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# Developing Vaccines in the Era of Reverse Vaccinology

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## INTRODUCTION

Vaccines are currently available for infectious diseases caused by various viruses and bacteria, and the prevention of disease and death by vaccination has profoundly improved the public health of many populations globally. However, vaccines are not yet licensed for use against many other important infectious diseases, and new or improved vaccines are needed to replace suboptimal vaccines and to address newly emerging pathogens. New vaccines are being introduced at a higher rate than ever before. Half of all new vaccines have been developed in the past 25 years, at a rate of approximately one per year over that time frame, compared with an average of one every five years before that.

For more than a century, vaccine development has followed Pasteur's principles: ''isolate, inactivate and inject'' the causative microorganism (1). The majority of vaccines currently licensed and available for human use include live, attenuated organisms and killed or inactivated organisms. A small subset is based on partially purified components of an organism and even fewer still are recombinantly produced vaccines. However, as we come to address the problems of organisms for which no effective vaccines are currently available, it will become increasingly important to turn to novel approaches offered by advances in biotechnology. Recently, vaccines against cervical cancer have been licensed on the basis of human papillomavirus (HPV) virus-like particles (VLPs) produced in yeast or insect cells (2). The ongoing discovery and application of innovative technologies should continue to revolutionize the way vaccines will be made in the future, generating vaccines against even the most challenging pathogens.

## TECHNOLOGICAL REVOLUTIONS IN VACCINE RESEARCH

The history of vaccine development can be marked by a number of milestones resulting from revolutions in technology. Earlier waves involved pathogen attenuation, inactivation, and viral cell culture, all supported by an increased understanding of the human immune response (3). However, recombinant DNA technology has probably been the single most innovative advance that has opened the door to new technological developments.

### Recombinant DNA Technology

Recombinant DNA technology was first successfully applied to vaccines over 25 years ago, with the production of a recombinant vaccine for Hepatitis B virus (HBV) (4). Although a vaccine based on purified HBV surface antigen (HBSAg), from the plasma of infected patients, was available since the 1970s, the expression in yeast of the HBSAg vaccine antigen provided the solution for a relatively simple, quick, and inexpensive process to produce a safer vaccine. This development also hallmarked the first VLP vaccine, as the recombinant HBSAg was able to self-assemble, facilitating purification and manufacture at the industrial level. Since this initial success, production of protein antigens using recombinant DNA techniques has become a standard practice for subunit vaccine development.

Along with the need for developing better-characterized (e.g., HBV), less reactogenic (e.g., acellular pertussis), or more potent vaccines (e.g., anthrax) to replace the existing vaccines, a more recent driver for developing recombinant subunit proteins has been the need to provide broader protection against multiple strains or serotypes of a bacterium (e.g., serogroup B meningococcus, pneumococcus). Strategies to increase the breadth of vaccine coverage include the engineering of multiple epitope-based vaccines, incorporating epitopes either from different antigens of the same pathogen or even from different pathogens into a single protein using synthesized genes.

Another major use of recombinant DNA technology has been in site-specific inactivation of toxins as a safe and efficient alternative to chemical inactivation. A successful example of this is the genetic inactivation of the pertussis toxin for incorporation into the acellular pertussis vaccine. Detailed structure-function analysis of the pertussis toxin allowed the identification of key amino acids responsible for the toxicity of the protein that could be mutated, thereby inactivating the toxin while maintaining antigenic conformation (5). This safe and immunogenic protein along with two other purified components, filamentous hemagglutin and pertactin, make up the acellular pertussis combined subunit vaccine and replaced the traditional killed whole-cell vaccine (6). Similarly, the site-directed mutation of toxins for use as the protein carrier in conjugated polysaccharide vaccines exemplifies an extrapolation of the diverse areas of vaccine design and development to