Application of Prime-Boost as a Novel Vaccination Strategy Against Microbial Pathogens

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The main public health strategy for reducing infection is vaccination. However, because immune responses can differ, due to the age-related and genetic characteristics of the individual, among other factors, novel strategies have been explored in the last decade to augment the immune response after immunisation. These studies have included increasing the dose in vaccines that require more than one dose, using new adjuvants and making the immunisation process more efficient. This review aims to discuss recent advances in prime-boost immunisation strategies with particular emphasis on the immunogenicities of different antigen preparations, combined antigens, adjuvants, security, simplicity and ease of use, including an evaluation comparing prime-boost to other routes that are used in vaccines. Substantial experience has been accumulated with different Neisseria species, such as *Neisseria lactamica, Neisseria meningitidis* and other pathogens. Currently, the prime-boost strategy has been investigated in situations where both priming and boosting immunisations were administered systemically. Moreover, measurements of efficacy have been focused on components of systemic immunity. Because for many pathogens the portal of entry is via mucosal surfaces, and while immunity at such sites can limit or even preclude infection, immunity is also important to evaluate prime-boost strategies for their effects on mucosal defence. Several experiments have shown that these strategies are safe and immunogenic and can offer significant advantages in terms of higher acceptability and higher immunogenicity at different ages compared to intramuscularly administered vaccines.

**Keywords:** prime/boost, microbial pathogen

Effective vaccines should generate protective immunity by producing antibodies and by establishing a durable T cell-mediated response [1]. In this theoretical context, an ideal vaccine is relatively easy to define; however, many vaccines are deficient and do not approach the ideal. Additionally, for many organisms, no vaccines exist. Producing a vaccine is a realistic strategy for obtaining protection but is not predictable. Many difficulties can delay successfully obtaining a vaccine. Among these hindrances, microorganisms possess evasion mechanisms that interfere with effective immune response. Further, for many organisms, it is unclear what immune responses provide effective protection [2]. However, recent advances in methods utilized to study the immune response to pathogens have provided a better understanding of immune mechanisms, including immunological memory [2,3]. In particular, the initiation of the immune response is important. Considering these aspects, the development of new adjuvants, vectors and new vaccine formulations allows the stimulation of protective immunity and should be considered when introducing vaccines for organisms resistant to treatments [4].

Thinking in a positive context, it is easy to define the properties of an ideal vaccine. Most of these properties are obvious, but many vaccines are far from ideal, and approaches used to produce routinely administered vaccines should be considered [5]. Protective immunity must be broadly protective against all variants that convey how to prevent a disease. Further, immunity is not effective in all vaccinated individuals, including children and the elderly. The ideal vaccine should be able to transmit maternal protection and may not necessarily need to be administered by injection; the vaccine should be cheap, stable (no requirement for a “cold chain”) and covered by insurance [6].

Recent research employing the sciences of biology, immunology, engineering, bioinformatics, genetics, and advanced technology will change in terms of our ability to direct science toward solving the problems of significant infectious diseases and immunological puzzles that slow or prevent vaccine development against threats such as many infectious diseases. One of the areas of current study that is extremely attractive is the inverse approach to the development of vaccines (i.e., reverse vaccinology), which uses the genome sequence of the pathogen [7]. The genome sequence provides a comprehensive catalogue of almost all of the antigens that the pathogen can express at any time. This approach starts from the genomic sequence; computer analysis can make an estimate of the antigens that are likely candidates for use. This approach could, therefore, open perspectives for identifying the antigens that can be inserted into potential vaccine candidates and that could provide protective immunity without knowing the amount of antigen, the immunogenicity during infection or the expression levels in vitro. This not only allows the identification of all of the antigens seen by conventional methods but also facilitates the discovery of antigens while working in a completely different direction [8]. However, even with all this technology, both immunisation schedules and new adjuvants should be taken into consideration [9].

