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Plant-produced Microbial Vaccines

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Preface

In recent years, plants have been increasingly explored for the production of biomedicines and vaccine components. The two main advantages of plant systems are low cost and a greater potential for scalability as compared to microbial or animal systems. An additional advantage from the public health point of view is the high safety compared to animal systems, which is important for vaccine production: there are no known plant pathogens capable of replicating in animals and in humans, in particular. A particular antigen or a protein has to be expressed in a plant using one of the many available platforms; this antigen/protein subsequently needs to be purified or processed, and later formulated into a vaccine or a therapeutic; these need to be delivered to a human or animal body via an appropriate route. Naturally, all these vaccines and therapeutics must be subjected to regulatory approvals prior to their use. Thus, the challenge is to adapt plant-based platforms for the production of cost-efficient biomedicals that can be approved by FDA for use as vaccine components or therapeutics, which will be competitive against existing vaccines and drugs.

This volume attempts to address the entire spectrum of challenges facing the nascent field of plant-based biomedicals, from the selection of an appropriate production platform to specific methods of downstream processing and regulatory approval issues. The chapter by D.C. Hooper is devoted to immunological issues that can arise for antigens produced in plants and delivered to a human or an animal via different routes. This chapter also discusses such specific topics as tolerance and immunomodulation, with particular reference to oral delivery of plant produced antigens. The chapter by Smith et al. discusses one specific example of a virus-based platform for the expression of peptides in plants, and related issues of downstream processing, that is, manufacture and purification of virus particle-based vaccines, and final product release and stability. Another production platform, via chloroplast-based expression of proteins, is discussed in the chapter by S. Chebolu and H. Daniell. The chapter presents several examples of various vaccine components and other biomedicals produced in plants, and these range from bacterial and viral proteins to human serum proteins and antibodies. The chapter by Ko et al. describes a particular application of the plant-based production of human antibodies used for passive immunization against rabies. It is an example of a classical transgenic technology modified for a specific expression of two antibody

chains. The chapter by R. Hammond and L. Nemchinov reviews the current status of plant production of veterinary vaccines, utilizing a great variety of platforms. The final chapter by C. Tacket presents case studies for the human trials of the first plant-produced candidate vaccines and discusses several regulatory issues that need to be addressed prior to their approval.

Production of vaccine components and other biomedicals in plants has a great potential in medicine and veterinary science. We hope that this volume will be a valuable contribution to this rapidly growing research field.

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Plant Vaccines: An Immunological Perspective

D.C. Hooper

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Abstract The advent of technologies to express heterologous proteins *in planta* has led to the proposition that plants may be engineered to be safe, inexpensive vehicles for the production of vaccines and possibly even vectors for their delivery. The immunogenicity of a variety of antigens of relevance to vaccination expressed in different plants has been assessed. The purpose of this article is to examine the utility of plant-expression systems in vaccine development from an immunological perspective.

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Introduction

From its genesis, vaccine development has primarily been an empirical science; originating with the chance discovery of Jenner that a relatively benign cowpox infection protected an individual against the considerably more deadly smallpox (Jenner 1798). Based on this observation and his own work on the germ theory of disease, Louis Pasteur pioneered the classic approach to vaccine development: attenuating the pathogenic agent such that its capacity to cause disease was limited but its antigenic structure unchanged (Pasteur 2002). During the heyday of vaccine development that followed, it was recognized that certain diseases caused by proteins, elaborated by bacteria and toxins, could be rendered apathogenic and used to vaccinate against the disease (Ramon and Zoeller 1927). Thus, by the beginning of the twentieth century, the basic attributes of a successful vaccine had been established. Essentially, the causative agent of a disease was modified to dissociate its ability to induce a protective immune response from its pathogenicity. Many of the vaccines for infectious diseases commonly in use today still consist of preparations of attenuated viruses or inactivated viruses and toxins (CDC 2002). The use of such reagents is termed active vaccination because their success is dependent upon the induction of an immune response in the recipient. This contrasts with passive immunization where the administration of preformed antibodies is used to confer immunity, as naturally occurs between the mother and offspring during pregnancy and early development.

