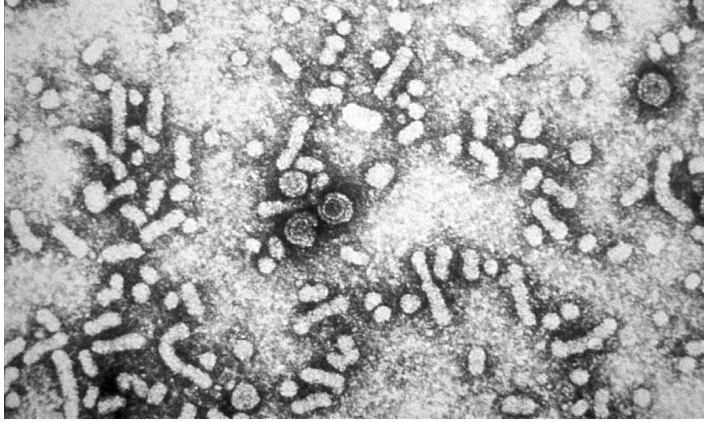

A transmission electron micrograph showing the ultrastructural morphology of Dane particles — spherical HBV virions of ~42 nm in diameter. Credit: CDC.

MILESTONE 11

# First recombinant DNA vaccine for HBV

In 1986, the Recombivax HB vaccine for hepatitis B was approved for human use in several countries, the culmination of research started by William Rutter, Pablo Valenzuela and colleagues in 1979 on the cloning of hepatitis B virus (HBV) antigens. It was the first vaccine to be produced using recombinant DNA technology and although it was only the third recombinant product to be approved for clinical use, it was also the most complex in forming nanoparticles that resemble patient-derived virus particles in both structure and immunogenicity.

Infection with HBV leads to the production of intact spherical virions of ~42 nm in diameter, also known as Dane particles, as well as the overproduction of 22 nm particles consisting exclusively of hepatitis B surface antigen (HBsAg). HBsAg is encoded by gene S, which contains three in-frame start codons that enable production of HBsAg proteins of three lengths (small, middle and large). The large HBsAg protein is the most abundant form found on the surface of infectious viral particles and is thought to have a crucial role in the binding of HBV to hepatocytes.

HBsAg was first identified in 1965 by Baruch Blumberg as an antigen found in the blood of an Aboriginal Australian and it was later shown by Blumberg and others to be associated with HBV infection and to be part of the virus itself. For his discoveries “concerning new mechanisms for the origin and dissemination of infectious diseases”, Blumberg was recipient of a joint Nobel Prize with Carleton Gajdusek in 1976.

> “The ability to produce immunogenic HBsAg in genome-free virus-like particles... allowed for the large-scale production of HBV vaccines unable to infect host cells”

Given the failure since its discovery to cultivate HBV in vitro, the first commercial HBV vaccine (Heptavax; licensed in 1981) was based on inactivated virus collected from the plasma of HBV-infected donors. However, plasma products at the time had been associated with HIV-1 and HCV transmission and vaccine supply was limited by the availability of chronic HBV carriers. Therefore, the use of recombinant DNA technology was an attractive option for development of a vaccine that solved both of these problems. Targeting HBsAg was also attractive, given that it was encoded by a single gene and thought to be closely involved in interactions with host cells.

In 1979, William Rutter, who had been involved in research on recombinant insulin and growth hormone, and colleagues, including Pablo Valenzuela at the University of California, San Francisco, successfully cloned HBsAg into *Escherichia coli* expression vectors, demonstrating the possibility of using recombinant HBsAg as an HBV vaccine. Using Dane particles isolated from human serum by one of their funders, Merck Sharpe and Dohme, the researchers synthesized double-stranded viral DNA and carried out restriction mapping and Maxam–Gilbert DNA sequencing to assemble the viral genome. Based on the 19 amino acids that had previously been identified at the amino terminus of HBsAg, they located the 892 bp genomic region encompassing the S gene. It corresponded to a single protein sequence, potentially forming a globular protein. They also identified three potential glycosylation sites that could account for the two sizes of polypeptide that can be removed from the viral surface coat by detergents.

A few years later, in 1982, the same group, together with colleagues from the University of Washington, cloned HBsAg into yeast expression vectors. They used a plasmid that placed the coding sequence under the control of a constitutive yeast promoter, which enabled a high level of HBsAg to be made, as verified by immunoassays. Remarkably, sedimentation as well as electron microscopy experiments showed that 22 nm particles were the predominant form of HBsAg secreted by the transformed yeast cells, similar to virus-infected human cells. Also like the 22 nm HBsAg particles from human cells, which had been shown previously to be ~1,000-fold more immunogenic than the unassembled HBsAg protein, the yeast-generated particles were recognized by the HBsAg-specific antibodies known at the time.

The ability to produce immunogenic HBsAg in genome-free virus-like particles (VLPs) was a breakthrough. It not only allowed for the large-scale production of HBV vaccines unable to infect host cells, but also created a blueprint for vaccines against other pathogens such as human papilloma virus (MILESTONE 14) and malaria and showed that a vaccine could be produced without the disease-causing pathogen itself. VLPs have also proved useful for applications such as antibody discovery, bioimaging and cell targeting. Recombinant DNA technology had lived up to its potential to transform basic research into applied research, whereby a living cell could be reduced to an information-processing machine and genetic engineering could become an integral part of both angles of research.

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