ~ 1 billion doses per year, yet this manufacturing capacity can only meet 1/10th of the global need (~ 10 billion, two doses for 70% of population). Second, effective vaccines are still not available for some diseases, including human immunodeficiency virus (HIV)

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infection, tuberculosis and malaria. Third, the efficacy of vaccines in the very young and old populations is much lower than that in their young adult counterparts.[2,3]

Dose sparing would be an attractive strategy to overcome the limited manufacturing capacity and reduce the cost of vaccination in developing countries. Therefore new vaccine delivery strategies and adjuvants that enable dose sparing have been extensively studied in recent years. Besides dose sparing, new delivery strategies and adjuvants are an essential part of our effort to develop future vaccines, in which the type, location and duration of the immune responses should be accurately controlled to ensure the efficacy and safety.[4,5] Moreover these new delivery strategies and adjuvants can also contribute to augment vaccine-induced immune responses in very young and old populations.

Tens of new chemical adjuvants have been developed in last decades, but only a few of them entered clinics, mostly due to the safety concern of administrating foreign chemicals into healthy recipients. Alternatively, the concept of laser-based vaccine adjuvant has been proposed and studied in recent years.[6–9] The key advantage of this technology over traditional chemical adjuvants is there would be no any foreign chemicals administered into our body, holding a great promise for future clinical applications. Because the early practice of laser adjuvant has been reviewed elsewhere,[10–12] in this, we will focus on the most recent advances in this field. Additionally, we will also summarize the progress on how laser technology facilitates the delivery of vaccines without incurring any unwanted side effects.

2. Facilitation of Cutaneous Delivery of Vaccines by Laser Technology

Most of current vaccines are administered through intramuscular injections, although the skin is known to be a more potent site for vaccination, because a large number of antigen presenting cells reside in the skin. In sharp contrast, there are fewer antigen presenting cells in the muscle in homeostasis state. Skin also contains abundant lymphatic and blood vessels, ensuring quick recruitment of immune cells from the circulation into the skin and fast migration of antigen-loaded antigen presenting cells from the skin into lymph nodes. In accordance to this, intradermal vaccination has been found to induce more potent immune responses than that induced by intramuscular injections of various vaccines, including influenza, Rabies, etc.[13]

Yet, intradermal injection of vaccines is frequently associated with severe local reactions. A number of studies showed that injection of influenza vaccines into skin by hypodermal needles caused swelling and erythema lasting for several days.[14,15] Additionally intradermal injection of Bacillus Calmette–Guérin (BCG) vaccines, a vaccine used worldwide to prevent childhood tuberculous meningitis and miliary disease, induced severe local reactions, leaving permanent scars on the skin.[16]

To resolve this issue, ablative fractional laser (AFL) was used to fractionally deliver vaccines into skin. AFL generates an array of microchannels in epidermis. These laser-generated microchannels are so small that they can be quickly healed in one or two days by surrounding healthy tissues. Vaccines can be delivered into these microchannels by applying vaccine solution on the surface of laser-treated skin or delivering vaccine powder accurately into each channels using epidermal powder delivery (EPD).[17,18] Amazingly, fractional delivery of vaccine into these well separated microchannels greatly reduced vaccine-induced skin reactions without compromising vaccine efficacy. For instance, fractional delivery of BCG vaccine into laser-generated microchannels resulted in faster and full recovery of the skin, whereas intradermal injection of BCG vaccine induced prolonged inflammation and permanent scars.[18]

Besides BCG vaccine, this laser-mediated fractional delivery can be also used for the transcutaneous delivery of novel anti-tumor or antiviral vaccines. Harnessing microchannels generated by the Precise Laser Epidermal System (P.L.E.A.S.E.), Terhorst *et al.* transcutaneously delivered a XCR1+ dendritic cell targeting anti-tumor vaccine, which subsequently induced robust anti-tumor immune responses in mice.[19] The laser-mediated fractional delivery is also beneficial for attenuated viral vectors, especially for vaccinia virus-derived vectors.[18] Vaccinia virus is traditionally delivered through physically damaged epidermis (by scarification) to induce protective immunity against smallpox. Although the majority of population is no longer receiving this vaccine after eradication of smallpox, vaccinia virus derived vectors are still the focus of the vaccine research.[20] Being a vector, it can induce