Vaccination by infection with an attenuated variant of a pathogen should provide the best long-term protection by eliciting the full range of immune effectors and immunological memory, the capacity to rapidly mount a recall response to an antigen. Nevertheless, even a vaccine that induces an incomplete immune response may be considered successful if it prevents disease, the primary objective of vaccination. For example, passive vaccination can be very effective at neutralizing a disease-causing toxin but does not induce immunological memory. Nevertheless, in rabies postexposure prophylaxis the passive administration of virus-neutralizing antibodies provides an underlying active immune response the additional time required to develop and clear the infection so that rabies with its lethal outcome is avoided (Hanlon et al. 2001). Similarly, active vaccination with a noninfectious vaccine is unlikely to generate a cytotoxic CD8 T cell response, which generally requires infection of target cells, but can be efficacious in protecting against an intracellular pathogen. In this case, the response would limit the capacity of an infectious agent to invade (antibody) as well as provide the immunological memory (T helper cells) that would accelerate the induction of the CD8 T cells or other cytotoxic effectors required for clearance. Under normal circumstances, vaccination is not expected to prevent subsequent infection with a pathogen, as this would require the maintenance of high levels of pathogen-neutralizing antibodies at the point of entry. While this is theoretically possible through IgA secretion in the gut, in practice even the clearance of an enteric virus does not prevent subclinical infection with the same virus (Weinstein and Cebra 1991). Thus the objective of

vaccination is to elicit an antibody response that interferes with the invasion of the target pathogen and to prime T helper cells such that the generation of a complete response encompassing humoral and cellular immunity is enhanced during the natural infection.

As understanding of immune function as well as the pathogenesis of different diseases has advanced, the uses of vaccination have expanded into areas distinct from protecting against infection. One of these areas is immunomodulation. An example of this would be passive immunization with Rh-specific antibodies to prevent sensitization of Rh-negative mothers against the Rh antigen, which has been used to modulate immunity for nearly 50 years (Kumpel 2002). Recently trials have been conducted to determine if active immunization can be used to treat autoimmune disease (Vandenbark et al. 2001; Cohen-Kaminsky and Jambou 2005; Li et al. 2005). Another rapidly growing use for vaccination is in cancer therapy. With the identification of antigens expressed exclusively or at higher levels than normal by transformed cells, a variety of active and passive anti-cancer vaccines are in development (Dredge et al. 2002; Vichier-Guerre et al. 2003; Lenarczyk et al. 2004; Bodey et al. 2000; Ko et al. 2005).

Molecular technologies enabling both antigens and antibodies to be expressed by plant-based systems have been developed. The purpose of this article is to examine the potential advantages and disadvantages of the *in planta* production of vaccine reagents from an immunological perspective.

Basic Immunology: Antigens and Immunogenicity

Antigens are the structures that are recognized by T cells, B cells and antibody in an immune response. The capacity of an antigen to induce an immune response is its immunogenicity. Most antigens are protein and not inherently immunogenic. This is likely to be at least partly due to the nature of antigen recognition. While B cells and antibodies can passively interact with intact antigens, T cells recognize antigens that have been processed and presented at the cell surface, an active process. For example, to stimulate the helper T (CD4) cells that promote the expansion and maturation of different immune effectors, antigen must be taken up, processed into peptides, and presented by specialized antigen-presenting cells (APCs) in the context of Class II major histocompatibility complex (MHC) antigens and second signals (Robinson and Delvig 2002). A protein that does not trigger uptake and presentation by APCs, as is the case for most self-proteins, may be nonimmunogenic yet still possess antigens. Under experimental conditions, these can be revealed by administering the protein together with an adjuvant to stimulate APC function (Schijns 2001). In reality, most natural immune responses are generated against invading pathogens and attributes of the infection likely provide the necessary immunogenic stimuli. Several toxins are among the limited group of noninfectious agents known to be highly immunogenic. Notably with respect to vaccine development, some of these remain immunogenic when their toxicity is attenuated.

Possible Advantages and Limitations of Using Plants to Produce Reagents for Active Vaccination

Plants as Expression Vectors

Edible plants expressing antigens that elicit protective immunity serve as the basis for the ideal vision of a vaccine that is inexpensive to both produce and deliver. While concerns about the impact of engineered plants on the environment and issues of profitability may dictate otherwise, the model plant-based vaccine could be grown locally using existing agricultural methods, harvested and fed to subjects. The plant approach would yield a reagent free of potential contaminants from the originating pathogen and other human or mammalian cell-based expression systems. Characteristics of the plant cells and tissues may be utilized to provide sufficient protection for the vaccine moiety to reach the small intestine for uptake and the induction of an immune response. Moreover, plant systems may be able to produce antigenic structures that are difficult to express in eukaryotic cell culture.

