Flagella as a Platform for Epitope-Based Vaccines

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The development of vaccines and their contribution to public health has been one of the most important achievements of immunology to date. The conventional vaccines are based on the entire pathogen, killed or attenuated, or proteins thereof in a form that does not cause infection but induces an immune response that leads to protection. Despite their success in eradicating hazardous infectious diseases such as smallpox, tetanus, polio and diphtheria, the performances of existing vaccines are not always satisfactory and there is a need to improve them. Among the crucial drawbacks incurred by the current procedures for vaccine preparation, one can include the difficulty of *in vitro* culturing of many viruses and most parasites and the biohazard due to incomplete attenuation or killing of the pathogen. In addition, the emergence of new strains as a result of genetic variation in viruses (such as human immunodeficiency virus and influenza) compels the design of new strategies for vaccines that will provoke cross-strain immunity.

One novel approach for vaccine development is the utilization of synthetic peptides which constitute relevant protective epitopes. Although no peptide or polypeptide vaccine is yet being used as an approved vaccine, several such experimental vaccines against viruses, cancer and allergy are undergoing clinical trials [1-4].

Peptide-based vaccines

Both humoral and cellular arms of the immune system recognize and react with only specific regions of the pathogen. This prompts the design of vaccines based on either naturally occurring immunogenic polypeptide(s) or synthetic peptides that correspond to immunodominant epitopes or highly conserved regions required for the pathogen's function. Peptide-based vaccines offer some advantages, including safety, low cost, and uniformity of different batches. The rationale of this strategy is to vaccinate with a minimal structure, consisting of a highly purified well-defined antigen, in order to stimulate an effective and predictable specific immune response while avoiding potential hazardous effects. However, this strategy must take into consideration the essential presence of both B cell and T cell epitopes, as well as the major histocompatibility complex restriction of the T cell response. Since the cellular immune response in humans is restricted to specific human leukocyte antigens, any single epitope-based vaccine will probably not be effective in a heterogeneous population. This can be overcome by the use of vaccines comprising several peptides, which would be effective in inducing all arms of the immune response as well as in broad MHC recognition. Furthermore, this approach allows the design of "tailored" vaccines by the selection of those epitopes restricted to the HLA that are most frequent in a specific population.

The main limitation of peptide-based vaccines is their low immunogenicity. However, this might be solved by conjugation to an appropriate carrier or expression in an efficient delivery system, and/or by administration with an appropriate adjuvant.

In this paper, we discuss our studies on peptide-based vaccines against the parasite *Schistosoma mansoni* and against influenza virus, in which the flagella of Salmonella serves as a platform for the selected epitopes.

The flagella expression system

The general concept of our approach is to use conserved epitopes derived from the pathogen as immunogens. Each epitope is expressed in Salmonella flagellin; the resultant recombinant flagella can be easily cleaved and purified for administration. This Salmonella is a vaccine strain and hence is not hazardous to the vaccinees [5,6]. Flagellin serves here as both a carrier and an adjuvant. The adjuvanticity stems from its association with the Toll-like receptor-5, which results in interleukin-12 and interferon-gamma secretion [7]. In addition, each flagellum is a polymer of over 20,000 copies of the flagellin monomer and thus acts as a multivalent antigen expressing multiple copies of the heterologous epitope. Pharmacokinetics studies indicated that the flagella remain at the site of administration for many hours and hence enable a prolonged exposure of the epitopes to the immune system. The foreign epitopes are efficiently presented to the immune system, as manifested by the production of specific antibodies both to the flagella and to the inserted foreign peptide, and by the protection induced against challenge infection. This approach was employed in our laboratory in several systems including the parasite *Schistosoma mansoni* and influenza virus. Other laboratories used the flagella carrier for vaccination purposes against various pathogens as well [8-10].

The parasite *Schistosoma mansoni*

A variety of immunodominant molecules have been described as candidate vaccines against schistosomiasis, including glutathione S-transferase, the muscle protein paramyosin, triose

MHC = major histocompatibility complex
HLA = human leukocyte antigen

Table 1. Protective imunity against parasite infection

Route	Antigen	% protection*
Intranasal	Fla-9B peptide	30–53%
	Fla	0–4%
	PBS	0%
Foot pad	Fla-9B peptide	± 3 0%
	Fla	0%
	Phosphate-buffered saline	0%

In three replicate experiments, 8–10 C57BL/6J mice were immunized intranasally three times with either recombinant (Fla-9B peptide) or native (Fla) flagella, and 4 weeks later the mice were infected with cercariae. At 7–8 weeks post-infection, liver perfusion was performed and the worms were counted. The percentage of protection was calculated from the number of worms in the immunized mice liver compared to that in untreated mice.
* $P < 0.05$.