very potent humoral and cellular immune responses against the foreign gene it carries. The vaccinia vectors have been used as carriers of HIV vaccines, in which replication-competent Tiantan vaccinia virus (rTV) carrying the gag, pol and gp140 genes induced potent immune responses and provided high level of protection in rhesus macaques.[21] However, to maximize its efficacy, these vectors need to be delivered by scarification, leading to uncontrollable skin damages. This may be the major reason why this highly effective vaccine vector is rarely used. On the other hand, fractional delivery of vaccinia vectors by laser-mediated EPD induced highly controllable skin damages, with more potent immune responses.[18]

Taken together, this fractional delivery strategy holds a great promise to improve intradermal skin vaccination. It is worthwhile to point out that AFC is not the only way to generate skin microchannels for fractional intradermal vaccine delivery. Technologies, like microneedle arrays, can also accomplish this goal. Our study showed cutaneous delivery of BCG vaccine as well as influenza vaccine by microneedles resulted in improved skin conditions as compared with intradermal injections.[22]

3. Increasing Blood Vessel Entry of Malaria Vaccine by Laser Illumination

Besides nonspecific ablation of superficial skin to generate microchannels, facilitation of vaccination could also be achieved in a more specific manner. In 1980s, researchers found illuminating skin with green laser resulted in blood vessel leakage due to the absorption of light energy by hemoglobin.[23] Oxygenated hemoglobin and hemoglobin inside red blood cells have a peak absorbance at 540 nm and 578 nm, respectively. Therefore upon laser illumination within these wavelengths, red blood cells carrying hemoglobin absorb laser energy and release heat to destroy capillaries in the skin. This treatment is named "selective photothermolysis" and widely used to treat vascular malformation in clinics for decades. Recently this laser treatment has been used to induce transient capillary leakage to increase the concentration of blood biomakers in the superficial layer of skin.[24]

Generally, entering circulation system is not required for most vaccines, but it is a crucial step for one promising malaria vaccine, PfSPZ (Sanaria Inc.), composed of radiation-attenuated sporozoites.[25] Malaria is a tropical disease caused by *Plasmodium falciparum* (pf) parasite which infected approximately 207 million people and cased 627,000 deaths in 2012 alone.[26] Vaccines are the most cost-effective strategy to control malarial epidemics, but currently the most advanced malaria vaccine, RTS, S, can only provide about 50% protection in humans.[27] Fortunately another promising malaria vaccine candidate, named PfSPZ, has been found to confer >80% protection in human volunteers.[25] Radiation-attenuated sporozoites could infect hepatocytes and synthesize early liver stage-specific antigens, which are important for inducing protective immunity against malaria infection. Conceivably, for this live-attenuated vaccine, stronger immunity is correlated with a greater amount of radiation-attenuated sporozoites reaching the liver. Therefore intravenous injection is used in current clinical trials to maximize the entry of sporozoites into the blood vessel and then the liver. Although intravenous injection is considered as the most efficient route for delivering sporozoites to the liver, it faces formidable technical hurdles in vaccination of a large population, especially infants and young children whose veins are hardly visible. Unfortunately it is infants and young children who suffer from malaria and need the vaccine most. On the other hand, intradermal injection is a more clinically acceptable route for vaccination and it also mimics the natural infection by mosquito bites. However, intradermal injection is far less efficient than intravenous route, probably because the entry of sporozoites into blood vessels is highly restricted in the dermis.[28] To achieve a similar level of protection, a substantially higher number of sporozoites is required for intradermal immunization than that for intravenous injection. Simply increasing the number of sporozoites per dose would increase the cost significantly, which would be problematic for a prophylactic vaccine needed by a large population in underdeveloped countries.

