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N. GIRIDHARAN
N. LAKSHMAIAH

National Institute of Nutrition
Jamia Osmania P O
Hyderabad 500 007, India

COMMENTARY

HBV: Have we found the ultimate answer?

S. P. Thyagarajan

Hepatitis B virus (HBV) continues to be the single most important cause of viral hepatitis throughout the world, along with hepatitis C virus, an important cause of chronic liver diseases and hepatocellular carcinoma[1]. The situation is further disturbing as seen from the recent reports that HBV could also be involved in extra-hepatic immunologically mediated diseases like primary biliary cirrhosis, polyarteritis nodosa and glomerulonephritis[2, 3].

The only known reservoir of this notorious virus is human beings themselves as healthy carriers of HBV in all the populations in the world. To date, there are nearly 370 million HBV carriers in the world, with the highest incidence of 10–20% in the tropical countries[1]. In India itself, a conservative estimate of 30 million carriers is projected to be present based on 3–5% HBV carrier rate in the population.

Studies on HBV exposure pattern in the population have clearly shown the predominant role of horizontal transmission besides vertical/perinatal transmission in the spread of HBV[4–6]. Reports have also shown the family clustering of HBsAg in 40–65% of family members of HBV carrier families, while the same was much lower in non-carrier families[5, 6]

Prevention and control of hepatitis B

All these informations on the notoriety of HBV led to public health urgency of an effective prevention strategy for hepatitis B. As per the conventional methodology adopted in the prevention of any infectious disease, production of hepatitis B vaccines was successfully attempted in the 1970s itself.

Plasma-derived hepatitis B vaccines

The first of the hepatitis B vaccines was manufactured using hepatitis B surface antigen (HBsAg) particles from the plasma of chronic HBV carriers by Krugman and Giles in 1973 (ref. 7). However, the commercial plasma-derived hepatitis B vaccine came into human use only in 1982. Current plasma-derived vaccines consist of highly purified formalin-inactivated and/or heat-inactivated alum-adsorbed hepatitis B 22 nm subviral particles of HBsAg that are free of detectable nucleic acid. The antigen is harvested from the plasma of asymptomatic, apparently healthy human carriers of HBV by a series of steps that may include precipitation, ultracentrifugation, gel filtration and/or affinity chromatography.

The methodology of this vaccine preparation is in brief as follows:

- Plasma from hepatitis B carriers
- Defibrination (with added calcium)
- Ammonium sulphate precipitation (concentration)
- Isopycnic banding (sodium bromide)
- Rate zonal sedimentation (sucrose gradient)
- Pepsin digestion pH2 (10-fold purification)
- Urea 8 M (denature–renature)
- Gel filtration (molecular sieve)
- Formalin 1:4000 (72 h/37°C)
- Vaccine, 20 mcg surface antigen/dose with 0.5 mg alum in 1 ml and thiomersal.

By these stringent procedures it is stated that all the known blood-borne viruses like retro, including HIV I and II, toga, lenti (HCV) and others are inactivated fully and their nucleic acid components removed.

Currently, there are more than a dozen manufacturers of plasma-derived vaccines worldwide. The commonest ones are Hepatavax B, manufactured by Merck, Sharp and Dohme laboratories, USA, and Hepavac-B by Korean Green Cross Corporation. These vaccines have been proved by several clinical trials as safe, immunogenic and effective hepatitis B vaccines.

Genetic recombinant hepatitis B vaccines

The widespread use of plasma-derived vaccines was curtailed by relatively expensive production costs and unfounded fear that resistant or unknown organisms could escape inactivation during the preparation of the plasma-derived vaccine. Elaborate procedures are necessary to purify the vaccine, and time-consuming tests must be performed to ensure that the vaccine is free from

Table 1. Current approaches in the hepatitis vaccine production

Source	Form	Status
Plasma	Subviral HBsAg particles	Licensed
	Micelle	
	Polypeptide	Investigational
Hepatoma cell line	Subviral HBsAg particles	Investigational
Recombinant plasmids		
Yeast		
E. coli		Licensed
Mammalian cells	Subviral HBsAg particles	Investigational
Recombinant viral		
Vaccinia	Vaccinia virus	Investigational
SV 40 in mammalian cells	Subviral HBsAg particles	
Synthetic	Peptides	Investigational
Anti-idiotypic antibody	Antibodies	Investigational

infectious hepatitis B and other blood-borne pathogens such as human immunodeficiency virus (HIV). The search for alternative, non-human sources of HBsAg has led to the development of recombinant DNA techniques to express HBsAg in yeast cells[8], from which the antigen is then purified and formulated into a vaccine[9].