Plant expression systems have been successfully used to produce relatively complex proteins including structurally intact, active antibody molecules. These consist of two heavy and two light chains with a total molecular weight of approximately 150 kDa. Thus antigen complexity is not a concern and, at present, a low-yield and weak immunogenicity are the major limitations of plant antigen expression systems. There are a number of approaches being investigated to increase the yield of foreign proteins expressed in plants (reviewed in Gleba et al. 2005; Ko et al. 2003) and any obstacle here will likely be overcome in the near future. On the other hand, attempts to improve the immunogenicity of antigens expressed by plants have met with only partial success and this objective is not without concerns, as discussed in the following sections.

Plant Viruses and Bacteria as Expression Vectors

In a somewhat different but related approach to the use of engineered plants, vaccine antigens may be expressed by plant viruses or bacteria. In this case, plants are used to produce the agent, which can then be administered in purified form or using the infected plant tissue as a vehicle. The expectation here is that based on their structures, the plant virus or bacterium may share some immunogenic attributes but none of the pathological properties of human pathogens. While plant bacteria can express relatively complex antigens, the use of plant viruses as expression vectors may be restricted to relatively simple antigenic determinants because of issues with virus assembly. The inability to express intact antigenic structures has repercussions for the utility of the construct. For example, short peptide sequences expressed in an appropriate context may be able to induce a limited T cell response but are

unlikely to trigger antibody production. Thus there would be no capacity to neutralize the target pathogen and the protective attributes, if any, would depend on a more rapid induction of a comprehensive immune response when an infection occurred. Yields of the vaccine antigen, in these expression systems only a fraction of the plant virus or bacteria, are also a concern. In addition, it is conceivable that the response to a weak antigen may be negatively impacted by prior tolerogenic exposure to native plant viruses and bacteria naturally present in the diet.

Our experience with the plant bacterium *Clavibacter xyli cynodontis* and an expression system using the coat protein of the alfalfa mosaic virus (AMV) expressed by tobacco mosaic virus highlights some of the differences between

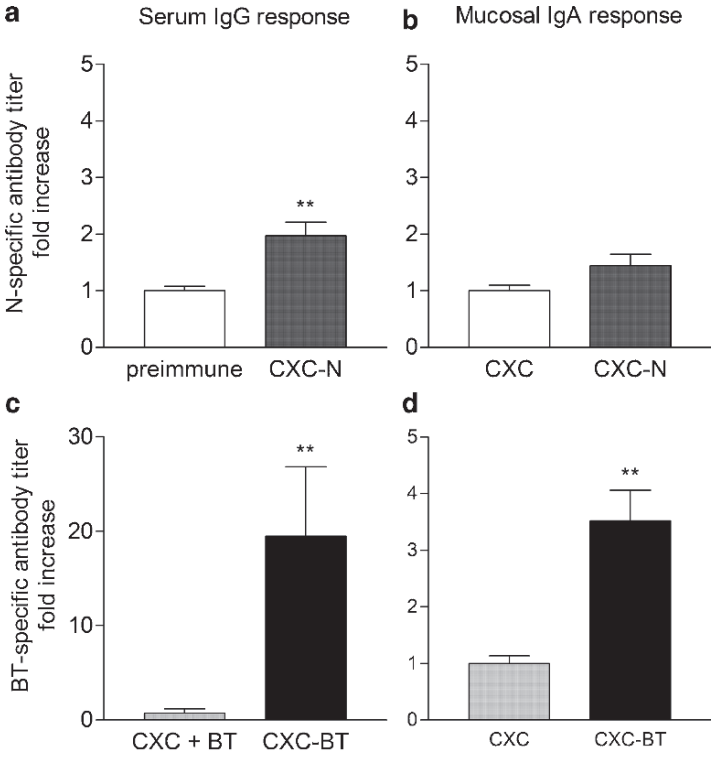

Fig. 1 Comparison of the immunogenicity of different *Clavibacter xyli cynodontis* constructs following parenteral and oral immunization. Groups of ten 8-week-old female Swiss-Webster mice were immunized with *Clavibacter xyli cynodontis* (*CXC*) engineered to express rabies virus nucleoprotein (N), *Bacillus thuringiensis* toxin (*BT*), or the native bacterium (*CXC*). Immunization consisted of a single dose of 5×10^6 CXC in saline intraperitoneally or two doses of 5×10^7 CXC in 10% sucrose at 2-week intervals *per os*. Serum and fecal pellet antibodies specific for N (**a, b**) and BT (**c, d**) were assessed 21 days after immunization by ELISA using antigen-coated plates and antibody isotype-specific secondary antibodies. The results are expressed as the fold-increase in antibody titers by comparison with preimmune levels. Statistically significant differences between the groups detected by the Mann-Whitney test are denoted by **, $p < 0.01$