To facilitate the entry of sporozoites into blood vessels, the inoculation site was treated with a low power laser (532 nm) at 1 J/cm^2. This treatment selectively increased permeability of blood vessels and significantly enhanced skin-to-liver delivery of intradermal-injected sporozoites by 7-fold.[29] A schematic diagram is shown in Fig. 1. More importantly, the laser-mediated enhancement of skin-to-liver

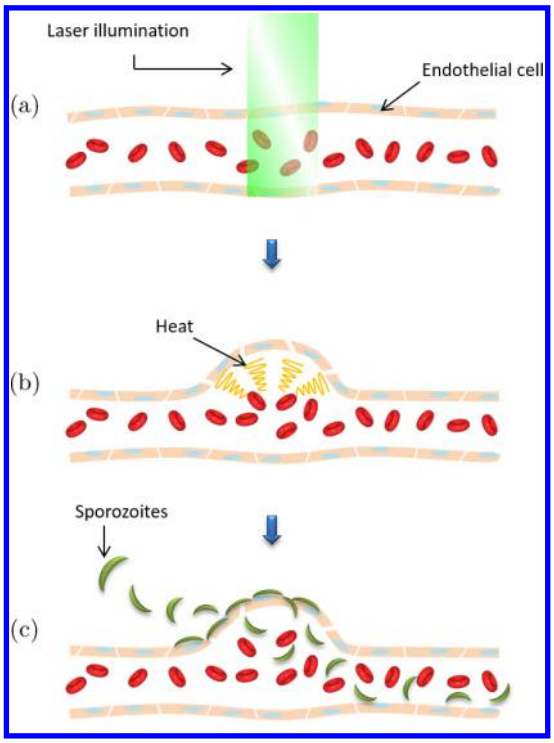

Fig. 1. Delivery of malaria vaccine is facilitated by laser illumination. (a) 532 nm laser illuminates the skin, penetrating through skin blood capillary. (b) The laser energy is specifically absorbed by hemoglobin inside red blood cells, and converted into heat, leading to a transient increase in permeability of capillary vessels. (c) The radiation-attenuated sporozoites malaria vaccine binds blood vessel walls and enters circulation system easily through these permeabilized blood vessels.

delivery resulted in much stronger sporozoite-specific immune responses than that induced by intradermal vaccination alone and conferred protection against malaria infection to a similar level as intravenous immunization.[29] If these early results can be confirmed in large animals and humans, laser-mediated intradermal delivery of radiation-attenuated sporozoites can serve as a more convenient and equally efficient alternative to intravenous vaccination. Moreover, the laser illumination can be combined with microneedle array to further simplify the vaccination in the future.

4. Laser Induced Micro-Sterile Inflammation Array as Vaccine Adjuvant

Another issue that hampers cutaneous vaccination is lack of safe adjuvants. Adjuvants could augment vaccine-induced immune responses, as well as modulate the type of immune responses. For example, Th1 immune responses are preferred to control intracellular pathogens infection (virus, intracellular bacteria, etc.), whereas Th2 immune responses are critical in the defense against extracellular pathogens (extracellular bacteria, etc.). Unfortunately, our previous studies showed most currently used or under developed adjuvants, including aluminum hydroxide (Alum), oil-in-water emulsion and toll-like receptor (TLR) agonists, were not suitable for cutaneous vaccination, because these foreign chemicals often induce severe and long lasting local reactions after being injected into the skin.[8–11]

To address this, the concept of using inherent "danger signals" to alert the immune system was proposed. The inspiration came from an old adjuvant, Alum. Alum has been used as a vaccine adjuvant since 1920s. Previous studies suggested that the adjuvant effect of Alum was attributed to its antigen deposit effect, meaning that Alum forms hydrogel with vaccines in the injection site, releasing antigens slowly and stimulating the immune system continuously. However, a number of recent studies challenged this traditional view. Hutchison *et al.* demonstrated that removing the injection site 2 h after immunization did not result in compromised immune responses, indicating that the antigen deposit effect may not be important for the adjuvant effect of Alum.[30] Meanwhile, a number of studies suggested the underlying mechanism owing to toxicity of Alum[31]: Alum kills host cells, and dead cells in turn release danger signals, including uric acid, genomic DNAs, etc.[31–33] These danger signals are designated as damage-associated molecular patterns (DAMPs). DAMPs could activate a number of immune pathways, like inflammasome and nucleic acid sensing pathway, inducing sterile inflammation that could subsequently enhance the adaptive immune responses.