Briefly, the genome of HBV is cloned and isolated in *E. coli* and the gene which codes for HBsAg is localized. The S gene (or Pre-S + S gene) fragments are inserted into an expression plasmid (Eg. pBR 322) and then introduced into a heterologous host such as yeast (*Saccharomyces cerevisiae* or *Hansennella polymorpha*), mammalian or insect cells. These cells permit an adequate expression of the derived protein in fermentation cultures. HBsAg is released from the cells by homogenization or disruption with glass beads, purified by clarification, ultrafiltration, chromatography, ultracentrifugation and then absorbed onto alum hydroxide, after which thiomersal is added as a preservative.

Engerix-B, the first genetic recombinant human vaccine, was produced by SmithKline Beecham Biologicals and launched commercially in June 1986. Since February 1984 to June 1986, 145 trials have been initiated with 94 investigators around the globe. Over 16,000 individuals of all ages in 80 countries participated, receiving almost 50,000 doses of vaccine.

Randomized double-blind studies were carried out to compare the reactogenicity and immunogenicity of yeast-derived hepatitis B vaccine versus the plasma-derived vaccines. The overall results showed that the side-effects were usually mild and there were no significant differences in adverse reaction in between the study groups. Both the vaccines were well-tolerated and highly immunogenic. The plasma-derived vaccine elicited higher response of anti-HBs geometric mean titre compared to the genetic recombinant vaccine[10–13].

Table 1 summarizes the current approaches in hepatitis B recombinant vaccines which are licensed; all the other types are still in the investigational status.

Hepatitis B immunoglobulin (HBIG)

Human plasma with measurable levels of high-titred anti-HBs upon purification led to the development of HBIG Immunoprophylaxis with HBIG is indicated in (a) exposure to HBV-containing material by percutaneous inoculation, oral ingestion or direct mucous membrane contact; (b) transient HBV exposure through intimate contact with the partners of acutely infected patients and (c) foetal/neonatal exposure to mothers with acute hepatitis B or HBV healthy carrier status.

Immunization schedules

Active immunization: Hepatitis B vaccination

The schedule of active immunization, consisting of hepatitis B vaccination is presented in Table 2.

Passive immunization: HBIG

HBIG is given as a means of post-exposure prophylaxis with BOB standard: 1:10,000 of anti-HBs at a dose of 0.5–0.7 mg/kg body weight under the circumstances enumerated in the previous section.

Combined immunization

Administration of both hepatitis B vaccine and HBIG is indicated to infants born to HBsAg-positive mothers who could be suffering from acute hepatitis B or could be healthy carriers of HBV. The regimen is: (1) one 0.5 ml dose of HBIG at birth within 24 hr, (2) three 20 mcg (1.0 ml) doses of the HB vaccine to be given as per the usual 0, 1, 6-month schedule, with the first dose being given within one week of birth It is shown that this strategy prevented 93% of children from acquiring chronic HBV carrier status from their mothers.

Impact of hepatitis B vaccination

As per WHO report, March 1994, 72 countries of the globe have adopted hepatitis B vaccine in their universal programme of immunization. Such vigorous application of hepatitis B vaccination to pediatric populations is beginning to have a discernible impact on hepatitis B in many countries by preventing vertical/perinatal and horizontal transmission of HBV as evidenced by the significant reduction in HBV carrier status over a period of time; the standard example being Taiwan, where such an exercise has brought down HBV carrier status from 18% to 8% from 1986 to 1993.

Unfortunately, strategies of vaccinating only high-risk populations have not resulted in control of hepatitis B even in some developed countries. The incidence of reported hepatitis has been increasing in the USA. The Centre for Disease Control estimates that 12,000 health care workers are hospitalized, with over 200 deaths due to liver cancer, cirrhosis and fulminant hepatitis[14].