Immunisation programs provide a vast amount of immunisation. The provision of programs and the vast amount of vaccines remain effective public health strategies for preventing disease [10]. While these strategies remain in place, however, there is still a need for new effective vaccination strategies for various pathogens [11,12].
The immune system recognises antigens present in vaccine preparations as foreign agents and processes them as such. When an individual is infected by the same agent, it quickly responds with a rapid and effective response, neutralising the agent before it enters into cells. The host also has a strategy to recognise and destroy infected cells before that agent can multiply to large numbers [13].

A vaccine is an antigenic preparation that enhances immunity to the disease(s) that it targets [14]. Vaccines normally contain antigenic components that resemble disease-causing microorganisms; they are often made from killed or attenuated organisms or their toxins. The agent stimulates the body's immune system to recognise the agent as foreign, then to process and "remember" it such that the immune system can more easily recognise and destroy any of these microorganisms upon subsequent contact.

Vaccines can be prophylactic (e.g., to prevent or mitigate the effects of future infection) or therapeutic (e.g., vaccines against cancer are also being investigated).

**Vaccination schedule**

In a vaccination schedule, for most vaccines, including the appropriate time range is important to take into account the number of doses that are either recommended or mandatory depending on the "location where the vaccine will be administered".

Vaccines are antigenic preparations used to produce active immunity against diseases to prevent or reduce the effects of infection [15]. Many vaccines require multiple doses for maximum effectiveness in producing an efficient immune response. For example, it is often recommended that the tetanus vaccine be re-administered every 10 years [11]. These processes are developed by government agencies based on studies to achieve maximum effectiveness. In the last two decades, the recommended vaccination schedule has grown rapidly and has become more complicated, as many vaccines have been developed recently [11].

**Delivery systems**

There is a considerable amount of current research regarding vaccine development and the ways to administer a vaccine. Current methods include the use of liposomes and ISCOM (immune stimulating complex) [16,17].

The recent development of new technologies for the administration of vaccines has resulted in the production of oral vaccines. The polio vaccine serves as a successful example: it was developed and tested on volunteers with no formal training, and the results were notably positive. With an oral vaccine, there is no risk of blood contamination. Oral vaccines are considered more stable and less prone to freezing damage, and this stability reduces the need for a "cold chain" (e.g., the resources needed to maintain the vaccines within a narrow temperature range from initial manufacture to the point of administration), which, in turn, lowers the cost of vaccines. Finally, a microneedle approach, which is still in the developmental stages, seems to be the vaccine of the future [18,19].

A number of innovative vaccines are also under development and in current use, such as using dendritic cell vaccines, which combine antigens with dendritic cells to present antigens to host white blood cells, stimulating an immune response. These vaccines have shown positive preliminary results for the treatment of brain tumours [20].

The use of new recombinant vectors, which combine the physiology of the genetic information of one organism with that of another, allows immunity to be created against diseases that have complex processes of infection [21]. Sequencing to identify bacterial proteins that are involved in complement inhibition would neutralise the key mechanism of bacterial virulence [22].

While most vaccines are created using inactivated or attenuated compounds from micro-organisms, synthetic vaccines are composed mainly or entirely of synthetic peptides, carbohydrates or antigens [23].

**Developing immunity**

For many vaccines, several immunisations are required to induce protection. Depending on the age of the vaccinee, up to five immunisations are required for vaccines, such as diphtheria, tetanus, and pertussis vaccine (DTP), which is administered three times during the first six months after birth followed by a fourth dose in the second year of life and a booster dose when the child is between four and six years old. Still, certain patients require additional doses of vaccine, even adults who have received full immunisation, such as tetanus-diphtheria (Td) for which a booster is recommended every 10 years throughout the life of the individual.

Clearly, different prime-boost approaches are likely to generate distinct types of immunity, and it is essential to ensure that only appropriate immune responses are targeted during immunisation. The factors that regulate distinct types of immune responses are poorly understood; depending on the route of delivery, it is difficult to predict in advance what type of immune response will be favoured. We found that four intranasal (i.n.) priming immunisations followed by an intramuscular (i.m.) boost significantly enhanced serum Opc-specific antibody titres. Studies are underway in our laboratory to determine the optimal number of doses and the quantity of Opc or PorB purified o
N. meningitidis are required for sensitising the mucosal immune system [24,25] and for stimulating the appearance of memory cells. We maintain that the quality of the produced antibody is another important factor to consider in choosing an adequate antigen.

