VACCINES

HEPATITIS B VACCINE MAKERS MULTIPLY

NEW YORK—Last July's announcement that the Food and Drug Administration (FDA) had approved the U.S.'s first genetically engineered human vaccine made the front page of *The New York Times*. Recombivax HB®—a yeast-produced hepatitis B subunit vaccine developed by Chiron (Emeryville, CA) and Merck Sharp & Dohme (West Point, PA), contains only the viral surface antigen (HBsAg), unlike the conventional whole-virus vaccine (also Merck's). Frank Young, the FDA's commissioner, said that "this development opens the door for the production of other vaccines that have so far been impractical, potentially unsafe, or impossible to make." And biotech industry analyst Peter Drake (Kidder Peabody, New York), said that "Chiron's hepatitis B vaccine represents a major psychological positive for the company and for the industry."

The vaccine market has a history of low profitability—because vaccines have traditionally been made from viruses, there has always been some risk that they would actually cause the disease rather than prevent it. This meant liability for the vaccine manufacturers. Also, conventional hepatitis B vaccine has been made from the plasma of chronic carriers of the hepatitis B virus (HBV); this population is limited, and the production process is costly and low-yielding. Merck's current vaccine sells for about $110 for a three-dose regimen. Add to that the fear—albeit unwarranted—that this vaccine might transmit AIDS, and it becomes clear why the vaccine has not gained a wide market.

But, now that the FDA has approved a recombinant, totally safe, vaccine, the whole picture has changed. As Philip J. Whitcome, director of strategic planning for Amgen (Thousand Oaks, CA), says, Merck clearly showed that the economics of the vaccine market can be changed, and it has now become very attractive. Kidder Peabody's Drake predicts the 1990 U.S. market for hepatitis B vaccine to be $100 million; of this, he says, the Chiron/Merck vaccine will capture 85 percent. Stuart Weisbrod, an analyst at Prudential-Bache Securities (New York), is slightly more conservative, giving it 60-percent market share.

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Immunofluorescent localization of rHBsAg particles within the endoplasmic reticulum of CHO cells. Once assembled, the particles are secreted into the medium.

Weisbrod claims that the market is totally elastic, and top end revenues really depend on how inexpensively the vaccine can be made. At $100 per regimen, the vaccine would reach only about 1 million people—those in the more affluent countries, where only 4–6 percent of the population has been exposed to the virus. If the vaccine's price could be brought down to $5, he says, then as many as 100 million could be treated—those in Third World nations where the incidence of HBV is disturbingly high (70–95 percent exposure). Weisbrod predicts that the company that first comes out with a recombinant mammalian cell-produced vaccine will take the largest percentage of the market—because the vaccine should be less expensive, and perhaps more efficacious, requiring a two- instead of three-dose regimen. Still, he concludes, "It's hard to out-market Merck."

Merck's predominance notwithstanding, the contenders for the hepatitis B vaccine market continue to emerge: SmithKline Biologicals (SKB, Rixensart, Belgium), for instance, has been marketing its recombinant hepatitis B vaccine, Engerix-B, since June 1986 (in Singapore). SKB received marketing approval in Belgium and Switzerland in late 1986. Walter S. Vandersmissen, SKB's international marketing director, says that the company expects other registrations in the next few months, and it is "exploring marketing avenues in the U.S."

SKB's vaccine, like Merck's, consists of recombinant hepatitis B surface antigen (rHBsAg, a product of the S gene) produced in *Saccharomyces cerevisiae*. HBsAg is a glycosylated polypeptide that can aggregate into immunologically active 22 nm particles; the particles consist of a major polypeptide (p25) and two others of higher molecular weight (gp33 and p39). All three share the 226 amino acid residues of the S region; p33 has 55 more residues at its amino end (pre-S2) and p39 adds yet another 110-odd residues to p33 (pre-S1). These pre-S regions may add to the efficacy of the vaccine.

Still, vaccines consisting solely of rHBsAg 22 nm particles produced in *S. cerevisiae* are at least as effective as the conventional, serum-derived-whole virus vaccine. Both Amgen and Biogen (Cambridge, MA) have developed rHBsAg vaccines, and both are in clinical trials. Amgen's partner in vaccine development (as for erythropoietin and interleukin-2) is Johnson & Johnson (J&J, New Brunswick, NJ). J&J recently completed phase I

trials with the vaccine. Both Drake and Weisbrod give Amgen/J&J a small—but significant—share of the market.

Biogen's partners in the hepatitis B vaccine game are the Wellcome Foundation Ltd. (Kent, UK) and Green Cross (Osaka, Japan). In contrast to Amgen, Biogen has transferred its technology and licensed the vaccine to both partners. Robert Gottlieb, Biogen's director of press relations, says that both Wellcome and Green Cross are in clinical trials with the vaccine. The Asian trials are further along, solidly in phase II. Gottlieb says that Biogen has a strong patent position on the surface antigen.

Scientists at Salk Institute Biotechnology/Industrial Associates (SIBIA, La Jolla, CA) and Phillips Petroleum (Bartlesville, OK) also use a yeast system to produce rHBsAg particles—but this yeast is the industrial strain *Pichia pastoris*. The scientists claim that their system can produce up to 400 milligrams of 22-nm particles per liter, significantly higher than the levels reported for *S. cerevisiae* systems.

