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COMMENTARY

Mucosal adjuvants: Opportunities and challenges

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ABSTRACT

Most pathogens access the body via mucosal surfaces. Mucosal vaccination is a highly effective and recommended method to prevent mucosally transmitted infections. Compared with immunization via intramuscular injection, mucosal immunization offers remarkable advantages, including non-invasiveness, low costs and reduced risk of transmission of blood-borne diseases, which make it more acceptable to human beings, especially to young children. However, only few mucosal vaccines are licensed for human, which is mainly due to the deficiency of safe and effective mucosal adjuvants. Adjuvants, as important components of most vaccines, are essential to enhance immunity and induce immune memory. The development of mucosal adjuvants, unfortunately, has been severely hampered by research strategies based on empiric trials and non-comprehensive methods for safety evaluation. Therefore, changing the research and development strategies of mucosal adjuvant field from empiricism based discovery to rational design based invention is highly demanded. The change of strategies mainly depends upon clarification of mechanism of mucosal adjuvant activity though a combination of life science, information science and materials science.

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Contribution of mucosal adjuvants

Mucosal adjuvants can be roughly divided into 2 categories: delivery vehicles and immunostimulatory molecules. It is worthy to note that some mucosal adjuvants are both immunostimulators and delivery systems, such as chitosan and its derivatives.[1] After decades of research, it has been proved that mucosal adjuvants can induce protective local and systemic immune responses, which is essential for effective mucosal vaccinations against a series of infectious diseases.[2-5] Furthermore, mucosal adjuvants play important roles in defenses against infections at distant as well as local sites. For example, CpG oligodeoxynucleotides (ODNs) act as potent mucosal adjuvants for nasal vaccinations against infections transmitted by blood transfusion and sexual behavior.[6-7] Additionally, mucosal adjuvants are potent enhancers of immune responses against tumor.[8-9] Among numerous mucosal adjuvants, Toll-like receptor (TLR) agonists and mutant enterotoxins are the 2 most attractive types because they are not only effective but also relatively safe.[2]

Safety evaluation of mucosal adjuvants

It is difficult to prove that a mucosal adjuvant is indeed safe. For instance, LTK63, a mutant of *Escherichia coli* labile toxin, was once thought to be safe. However, it has been demonstrated that intranasal administration of LTK63 can induce transient facial nerve paralysis (Bell's palsy).[10] It is unclear whether mucosal adjuvants are indeed safe for humans, even for animals in models for preclinical research, which is a major hurdle for the utilization of mucosal adjuvants. Although more and more researchers begin to focus on safety assessment of mucosal adjuvants, existing methods of evaluation are still not rigorous enough. The problem is mainly reflected in the following 3 aspects: (i) lack of standardization: Safety evaluation of mucosal adjuvants mainly depends on knowledge structure and experience of researchers themselves, but not a standardized evaluation methodology based on a broadly acknowledged scientific theory. (ii) lack of comprehensiveness: Most current safety evaluation strategies are too simple. Therefore, it needs to be reconsidered that whether conclusions based on these strategies are authentic. To date, strategies based on animal models are main methods for safety evaluation of novel mucosal adjuvants. Body temperature, body weight, food consumption, mediators of toxicity (such as some proinflammatory factors) and tissue sections of pivotal organs (brain, lung, heart, kidneys, liver, reproductive organs and immune organs) are common indicators. However, these indicators have not been fully applied. Much attention has been focused on early-onset damages following vaccination. More attention is needed to be paid to late-onset damages, carcinogenicity and health conditions of offspring of experimental animals. Furthermore, safety evaluation based on animal models under special physiological condition is not enough. For example, in most mice models, experiment objects are young adults (6–8 weeks old), while little attention has been paid to neonatal mice, old mice and pregnant mice models. (iii) lack of specific pertinence: There is no doubt that the delivery routes of mucosal adjuvant are closely related to rational design of methods for the safety evaluation. For example, particular attention should be paid to damages in central nervous system and respiratory system for safety

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assessment of nasal adjuvants. Furthermore, the results of the assessment depend on multiple factors, and any change of factors (such as animal species, vaccine antigens, delivery routes, etc.) in the evaluation system may affect the conclusion. Even if a mucosal adjuvant has been proven to be safe in an evaluation system, the safety of the adjuvant has to be proved again in another evaluation system. Although guidelines on the nonclinical evaluation of vaccine adjuvants released by World Health Organization have been playing positive roles,[11] there is still an urgent need of a more scientific and systematic approach for safety evaluation of mucosal adjuvants.

