Modulation of immune responses following antigen administration by mucosal route

Eva Medina, Carlos Alberto Guzmán *

Department of Microbial Pathogenesis and Vaccine Research, Division of Microbiology, GfB-German Research Centre for Biotechnology, Mascheroder Weg 1, D-38124 Braunschweig, Germany

Received 19 July 1999; accepted 13 October 1999

Abstract

Most microbial infections are either restricted to the mucosal membranes or the etiologic agents needed to transit the mucosa. Thus, it is desirable to stimulate a mucosal response following vaccination, to block both infection and disease development. Attenuated vaccine carriers mimic natural infections, triggering also mucosal responses. Similar results can be achieved by administering antigens with appropriate adjuvants. However, the delivery of antigens per se is not sufficient to engender a protective response. A successful immunization requires the elicitation of an appropriate type of immune response (e.g. antibodies vs. cell-mediated immunity, Th1 vs. Th2 helper pattern). Therefore, a successful vaccination strategy demands the choice of adequate antigens, and their appropriate delivery and/or formulation to promote the required quality of immune response. Different strategies to optimize the immune responses elicited following vaccine administration by the mucosal route are discussed. © 2000 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: Adjuvant; Antigen delivery; Immune response; Live carrier; Mucosal vaccine

1. Introduction

Infectious diseases are the major cause of morbidity and mortality, accounting for a third of the deaths which occur in the world each year. They are also highly expensive in terms of health-associated costs of infected patients and loss in productivity at work. The main strategies used to prevent them are therapy and prophylaxis. Between these two options, vaccination has become the most cost-effective measure to combat infectious agents. However, there are still many diseases for which vaccines are not yet available or the available vaccines are not completely satisfactory due to low efficacy, poor stability and/or high cost. There is thus an urgent need for both new and improved vaccines.

Most infectious diseases are either restricted to the mucosal membranes or the etiologic agents need to transit the mucosa during the early steps of the infection [1]. Therefore, it is desirable to obtain a local mucosal immune response as a result of vaccination to block both infection (i.e. colonization) and disease development. Parenterally administered vaccines mainly stimulate systemic responses, whereas vaccines administered by a mucosal route mimic the immune response elicited by natural infections and can lead to efficient mucosal and systemic responses [2]. Administration of vaccines by a mucosal route is also associated with lower rates of side effects, higher acceptance by the public and lower delivery costs, being particularly suitable for mass immunization programs.

2. Administration of vaccine antigens by the mucosal route

The delivery of antigens by the mucosal route is associated with two major problems: (i) antigens delivered by this route are generally poorly immunogenic, and (ii) some antigens need to be protected against degradation. To overcome these problems, different strategies have been used, such as their entrapment into biodegradable microspheres or liposomes, their production by attenuated viral/bacterial carriers or transgenic plants, and/or their administration with mucosal adjuvants.
2.1. Microparticles

Microparticles are polymeric particles, such as poly-lactic-co-glycolide, which can be used as a delivery system. Vaccine antigens are incorporated either adsorbed or chemically bound to the matrix. Microparticles can protect either vaccine antigens or plasmid DNA from degradation. In addition, their uptake by professional antigen presenting cells (APC; e.g. macrophages, dendritic cells) facilitates antigen presentation, leading to the elicitation of efficient responses both at systemic and mucosal levels. The stability of the antigen and the modulation of its release seem to be critical factors for a successful outcome using this approach [3]. Bacterial [4] and viral proteins [5], conjugated vaccines [6] and DNA [7,8] have been encapsulated in particles and shown to stimulate the elicitation of antigen-specific serum antibody responses and mucosal IgA.

2.2. Adjuvants

Adjuvants are compounds that, when combined with an antigen, potentiate the elicited immune response. Over the last few years, many efforts have been invested to find novel adjuvants with an ability to enhance not only systemic, but also mucosal immune responses. A number of adjuvants have been shown to effectively enhance immunogenicity of antigens delivery by a parenteral route. Unfortunately, only a few molecules such as the heat labile toxin from Escherichia coli and the cholera toxin of Vibrio cholerae have been described which can exert a potent activity as mucosal adjuvants [9,10]. Their toxicity and potential side effects hinder their use in human vaccination [11]. Nevertheless, non-toxic derivatives of these molecules, which retain their adjuvanticity, have been generated by site-directed mutagenesis [10,12,13]. However, intranasal administration of the genetically inactivated heat labile toxin still results in the alteration of the respiratory mucosa, inflammatory infiltration of the meninges and passage of the toxin into the brain (N. Garcia, presentation at the World Vaccine Congress, Geneva, 26–28 September 1999).

