which recombinant DNA technology has been applied. Ultimately, recombinant DNA technology is forming the basis of a wide range of new platforms for vaccine delivery and exploitation of expanding immunological knowledge, examples of which are discussed below.

Engineering Live Attenuated Vaccines

The use of recombinant DNA has been applied to the genetic attenuation of bacteria and viruses for use in live attenuated vaccines. Previously, attenuation of bacteria or viruses was achieved through serial in vitro passage or random chemical mutagenesis, with the potential accumulation of unknown and uncharacterized mutations and the risk of reversion. By contrast, recombinant, attenuated vaccines are specifically engineered to inactivate defined target functions with nonreverting mutations. Furthermore, the present requirements for licensing new vaccines are more rigid than that in the past and call for strains that are well defined and carry precisely defined mutations.

The rational design behind genetically engineering a recombinant vaccine strain should find the right balance between attenuation and immunogenicity. Initial attempts to generate recombinant, attenuated vaccines gave rise to unacceptable levels of reactogenicity in humans, and many promising candidate strains failed in clinical trials. To achieve balanced attenuation, different combinations of mutations can be introduced, which group into two main types, those that target critical housekeeping functions and those that target disease-related virulence factors. For example, auxotrophic mutations have been engineered by deleting genes in essential metabolic pathways. One of the most commonly used mutations for generating vaccines for intracellular pathogens such as *Samonella* and *Shigella* are *aro* mutations, which disable the shikimate pathway essential for the biosynthesis of aromatics including the aromatic amino acids. A second method is the targeting of critical virulence genes such as those encoding expression of the *Salmonella* pathogenicity island 2 (SPI2) type III secretion systems of *Salmonella* or the *ctx* toxin of *Vibrio cholerae*. Currently, candidate live vaccine strains are being constructed that combine different mutations, which optimize the balance between attenuation, reactogenicity, and immunogenicity, highlighting the need to understand the organisms' physiology and interaction with the host as well as protective immune responses to the disease.

Live attenuated virus vaccines are also being designed through reverse genetic approaches. For instance, attenuated dengue viruses have been generated by sequence modification or deletion and, alternatively, by producing recombinant antigenic chimeras between two related viruses (7). Recombinant DNA techniques could also facilitate the rapid generation of genetically attenuated viruses from emerging infections, such as metapneumovirus (8), or in response to the emergence of a new influenza variant. The use of reverse genetics enables rapid production of reference influenza vaccine viruses, and this has been exploited for the generation of an inactivated whole-virion-based vaccine for the influenza H5N1 reference vaccine strain in response to the latest pandemic flu threat (9,10).

Live Attenuated Bacteria as Vectors

There is increased interest in the use of live attenuated bacterial vaccines (LBVs) as carriers for the presentation of heterologous antigens for the engineering of live, recombinant mucosal vaccines. LBVs allow vaccination through mucosal surfaces and specific targeting of professional antigen-presenting cells located at the inductive sites of the immune system (11). Both humoral and cellular immune responses can potentially be primed by this approach.

Bacterial species that are being investigated as vector vaccines include attenuated strains of *Salmonella enterica* serovar *typhi* and serovar *typhimurium*, *Shigella*, *V. cholerae*, *Listeria monocytogenes*, bacille Calmette-Guerin (BCG) derived from *Mycobacterium bovis*, and *Yersinia enterocolica*. Other bacterial vectors have included nonpathogenic strains derived from the normal flora such as *Streptococcus gordonii*, *Lactobacillus casei*, and *L. lactis* (12).

Salmonella strains are of particular interest since these strains can be administered orally and may induce mucosal as well as systemic immune responses. Furthermore, more than 20 years of experience with a licensed live attenuated *Salmonella* vaccine, *S. typhi* Ty21a (Vivotif®, Berna Biotech, a Crucell company, Berne, Switzerland) is available and indicates that this strain is safe and effective in vaccination against typhoid fever (13). The generation of new carrier strains as well as improved systems of in vivo expression and localization (e.g., surface vs. internal localization and/or targeting to different cell compartments) of heterologous proteins is the focus of many groups' efforts (14,15).

A further novel approach exploits intracellular bacteria as delivery vectors for DNA vaccines (11). Some bacteria have been shown to deliver DNA vaccines to human cells in vitro and have provided evidence for in vivo efficacy in several experimental animal models of infectious diseases and cancers.

Conjugate Vaccines

The use of protein-conjugated polysaccharides as a tool for the prevention of diseases caused by encapsulated bacteria has proved to be highly successful in the development of effective vaccines against *Haemophilus influenzae*, *Neisseria meningitidics*, and *Streptococcus pneumoniae*. Subunit vaccines based on purified capsular polysaccharide can elicit protective immune responses in adults and are the basis of licensed multivalent pneumococcal and meningococcal polysaccharide vaccines. However, these have not been widely exploited as they were shown to result in short-lived protective immunity and to be ineffective in infants, largely because of their inability to engage a T cell–based immune response. The development of conjugate vaccines derived from polysaccharides of the capsule chemically conjugated to tetanus toxoid or diphtheria CRM197 carrier proteins resulted in a T-dependent antigen capable of protecting young children and providing long-term immunological memory. The recent success of the heptavalent conjugated vaccine (Prevenar) against pneumococcus, which protects against the seven serotypes that most commonly cause invasive pneumococcal disease in infants and young children, is an example of the development of an improved conjugate vaccine (16).

To date, the polysaccharide used in large-scale vaccine production has been purified from the pathogen itself, grown in large quantities—an approach that is costly and difficult to control. The large-scale production of a conjugate vaccine containing synthetic polysaccharides has been recently achieved (17). Through simplification of the carbohydrate chemistry involved, the first large-scale production of a *H. influenzae* type b vaccine, consisting of synthetic polysaccharide conjugated to tetanus toxoid protein carrier, was demonstrated. This vaccine has been shown to be as efficient as