These discoveries raise a question: why we need foreign chemicals, like Alum, to induce cell deaths, but not a much safer physics treatment that would not leave any foreign chemicals in our body. To prove this, we treated skin cells with high temperature (65–95°C), and injected them back into the skin with influenza vaccine. Interestingly we found this treatment induced higher immune responses compared to vaccine alone.[34]

These results revealed that skin tissue injury can serve as a vaccine adjuvant. Nonablative fractional

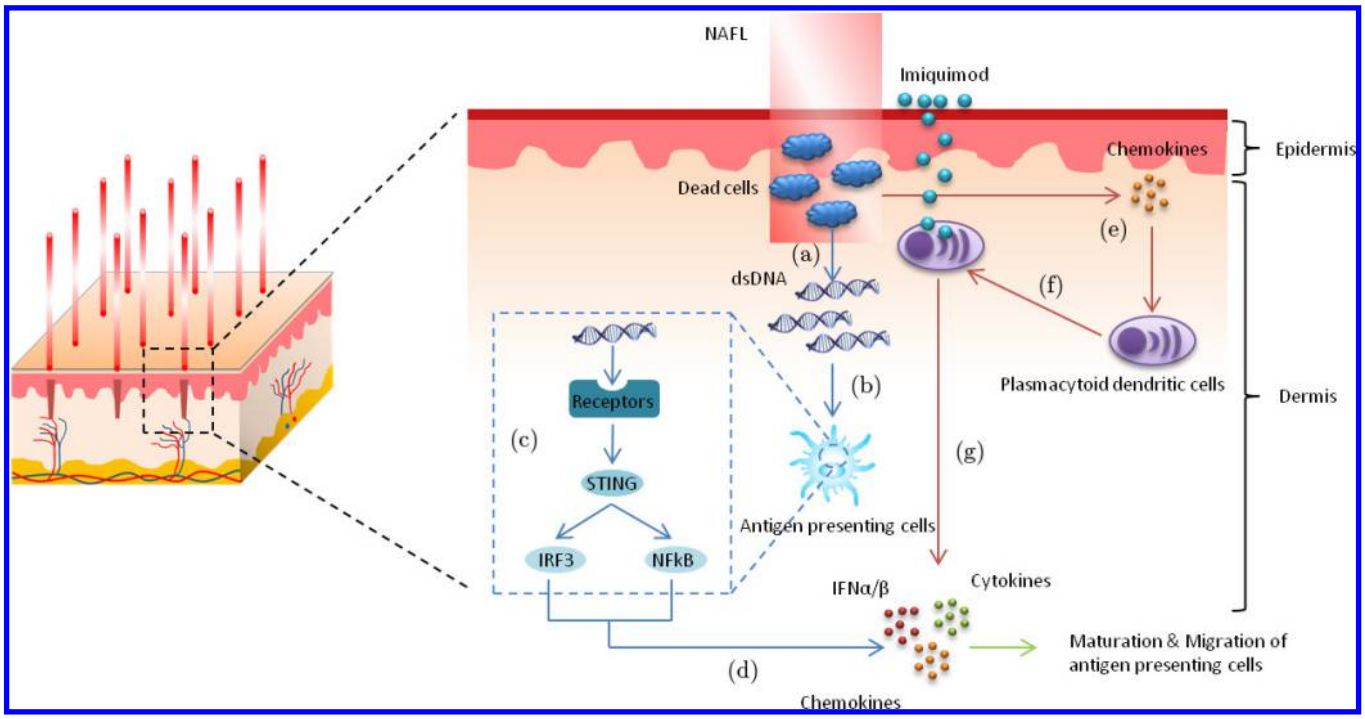

Fig. 2. Micro-sterile inflammation array-based adjuvant. An NAFL induced micro-sterile inflammation array is shown on the left, and one of the micro-injured zones is enlarged on the right. (a) NAFL treatment kills skin cells which release dsDNA subsequently. dsDNA is taken up by antigen presenting cells (b) and recognized by DNA receptors (c). Upon ligand binding, activation signals by the receptors are transduced to STING, followed by activation of IRF3 and NFκB. (d) Type I interferons, proinflammatory cytokines and chemokines are produced to enhance the maturation and migration of antigen presenting cells. NAFL can be also combined with plasmacytoid dendritic cell activator, Imiquimod. (e) NAFL treatment first induces expression of chemokines in the skin. (f) These chemokines recruit plasmacytoid dendritic cells from circulation system into the skin. (g) The plasmacytoid dendritic cells are subsequently activated by topically applied Imiquimod cream, and release a number of factors to enhance the maturation and migration of antigen presenting cells, leading to enhanced adaptive immune responses.

laser (NAFL) can controllably induce skin injury, yet leading to a younger looking skin, a mature technology used in the cosmetic industry for decades with excellent safety profile.[35] The NAFL generates an array of micro-injured zones each as small as 200 micrometers in diameter rather than damage a single large area of skin as illustrated in Fig. 2. These micro-injured zones induce tiny sterile inflammation zones well separated by healthy skin and these tiny sterile inflammation zones can be resolved quickly, as short as 2 days, ensuring its safety.[34] Interestingly, this transient inflammation is sufficient to enhance the immune responses induced by a number of vaccines, including model vaccine ovalbumin, Hepatitis B vaccine, and influenza vaccine. Vaccination of influenza vaccine with this micro-sterile inflammation array induced more potent protection against a viral challenge.[34]