Interestingly, we have also shown recently that the fibronectin binding protein I of Streptococcus pyogenes is an efficient mucosal adjuvant able to substantially improve cellular, humoral and mucosal responses when coupled or co-administered to an antigen administered by the intranasal route [14,15]. Although the use of this molecule promotes a dominant Th2 response, efficient cytotoxic T lymphocyte responses were also stimulated [14].

The ability of cytokines to regulate and enhance immune responses makes them attractive molecules to be included in vaccine preparations. Co-administration of cytokines has been shown to be effective as vaccine adjuvant in many different experimental settings. Examples are provided by co-administration of cytokines such as IL-1, IL-2, IL-10, IL-12, GM-CSF and IL-6, which resulted in an enhanced immunogenicity of vaccine preparations against bacteria, viruses and tumors [16–20].

Interestingly, bacterial DNA containing immunostimulatory motifs (unmethylated CpG dinucleotide) can trigger an innate immune response characterized by the production of predominantly Th1-type cytokines. These motifs have been shown to enhance the immune response to different vaccine antigens [21,22].

2.3. Viral particles

Recombinant viral or virus-like particles offer new approaches for vaccine development. Progress in recombinant DNA techniques has made the insertion of foreign epitopes into proteins with inherent multimerization capacity possible, such as viral capsid or envelope proteins. For example, the core protein of hepatitis B virus, because of its highly symmetric structure and immunological properties, constitutes an attractive candidate as a carrier for foreign epitopes able to induce both B and T cell immune responses against the inserted epitope [23,24]. The RNA phage Qbeta coats have also been shown to enhance antibody responses against expressed vaccine antigens [25].

2.4. Liposomes

Liposomes are lipid vesicles formed when phospholipids are exposed to an aqueous environment. They arrange themselves in bilayers and close up, forming vesicles. During this process, any antigen present in the aqueous phase will be retained within the vesicle. Liposomes have been extensively used as a delivery system for vaccine antigens against bacterial [26,27] and viral [28,29] infections and cancer [30,31]. Liposomes not only protect the antigens present in the formulation, but can be used as immunoadjuvants since they can potentiate humoral and cell-mediated immunity against the encapsidated antigens [27,32,33]. Immunopotentiating reconstituted influenza virosomes (IRIV) are liposomes which carry the two glycoproteins of the influenza virus on their surface. Several vaccines such as hepatitis A and B, tetanus toxoid and diphtheria toxoid have been developed based on IRIV, which have proved to be safe and highly immunogenic [34,35].

2.5. Immunostimulating complexes (ISCOMs)

The ISCOM is a delivery system designed for both parenteral and mucosal administration. ISCOMS are complexes built up by cholesterol, lipid, immunogen and saponin. Administration of ISCOMS by either the intranasal [36] or oral route [37,38] has been demonstrated to promote antigen-specific antibody responses and induce helper and cytotoxic T cell responses as well as local production of secretory IgA.
2.6. Transgenic plants

Production of protein-based vaccines has been shown to be possible using transgenic plants [39]. Antigen production in food plants (e.g. banana trees, potatoes) enables direct mucosal delivery through the consumption of the recombinant plants. Advances in recombinant DNA technology have facilitated the introduction of a variety of vaccine-relevant genes into plants, facilitating the development of vaccine prototypes against infectious and non-infectious diseases [40–42]. The possibility to express the vaccine antigens in seeds expands the potential uses of this approach, mainly in the veterinary field, facilitating both storage and conservation. The potential of transgenic plants as oral delivery system for immuncontraceptive vaccines for herbivore species has also been explored with promising results [43].