Research and development of mucosal adjuvants

The mucosal epithelial barrier is a major obstacle to effective mucosal vaccination because of the limitation to bioavailability of vaccine antigens for sampling by antigen presenting cells (APCs).[12] Therefore, the ability of adjuvants to promote bioavailability of vaccine antigens is essential for effective mucosal vaccination. Various delivery enhancers have been used for mucosal immunization.[13] It is particularly noteworthy that some substances, such as polyethyleneimine and chitosan, are not only relatively safe penetration enhancers but also potent immunostimulants.[14]

Another key factor that needs to be considered is targeting ability of mucosal adjuvants, which is essential for efficient mucosal vaccination and reduction of unnecessary waste and undesired effects. Some immune cells and immune receptors on them are ideal targets for development of rationally designed mucosal adjuvants. Dendritic cells (DCs), as the most important APCs, are critical for optimal vaccination.[15] In addition, long term protective immunity, an indicator for measuring the effectiveness of mucosal vaccination, is closely related to activation of memory B cells, memory T cells and memory natural killer (NK) cells.[16-18] Furthermore, mast cells have been shown to be able to induce protective immunity against pathogens.[19] In summary, APCs, immune memory cells and cells with newly discovered anti-infection functions are particularly noteworthy.

For decades, thousands of mucosal adjuvants have been developed. However, few of them have been demonstrated to be safe enough for human use. From this perspective, the safety problem of mucosal adjuvants may be a more urgent challenge when compared with effectiveness of mucosal adjuvants. In fact, most mucosal adjuvants are potentially toxic. Effectiveness and toxicity of most mucosal adjuvants seem to be intrinsically linked, which suggests that an effective mucosal adjuvant with absolute safety can hardly been achieved, and the pursuit of an optimised effectiveness/toxicity ratio is more realistic. In addition, storage of most existing mucosal adjuvants is highly dependent on the cold chain, but the cold chain maintenance is still a big problem around the world, especially in developing countries. Mucosal adjuvants without excellent thermal stability can become ineffective or even harmful in practical applications. Therefore, the thermal stability is an important factor that needs to be considered in the design process of mucosal adjuvants.

Information technology is an important means to overcome difficult problems mentioned above. However, development of mucosal adjuvant field is severely restricted because of low utilization of information science. Gratifyingly, a web-based resource has been built to aid in the design of ODNs-based adjuvants.[20] Moreover, big data analysis has been implemented in adjuvant field to advance our understanding of the mechanisms of actions of adjuvants.[21] To date, the value of information science has been demonstrated in many areas, and more information science based tools for designing mucosal adjuvants are needed to be developed.

Except for new technologies, new concepts are also helpful to the development of mucosal adjuvants. In fact, some old concepts have limited the birth of novel adjuvants. For example, it is a widely accepted concept that adjuvants increase immune responses largely though promoting inflammation. However it has been demonstrated that limited generalized inflammation induced by adjuvants is helpful to achieve optimal efficacy.[22] More importantly, a polysaccharide-based adjuvant has been shown to be potent to increase humoral and cellular immunity through a noninflammatory mechanism.[23] Moreover, the role of TLR in activation of innate immunity was considered central to the antibody-enhancing effects of adjuvants.[24] But some recent studies challenged this view. It has been demonstrated that MyD88 and Toll/IL-1 receptor domain-containing adaptor inducing IFN-β (TRIF), the critical signaling components for TLR, are not necessary for antibody responses.[25,26] These new findings provide new insights into development of mucosal adjuvants.

Finally, the selection of adjuvants for a mucosal vaccine is as crucial as the invention of adjuvants. Irrational mix of a mucosal vaccine and an adjuvant is useless or even harmful.[27] Therefore, we need to not only pay attention to substances with adjuvanticity, but also focus our attention on antigen–adjuvant interactions. It is the core task for researchers to elucidate mechanisms of action of mucosal adjuvants by a strategy that combing a variety of disciplines, and then to accomplish the goal of precision vaccination: the optimal combination of mucosal vaccine, adjuvant and delivery route targeting a specific disease.

Abbreviations

APCs	antigen presenting cells
DCs	dendritic cells
NK	natural killer
ODNs	oligodeoxynucleotides
TLR	Toll-like receptor
TRIF	Toll/IL-1 receptor domain-containing adaptor inducing IFN-β

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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