Recently, a great deal of attention has been directed toward needle-free immunisation strategies as alternative methods for vaccine delivery. Both mucosal (i.n., oral, and rectal) and transcutaneous immunisation in the presence of an appropriate adjuvant have been shown to induce humoral and cellular immune responses in both the systemic and mucosal compartments of immunised animals. Alternating routes for delivery of the priming and booster doses in immunisations, which are known as prime-boost strategies, have also been examined. Such prime-boost strategies could be particularly important in an imminent or post-release bioterrorism event if it is possible to administer a parenteral priming dose and simultaneously distribute a follow-up patch, pill, or nasal applicator that could be self-administered. Such vaccine strategies would greatly improve national preparedness [14].

An effective vaccine usually requires more than a one-time immunisation in the form of a prime-boost [11]. Traditionally, the same vaccines are given multiple times as homologous boosts. New findings have suggested that prime-boost can be used with different types of vaccines containing the same antigens. In many cases, these heterologous prime-boosts can be more immunogenic than homologous prime-boosts.

The heterologous prime-boost strategy represents a new method of immunisation and will stimulate a better understanding of the immunological basis of vaccines[14].

While it is not entirely clear why some vaccines require more immunisations than others, it is well accepted that multiple immunisations (i.e., prime-boosts) are crucial for even the most successful vaccines. This principle applies to live-attenuated vaccines (e.g., the oral polio vaccine), inactivated vaccines (e.g., the hepatitis A vaccine), recombinant protein subunit vaccines (e.g., the hepatitis B vaccine), and polysaccharide vaccines (e.g., the Haemophilus influenzae type b vaccine). For these vaccines, the prime-boost is homologous because the same vaccines given in the earlier priming immunisations are used for subsequent boost immunisations.

**Prime–boost vaccines against pathogens**

A 1992 landmark Science report was among the first to employ the heterologous prime–boost immunisation technique in a non-human primate model [26]. In that study, Macaca fascicularis were first immunised with recombinant vaccinia virus expressing SIVmne gp160 antigen and were subsequently boosted with gp160 protein produced in baculovirus-infected cells. Animals were protected from intravenous challenge with SIVmne viruses. This experiment led to one of the most promising reports of protection in the early HIV vaccine development efforts.

In several works, that pivotal report established a key principle for the use of heterologous prime–boost immunisations: that is, to elicit both humoral and cell-mediated immune responses [12] . Modern immunology has established that such a balanced immune response is important for protection against several types of pathogens. Traditional vaccines, particularly inactivated and subunit vaccines, are not notably effective in eliciting T cell responses.

Over the past several years, the use of heterologous prime–boost approaches in vaccine research has gained significant momentum against a wide range of pathogens. Several features have become apparent in this trend.

For tuberculosis vaccine development, qualitatively and quantitatively different cellular immune responses have been elicited in rhesus macaques receiving a recombinant Bacille Calmette-Guérin (BCG) prime followed by an adenovirus type 35 vector boost that expressed a fusion protein composed of Ag85A, Ag85B, and TB104 [27]. Alternatively, BCG can be used as a boost following priming with a DNA vaccine. In one study conducted in calves, a DNA prime with Ag85B, MPT64, and MPT83 antigens followed by a BCG boost was able to elicit higher immune responses and better protection than BCG alone against Mycobacterium bovis challenge [28].

Second, a well-designed heterologous prime–boost approach can expand the scope of immune responses. When mice were primed with a DNA vaccine expressing ESAT6 and later received the same antigen in the form of recombinant protein as a boost, the production of Th1-type cytokines increased significantly, as did the IgG2 to IgG1 ratio [29]. In another murine study, a prime with a DNA vaccine expressing the gD antigen of herpes simplex virus type 2 (HSV-2), which preferentially induces Th1-type cellular immune responses, and a boost with recombinant gD protein, which mainly induces Th2-biased responses, led to significantly enhanced antibody levels, T cell proliferation, and Th1 cytokine production [30].