3. Live bacterial vectors

Live attenuated vectors are one of the most efficient delivery systems for stimulation of the mucosal-associated immune system. They have extensively been used to express heterologous antigens from pathogenic microorganisms to induce both local and systemic immune responses. Additional advantages of live vectors are the low costs and simplicity associated with batch preparation in bioreactors, which in turn facilitates technology transfer. Although available delivery systems enable the elicitation of different types of immune responses, their enormous potential and versatility in this regard have hardly been exploited.

Among the available systems (Table 1), the use of attenuated Salmonella strains as carriers probably constitutes the most studied strategy [44–46]. Safe Salmonella carriers can be generated by introducing defined non-reverting mutations into the chromosome. Although a number of attenuated mutants have been constructed and even characterized in animal models with regard to their virulence, only few of them have been evaluated as vaccines carriers. Mutants deficient in the biosynthesis of aromatic amino acids (e.g. aroA, aroC and aroD) [47] or purines (e.g. purA and purE) [48], the production of adenylate cyclase (cya) or the cAMP receptor protein (crp) [49], with mutations affecting the global regulatory system phoP/phoQ [50] or harboring mutations in the seeC and seeD genes [51] have been the most widely characterized. Attenuated Salmonella can trigger efficient humoral, T helper and cytotoxic responses against the carrier itself or co-expressed heterologous antigens, being stimulated mucosal as well as systemic immunity [52–55]. Interestingly, their use can find application not only in the classical field of anti-infective vaccines, but also in the immunoprophylaxis of tumors [56].

Listeria monocytogenes is an intracellular pathogen able to enter the cytoplasm of the host cell. Attenuated strains of L. monocytogenes have been shown to be very effective delivery systems for heterologous antigens, being able to target antigens to both the MHC class I and class II presentation pathways [57–59]. The ability of L. monocytogenes to stimulate the production of Th1-type cytokines makes this bacteria a very attractive delivery system for the development of vaccine against viruses, tumors and intracellular parasites [60–64]. Among other potential bacterial carrier strains, deletion mutants of V. cholerae [65] or Shigella spp. [66], invasive E. coli [55,67] and commensals such as Streptococcus gordonii [68] or Lactobacillus spp. [69] have been shown to elicit high titers of antibodies against the heterologous antigens and/or protective immunity in vaccinated animals.

4. Modulation of the immune responses stimulated by live carriers

The development of strategies to stimulate appropriate effector populations according to particular needs would increase the potential of attenuated carriers in vaccinology. In fact, the elicitation of an inappropriate type of immune response can even lead to immunopathological reactions, which can worsen symptoms following natural infections. Different approaches can be used to modulate the immune response elicited. The co-expression or co-delivery of cytokines offers almost unlimited possibilities for the manipulation immune responses according to the spe-

### Table 1
Most commonly used live bacterial carriers

<table>
<thead>
<tr>
<th>Strain</th>
<th>Localization</th>
<th>Potential as carrier for DNA vaccines</th>
<th>Immune responses stimulated*</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella spp.</td>
<td>Phagosome</td>
<td>+</td>
<td>S, M, Ab, Th, CTL</td>
<td>[44–56,70,71]</td>
</tr>
<tr>
<td>Shigella spp.</td>
<td>Cytoplasm</td>
<td>+</td>
<td>S, Ab, Th</td>
<td>[66]</td>
</tr>
<tr>
<td>Listeria spp.</td>
<td>Cytoplasm</td>
<td>+b</td>
<td>S, M, Ab, Th, CTL</td>
<td>[57–64,72]</td>
</tr>
<tr>
<td>Vibrio spp.</td>
<td>Extracellular</td>
<td>–</td>
<td>S, Ab</td>
<td>[65]</td>
</tr>
<tr>
<td>S. gordonii</td>
<td>Extracellular commensal</td>
<td>–</td>
<td>S, M, Ab, Th</td>
<td>[68]</td>
</tr>
<tr>
<td>Lactobacillus spp.</td>
<td>Extracellular commensal</td>
<td>–</td>
<td>S, M, Ab, Th</td>
<td>[69]</td>
</tr>
</tbody>
</table>

*Abbreviations: Ab, antibodies; CTL, cytotoxic T lymphocytes; M, mucosal responses; S, systemic responses; Th, T helper.
*Only in vitro studies.
pecific needs, however, this approach will not be further expanded (see Section 2.2), because it is considered beyond the scope of this review.