phosphate isomerase and others [11,12]. A protective surface antigen, denoted 9B, was described in our laboratory. It is an abundant antigen in the early stages of the parasite life cycle that confers 45% and 65% protection when administered in complete Freund's adjuvant [13] or in proteosomes [14]. A single 14-residue epitope derived from this protective surface protein of the parasite *Schistosoma mansoni*, denoted 9B, was expressed within the Salmonella flagellin. Immunization of mice with this construct led to significant protection against challenge infection, as manifested by an average reduction of 42% in worm burden in the liver [Table 1]. It is noteworthy that effective protection was achieved by intranasal immunization without an additional adjuvant. This is probably due to blocking the pathogen at the first stage after its invasion to the host (the lungs stage) before it spreads and causes damage to the liver. The mechanism leading to the observed protection involved both innate immunity parameters, such as complement-mediated lysis of parasite, and humoral response specific to the peptide and to the intact schistosomula [15].

Influenza virus

Our most extensive work was performed with the influenza virus. The efficacy of vaccination towards influenza virus is limited by the frequent modifications and antigenic variations of its glycoproteins. Hence, current vaccines are produced annually according to the circulating strains that are expected to prevail in the following season. Efforts in our laboratory are directed towards the rationally designed vaccine that will induce long-term protection against the various strains of the virus.

In a previous study Levi and Arnon [16] selected conserved epitopes of the hemagglutinin and the nucleoprotein that were previously defined as B and T cell-inducing antigens. These epitopes were expressed in the flagellin of a Salmonella vaccine strain and the recombinant flagella were used for intranasal immunization without any external adjuvant. Such immunization protected mice against infection and was efficient in aged mice in which the immune system is comparatively weak [17].

Towards the design of vaccine for human use, four influenza epitopes were chosen according to their ability to activate the humoral and cellular arms of the human immune system.

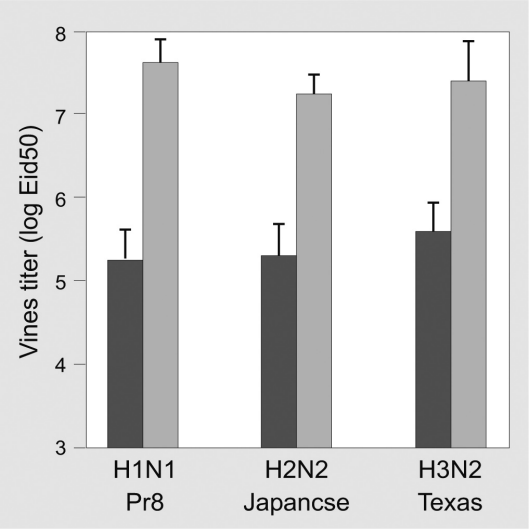

Figure 1. Protective vaccination of human/mouse radiation chimera transplanted with human peripheral blood mononuclear cells and immunized once intranasally with the recombinant flagella mixture expressing the influenza eitopes. Seven days after immunization the mice were infected with one of three influenza strains: A/PR8 (H1N1) or A/Japan/57 (H2N2) or A/Texas/1/77 (H3N2). Only the transplanted and vaccinated mice (dark bars) were able to resist the challenge infection, and the virus titer in their lungs was significantly reduced as compared to non-transplanted and vaccinated mice (light bars).

These epitopes are conserved and shared by different strains of the virus and they can be presented to the immune system by different and most frequent HLA molecules in the population. Employing the human/mouse radiation chimera in which human peripheral blood mononuclear cells are functionally engrafted into irradiated mice [18], an efficient protective response was observed in the transplanted immunized mice, but not in the control groups of either non-transplanted immunized mice or transplanted but non-immunized mice.

Intranasal immunizing of human/mouse chimera with this mixture of flagella without additional adjuvant resulted in generation of specific antibodies against the influenza virus in the serum and in the lungs. These mice were protected against sub-lethal and lethal infection challenge by the virus. Following a single intranasal vaccination, the chimeric mice were infected with different strains of influenza virus, including H3N2, H2N2 and H1N1. The reduced viral load in their lungs indicated cross-strain protection, as depicted in Figure 1 [19].

These cumulative data indicate the feasibility of the use of flagella as a carrier and adjuvant for foreign epitopes. The resultant recombinant vaccines were not only efficient, but also safe and well tolerated by the vaccinated animals.

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