Third, the prime–boost vaccine approach can also improve the effectiveness of existing vaccines. One example is the use of DNA prime, which increased antibody response levels in animals that later received boosts with inactivated rabies vaccines [31]. Similarly, DNA prime increased the titres and longevity of hyperimmune sera in animals that were immunised with the recombinant PA antigen against anthrax [32]. By adding a DNA prime, mice boosted with the licensed hepatitis B surface protein vaccine were able to produce stronger and more homogeneous antibody responses in a study group compared to groups only receiving recombinant protein alone. Higher IL-12 and IFN-γ secretions in splenocytes were also observed [32].
Mechanisms of heterologous prime–boost vaccines

A fundamental but still mysterious question is why the heterologous prime–boost is more effective than homologous prime–boost, even when the same vaccine components are used for each. One way to study this question is to determine the importance of the order of administration of heterologous prime–boost vaccines. Using a Mycobacterium bovis model, it was demonstrated that the order of prime–boost vaccination of neonatal calves with BCG and DNA vaccine, encoding Hsp65, Hsp70, and Apa, was not crucial for enhancing protection against bovine tuberculosis [33].

The immunogenicity of heterologous prime–boost can be further improved by including other factors that may further facilitate or enhance the effects of vaccines. For example, including plasmid cytokines and colony-stimulating factors could enhance the immunogenicity of DNA-primed viral vector-boosted HIV-1 vaccines [34]. The potency of the DNA vaccine prime can be enhanced by using a microparticle-based formulation followed by a protein boost [35]. However, it is not clear whether using different adjuvants for a protein vaccine boost will make any difference.

Heterologous prime–boost vaccination using both traditional and novel immunisation approaches provides exciting opportunities to elicit unique immune responses to allow for improved immunogenicity and/or protection. Research has shown that the heterologous prime–boost can take various forms and that the order of prime–boost administration may be important, although this finding may be antigen-dependent and may be influenced by the host species and the type(s) of immune responses to be achieved. Future studies will need to focus more on the mechanisms behind the heterologous prime–boost vaccination approach and solve practical issues related to a two-component vaccine, including of vaccines costs and any currently unidentified safety issues.

Prime-boost and Neisseria

Most vaccines are delivered parenterally by means of injections, which is the traditional method for presenting an accurate amount of antigen to the immune system. However, the majority of infectious diseases are caused by pathogens entering the body via mucosal surfaces, which have an immune system designed to initiate local and systemic immune responses.

Outer membrane proteins can induce a functional immune response against serogroup B Neisseria meningitidis. However, none of the vaccines that have been developed to date can induce universal protection, due to the great heterogeneity of the surface proteins of the outer membrane. The modest cross-reactive immunity induced by the OMV vaccines has fuelled the search for an outer membrane antigen (or group of antigens) that induces functional antibodies and is present in all meningococcal strains. Such antigens, were they present on all strains irrespective of the serogroup, might form the basis for a truly universal meningococcal vaccine, which would eliminate the potential problem of capsular switching on pathogenic strains following polysaccharide vaccination [38], [39], [40], [41].

In the hexavalent meningococcal B OMV vaccine (HexaMen), two of the six Porin A (PorA) proteins present in the vaccine are weakly immunogenic in mice and humans. Therefore, the researchers used a prime-boost strategy in which they observed an increase in antibody avidity, indicating that affinity maturation had occurred. The study showed that specific priming, rather than specific boosting with monovalent OMVs, yielded a significant rise in the serosubtype-specific immune response against a weakly immunogenic PorA with high-avidity antibodies in an extended immunisation schedule [42].

In our study, [43] we demonstrated that the i.n. immunisation of neonatal mice in a prime-boost model with N. lactamica NOMVs could be valuable in the design of vaccines for the prevention of meningitis. As we know, understanding the basis for this observation could lead to the identification of those N. lactamica antigens that mediate cross-reactive protective immunity and the development of natural immunity.