Most other hepatitis B vaccines in development are produced in mammalian cell systems. Genentech (South San Francisco, CA), for instance, produces 22-nm rHBsAg particles secreted by Chinese hamster ovary (CHO) cells, stably transfected with the HBV S gene. Genentech scientist Eric Patzer and associates (see *Bio/Technology* **4**:630, July '86) have demonstrated that "the recombinant vaccine is more immunogenic in chimps than previously reported vaccines of either plasma-derived HBsAg or yeast-derived recombinant HBsAg."

The first clinical trials of Genentech's vaccine were conducted by the NIH in 1985. Genentech has licensed the product to Mitsubishi Chemical (Tokyo, Japan)—which has started phase I clinicals—and to Institut Merieux (Lyon, France).

Integrated Genetics (Framingham, MA) uses mouse cells and a bovine papilloma virus expression system to produce and secrete rHBsAg 22 nm particles. The protein, according to Stanley C. Erck, president of corporate development, is identical to that isolated from serum. Erck says that Connaught Labs (Ontario, Canada) and Daiichi Seyaiku (Tokyo), it's two partners, are both in phase II clinicals with the vaccine.

Scientists at the Institut Pasteur (Paris) also use CHO cells to express rHBsAg genes. These particles, however, carry a receptor for polymerized human serum albumin, probably coded for by the pre-S region. There is increasing evidence that the pre-S region carries an immunodominant epitope of HBV. This epitope, according to Marie-Louise Michel and colleagues (see *Bio/Technology* **3**:561, June '85), is recognized by human antibodies elicited by HBV infection. Moreover, antibodies directed against this receptor epitope may interfere with the virus' binding to liver cells, thus preventing its uptake. (HBV is heavily implicated in liver cancer.)

Other groups investigating pre-S regions and polyalbumin receptors include Chiron's Pablo Valenzuela and associates (using a yeast system; see *Bio/Technology* **3**:317, April, '85), and Takeda Chemical Industries' Kazuaki Kitano and colleagues (also a yeast system).

Endotronics' (Coon Rapids, MN) scientists also feel that the pre-S region may increase the efficacy of a hepatitis B vaccine. Endotronics bought patent applications and technology to a mammalian cell-derived vaccine from Celias (U.K.) in early 1986; since then Endotronics has expanded the technology. John Salstrom, the company's director of molecular biology research, says that Endotronics is using CHO cells and a proprietary cell line to produce a vaccine that is enriched for the pre-S region. Meanwhile, Endotronics' first vaccine, that it acquired with the Celias technology, is already in phase I clinicals.

Salstrom forsees even more efficacious vaccines in the third- and fourth-generation products. He says the third generation vaccine will be "antibody-potentiated." If antigen and antibody are mixed together, then one can decrease the amount of antigen by at least 100–1000-fold; because antibody is much cheaper to produce than antigen, the price of the vaccine would plummet. A fourth generation vaccine would be even cheaper, consisting solely of anti-idiotypic antibody.

—Jennifer Van Brunt

RESEARCH PAPER ANALYSIS

PINE REGENERATED VIA SOMATIC EMBRYOGENESIS

As reported in this issue by Praymod Gupta and Don Durzan of the University of California (Davis), the loblolly pine can now be added to the very short list of gymnosperms (or conifers) that can be regenerated in culture via somatic embryogenesis. Conifers represent major sources of softwood and fiber, and reforestation efforts with clones or selected lines propagated via organogenesis are highly labor-intensive. Somatic embryogenesis (polyembryogenesis) offers forestry the welcome prospect of large numbers combined with cost-efficiency.

The paper by Gupta and Durzan also demonstrates that the techniques of freeze-preservation and encapsulation, just now being developed for somatic embryos of flowering plants, are also applicable to conifers. Both are methods for short- and long-term storage; encapsulation has the added potential of providing a method of dissemination.

Of special interest in the report is the use of various staining regimes to identify specific cell masses that have embryogenic potential. Plant cells, whether grown in agitated or stationary cultures, typically are composed of mixed populations. In some instances, cells and cell masses may look different under the microscope, varying in size, shape, or degree of vacuolation. When these cells are plated out or individually isolated, the inconsistencies often foretell differences in developmental potential. In other instances, cells may appear similar but behave differently.

In almost all cases, however, only a certain percentage of cells goes on to regenerate plants. The task, then, is to identify embryogenic cells in the population of any one culture or among replicate cultures, avoid their loss during subculture, and then enrich their number, particularly prior to embryo maturation and plantlet formation. Identifying and selecting the proper cells or callus segments, for example, has proved essential in demonstrating and using somatic embryogenesis in the cereals. And it is critically important in commercial operations where regeneration efficiencies, numbers of cultures and plants, and operating costs are critical. Researchers are now considering a number of "high-tech" strategies, such as using molecular probes targeted to embryo-specific RNA, or antibodies directed to embryo-specific proteins. Gupta and Durzan's use of standard stains (acetocarmine, Feulgen, and Evan's blue) offers an inexpensive and readily available tool for screening, selecting, and studying embryogenic populations.

—Philip V. Ammirato