The presence of mutations affecting the virulence properties of the carrier, synthesis of essential nutrients, regulatory factors or metabolic enzymes may influence the course of the infection, thereby affecting the quality of the immune response elicited. In this regard, it has been demonstrated that different qualities of immune response can be elicited following vaccination with Salmonella carriers, containing different attenuating mutations [51,70].

Interestingly, the quality of the immune response generated against a recombinant antigen delivered by a live vaccine carrier is not entirely determined by the bacterial vector used or the nature of the immunogen, but can also be influenced by the particular promoter chosen to control antigen expression. In fact, the use of different promoters enable to change a mixed Th1/Th2 response pattern to a dominant Th1 response (unpublished data). The expression of heterologous antigens under the control of different promoters provides the base to design novel vaccination strategies using Salmonella as a delivery system. Based in the available information about the quality of immune response required to protect the host against specific agents, it would be possible to select the most suitable promoter to drive the expression of the vaccine antigen in order to promote the specific type of T helper response required to achieve protection [56].

The form of display or topology of the vaccine antigen seems to play also a critical role in vaccine efficacy. A better protection against *L. monocytogenes* infection had been obtained by the secretion of antigens than by using vaccine constructs in which the antigens were somatically displayed [71]. Various approaches have been used to achieve export of the vaccine antigen. Among them, the construction of chimeric proteins between the listeriolysin or anchorless ActA of *L. monocytogenes* and the vaccine antigen [60,72], or the use of the secretion signals of the hemolysin A protein from *E. coli* (type I secretion system) to achieve protein export [71,73–75]. Modulation of the immune response during vaccination can also be achieved by the delivery of vaccine antigens to the host cell cytosol using type III secretion systems [76].

5. DNA immunization

DNA vaccines have been designated as a third generation of vaccines. They can stimulate cytotoxic T cell responses as well as helper T cell and humoral immunity [77,78]. In addition, the immune response can be enhanced or modulated when administered in combination with DNA-encoding cytokines [17,18,20]. DNA immunization has been shown to be effective at conferring protection against viruses, bacteria, parasites and cancer [77–81]. The most frequently used vectors for genetic immunization are plasmid DNA engineered to express the genes of interest in eukaryotic cells [82].

Different strategies have been used for the delivery of DNA vaccines such as the use of naked nucleic acids, encapsidated DNA, DNA delivery by live carriers, etc. One of the strategies recently described for DNA vaccination consisted in the in vivo production of vaccine antigens in the context of an alphaviral replicon [83]. This type of construct can efficiently trigger cellular and humoral immune responses. The advantage of this approach is that expression mediated by the alphaviral vector is transient and lytic, avoiding potential problems such as chromosomal integration. Professional APC play a key role in the induction of immune responses triggered by vaccination with DNA. The use of attenuated bacterial carriers as a delivery system for DNA vaccines might facilitate the targeting of these cells at the level of the inductive sites [84]. Promising results have been obtained using as carriers attenuated *Salmonella* strains [84,85] or invasive intracellular pathogens such as *Shigella* spp. [86] or self-destructing *L. monocytogenes* [87]. This approach seems to promote dominant Th1 responses.

6. Conclusions

The studies carried out in the last few years contribute to expand our understanding of the underlying events to the generation of mucosal immune responses. Many strategies can be used to stimulate both systemic and mucosal responses following antigen administration by the mucosal route. However, these systems promote slightly different types of host responses. In addition, independently of the intrinsic nature of the immunogen, any of the selected delivery systems can be further modulated. In fact, we are now learning which strategies can be exploited to fine-tune the elicited responses.

This knowledge can be exploited to favor the rational development of vaccines for the therapy and/or prophylaxis of infectious and non-infectious diseases, by selecting the optimal system/setting to promote the quality of immune response required to achieve protection. In the near future, we will see the emergence of a new generation of mucosal vaccines with optimized efficacy, low reactogenicity and high public acceptance which will be amenable for use in mass immunization programs.

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