It has been shown that whole killed meningococci and pertussis bacteria possess mucosal adjuvanticity for admixed particulate antigens, which are weakly immunogenic by the nasal route [44]. Meanwhile, we have demonstrated good immunogenicity in our studies [45].

In another study in our laboratory, we used a prime-boost strategy with purified antigens of outer membrane of Neisseria meningitidis [25]. The nasal route for vaccination offers both mucosal and systemic immunity for the prophylaxis of respiratory diseases. To induce maximal protective mucosal immunity, using a mucosal adjuvant is essential. Current studies are searching vaccine antigens that are derived from the outer membrane protein (OMP) of Neisseria meningitidis. This study investigated the immunogenicity of intranasally administered class 5C protein of Neisseria meningitidis B, employing purified LPS from immunotypes L3, 7, 9 or L8, cholera toxin and whole cells of Bordetella pertussis as mucosal adjuvants for the development of a new meningococcal vaccine. The study showed that the nasal delivery of class 5C protein with mucosal adjuvants has considerable potential in the development of a mucosal vaccine against serogroup B meningococci in a prime-boost immunisation in the presence of the adjuvants used. A monoclonal antibody against class 5C protein using popliteal lymph nodes was obtained.

We also evaluated this strategy in a rabbit model using a prime boost immunization in 1 [46,47]. A protocol was proposed to study the immunogenicity of killed whole cells of N. lactamica, N. meningitidis, N. sicca or N. meningitidis (carrier-isolated) by i.n. immunisation, considering the natural entry route of the pathogen. Adult rabbits were inoculated i.n. with oropharynx-isolated N. lactamica, N. meningitidis, N. sicca or N. meningitidis. The rabbits...
developed levels of specific IgG antibodies in their sera as determined by ELISA using whole cells of homologous and heterologous strains. Sera from rabbits immunised with *N. lactamica*, *N. meningitidis*, *N. sicca* or *N. meningitidis* c strain showed IgG antibodies reactive to 5- to 190-kDa antigens on immunoblotting with high avidity.

In our recent study, we investigated the adjuvant properties of toxoids Stx1 and Stx2 (STEC) from *Escherichia coli* and native outer membrane vesicles (NOMV) of *Neisseria meningitidis* B, comparing two methods of immunisation: prime-boost or intramuscular vaccination in BALB/c mice. The prime-boost was effective; however, it performed no better than two intramuscular doses. This study may contribute to the development of new technologies and strategies directed against *N. meningitidis* B and employing toxoids as adjuvants [48]. We also investigated the use of the Stx2 toxoid from *E. coli* as an adjuvant for a possible future vaccine model and as an antigenic stimulant protein of the external membrane of meningococci (OMP) carried in liposomes [49].

**Conclusion**

We must bear in mind that new technologies are assisting in the rapid development of new advances in antigen preparations. We must use these developments to increase agility and implementation in the short, medium or long term.

It is important to remember that genetic factors influence various aspects of the context of the immune response; thus, polymorphisms in immune response genes can result in variation in the immune response to vaccines and biological products. Defining these polymorphisms can provide an opportunity to determine who is at risk of a serious result of an infectious disease, the probability of a serious adverse event of a vaccine, the number and dose of a vaccine required to induce immunity, and even the impact of the driving development of new vaccines.

We believe that the issues discussed above represent significant factors that may impact the field of vaccinology. Once understood and addressed, these areas become opportunities to increase immunisation rates in populations at risk, particularly in the elderly, to reduce the cost of health care in a setting of a population structure consisting of more older individuals, and to improve public health. Each of these issues is critical for understanding the economic, social and scientific drives for vaccine development and use within the population. These issues present themselves as a puzzle in the process of recognising the threat of infectious diseases, vaccine development, and public use of vaccines. Despite the best available technology and the development of better vaccines, such vaccines are useless if not used and trusted by health professionals and by the public. These concepts discussed above could improve the safety of vaccines, allowing screening for susceptibility to adverse events and improving confidence in vaccines and public health strategy. In “sun”, we believe that the future is both bright and promising for vaccinology as a new science and as technological advances continue, particularly in genetics and immunology. If successful, human health is likely to benefit substantially.

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