Our further investigation revealed that dsDNA released by laser-damaged host cells is one of the major mechanisms underlying NAFL-induced adjuvanicity.[22] As mentioned above, Alum adjuvant induces the release of genomic dsDNAs from dead cells, which in turn activate the DNA sensing pathways.[31,32] The dsDNA can be recognized by cytosolic receptors, including cyclic GMP–AMP synthase (cGAS), etc.[36] Upon binding to the dsDNA sensor, the activation signal is transduced to the adaptor protein Stimulator of interferon genes (STING), followed by activation of Type I interferon transcription through a TBK1–IRF3-mediated pathway or by activation of pro-inflammatory cytokines through NFκB pathway. Apart from dsDNA, tissue injury could also induce DAMPs like uric acid to activate inflammasome,[33] a complex activates capase-1 to cleave prematured interleukin-1 family (IL-1β, IL-18 and IL-33) into their active forms.[37] Moreover, TLR are also potential targets of DAMPs. Upon ligand banding, TLRs transduce signals through MyD88 or TRIF protein to activate NFκB pathway or IRF pathway,

respectively.[38] However, by using mice deficient in one of these pathways, our results clearly showed the dsDNA-sensing, but not TLRs- or inflammasome-sensing pathway participated in augmentation of the immune responses by NAFL.[22] The adjuvant effect of NAFL is summarized in Fig. 2(a)–2(c).

The dsDNA released from dying host cells appears to have a universal role to augment adaptive immune responses. Besides aforementioned Alum adjuvant, sensing of dsDNA was also suggested to be a key to the immunogenicity of DNA vaccine. In support, B and T cell-mediated immune responses induced by DNA vaccine were greatly impaired in STING- or TBK1-deficient mice.[39,40] Moreover, the transfer of tumor derived dsDNA and subsequent activation of STING–IRF3 pathway have been shown to sufficiently augment $CD8^+$ T cell responses against tumor cells.[41] Alum adjuvant not only induces release of dsDNA but also contributes to transfect dsDNA into cells.[32] However, in most of the studies, including our study on NAFL adjuvant, how dsDNA entered cytosol remains unknown. Further studies in understanding this process are essential to maximize the effect of dsDNA-mediated immune augmentation as well as the immunogenicity of DNA vaccine.

Apart from intradermal injections, NAFL could be combined with other cutaneous vaccine delivery technologies. One of most promising technology is microneedles, especially biodegradable microneedles.[42] As we mentioned previously, fractional delivery of vaccines by biodegradable microneedle array could greatly reduce the skin irritation induced by cutaneous delivery of vaccines. Biodegradable microneedle also offers additional advantages over traditional vaccination strategies, such as painless, sharp-hazard-free and self-applicable. However, delivering a clinically relevant dose of vaccine by these microneedles is always an issue, because polymerization matrix must occupy the microneedle shaft to provide sufficient mechanic strength.[43] Unfortunately, this limitation could not be resolved simply by increasing the length of the microneedles or the density of needles, since long microneedles or increased density caused for pain and severe irritation, deviating from the ultimate goal of using microneedles.[44] Our study revealed that NAFL adjuvant might be able to address this dilemma. Pretreatment of the inoculation site with NAFL augmented the efficacy of microneedle-delivered influenza vaccine by at least 4-fold, holding a great potential to spare the vaccine dose required for cutaneous vaccination.[22] In addition, NAFL broadens the protection spectrum of microneedle-delivered influenza vaccines. Immunization of influenza vaccine (A/Puerto Rico/8/1934 H1N1 strain)-loaded microneedles, along with NAFL treatment not only fully protected mice from the challenge of the homologous virus strain, but also resulted in a significantly higher survival rate when challenged by genetically distant H1N1 strain (A/California/7/2009 and A/New Caledonia/20/1999) and heterosutypic H3N2 strain (A/Aichi/2/68).[22] Cross-protective immunity is extremely important for seasonal influenza vaccines because the mismatch between immunizing viral strains and circulating viral strains occurs frequently, reducing the efficacy of seasonal influenza vaccines substantially. Such a mismatch took place recently in the flu season of 2009–2010, 2012–2013 and 2014–2015, diminishing the efficacy of vaccines, especially in elderly population (>65 years of age).[45,46]

NAFL can be used as a standalone vaccine adjuvant alone or along with other chemical adjuvants. As shown in Fig. 2(e)–2(g), the base of the combination is NAFL-induced micro-sterile inflammation array could recruit a large number of antigen presenting cells, especially plasmacytoid dendritic cells, into the skin via releasing a number of chemokines. Plasmacytoid dendritic cells have been demonstrated to be pivotal in inducing immune responses against influenza virus.[47] In homeostasis state, there are few plasmacytoid dendritic cells residing in the skin, but they are actively recruited to the skin following NAFL treatment.[34] An increased number of plasmacytoid dendritic cells in the skin provides an opportunity to activate these important cells locally by topical application of immune stimulators, rather than intradermal injection of them, which would induce severe local reactions and systemic side-effects. For instance, the Imiquimod cream (Aldara®),[48] is clinically used as a topical treatment for genital/perianal warts, superficial basal cell carcinoma and actinic keratosis.[49] It is a potent activator of plasmacytoid dendritic cells, binding to TLR-7. When Imiquimod was applied on NAFL treated area, it activated plasmacytoid dendritic cells accumulated in the skin and strengthened adaptive immune responses. This combination lead to a 7-fold increase of antibody titers over traditional influenza vaccination in hemagglutination inhibition (HAI) assay, a gold

standard to evaluate the efficacy of influenza vaccination.[34] The result indicates this immunization strategy can offer a significant dose sparing, which may greatly reduce the cost of vaccines and speed up the vaccination of whole population during a pandemic. Besides dose sparing, this immunization strategy may also help the elder population. Intradermal or intramuscular injection of influenza vaccine only induced insufficient immune responses owing to the immunosenescence in elderly.[3] Encouragingly, addition of NAFL/Imiquimod adjuvant system greatly reversed the immunosenescence and conferred high level of protection against lethal viral challenges in old mice.[34]

5. Conclusion

A great deal of progress has been made recently on how vaccination can be facilitated by lights. A number of new concepts emerged, such as fractional delivery and micro-inflammation array, greatly bolstering our understanding of the nature of the skin immune system. These concepts hold a great promise to solve several key issues in today's vaccine field, leading to a safer and more efficient vaccination. Because these concepts/strategies were only tested in mouse and pig models, a more clinically relevant model like monkeys and clinical trials are urgently needed in the near future to fully realize their potentials.

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