Aggarwal and Naik[15] while discussing the appropriate strategy of hepatitis B vaccination in India have worked out the relative health advantage of universal immunization policy over selective

Table 2. Schedule of active immunization

Group	Initial	1 month	6 months
Younger children (birth to 10 years)	0.5 ml (10 mcg)	0.5 ml	0 5 ml
Adults and older children	1 0 ml (20 mcg)	1.0 ml	1.0 ml
Dialysis patients and immuno-compromised patients	2.0 ml*	2.0 ml	2.0 ml

*Two 1.0 ml doses given at different sites. Only intramuscular route; deltoid muscle is the preferred site. For infants and children injections are given on the anterolateral aspect of thigh. Specific immunity lasts for at least 5 years. One booster dose is advised after 5 years.

immunization of high-risk groups. Universal immunization has a protective efficacy of 92% and a carrier prevention rate of 341 2/10,000 infants born, with a cost of US$ 126 per carrier prevented. On the other hand, selective immunization policy would have a protective efficacy of 12% and a carrier prevention rate of 44 8/10,000 infants born, with a cost of US$ 495 per carrier prevented. However, the overall cost involved in the implementation of universal hepatitis B immunization in India would work out to be much higher indeed. Our country's birth rate being 30 per 1000 population, the estimated number of total live births per year is 25.5 million. As the expenditure on immunization of each child is estimated at US$ 4.3, the total expenditure on HBV immunization will be approximately US$ 110 million or Rs 351 crores per year[15]. This may appear to be a formidable sum but is surely not impossible for the country to mobilize, considering that our GNP (1990–91) estimate is Rs. 468,426 crores per year[16]. The benefits of such a policy are likely to be apparent in about three to four decades in the form of markedly reduced numbers of patients with chronic liver disease, hepatocellular carcinoma and post-transfusion hepatitis, a lower cost of their medical care and an increased life expectancy of our population[17]. In the background of this information, it has to be summarily decided to include hepatitis B vaccine in the expanded programme of immunization (*EPI*) in India.

India has an extensive primary health care network including a good coverage of vaccines under the EPI. Because of its flexible dose schedule, HBV vaccine can easily be integrated with the existing programme, without requiring additional visits. Thus, the first dose can be given with BCG, the second with the first dose of diphtheria, pertussis, tetanus (DPT/oral polio vaccine (OPV))[15] or any other acceptable schedule as decided by the national health policy planners.

The cost factor involved in its implementation could be managed effectively by going in for indigenous manufacture of plasma-derived hepatitis B vaccine until genetic recombinant technology transfer materializes. Until such time, the high-risk population of our country, which includes medical, paramedical and dental personnel, patients exposed to blood and blood products and children born to HBsAg-positive mothers might have to be compulsorily vaccinated with immediate effect without compromising on the cost involved.

Does it mean we have found an answer for hepatitis B?

In spite of having one of the most successful viral vaccines next to smallpox vaccine, we are yet to conquer the disease hepatitis B and control its spread to different populations the world over. Three major hurdles have been recognized to impede the finding of a complete answer to this global problem. They are:

(a) *Prohibitive cost of hepatitis B vaccines.* Even though the current pricing of hepatitis B vaccine is too high for the developing and underdeveloped world to accommodate in their health budget for inclusion under their EPI programmes, efforts are on for evolving cost-effective vaccines. In India both governmental and private organizations have started research and development ventures on indigenous manufacture of hepatitis B vaccines, either plasma-derived or genetic recombinant types. The plasma-derived hepatitis B vaccines are being attempted by M. S. University, Baroda, Bharath Immunological & Biologicals Ltd., Delhi, along with Dr ALM PGIBMS, Madras University, and Span Diagnostic Ltd, Surat. The genetic recombinant type of hepatitis B vaccines are being designed at NII and ICGEB, New Delhi, Cadila Laboratories, Ahmedabad, and Transgene Biotek Ltd, Hyderabad. Hence, this problem could be anticipated for an optimistic solution in the near future.

(b) *HBV mutants.* Based on the sequencing of the entire HBV genome, five genotypes have been identified but all of these belong to one serotype. There has been much interest recently in genetic variants of HBV and their impact on clinical disease and vaccine efficacy[18, 19].

(i) *Precore/core mutants.* Variants of HBV with specific mutations within the precore and/or core region have been identified, principally in the Mediterranean region and Asia[20, 21]. These precore/core mutants are thought to be associated in some cases with fulminant acute hepatitis and more severe chronic hepatitis.

(ii) *Vaccine-induced escape mutants.* A second type of HBV mutant virus containing one or more mutations in the S-gene encoding the envelope protein has also been reported[18]. These S-gene mutant viruses have been thought to represent neutralization escape mutants of HBV, since they have been recovered from individuals actively or passively immunized against the virus. The mutations found in such mutants were located in the region of the S-gene that is thought to encode the major group reactive epitopes involved in neutralization of the virus. These and similar variants of HBV have been identified in North and South America, Europe, Asia and Africa, usually in infants who develop hepatitis B despite perinatal immunization or in chronically infected individuals receiving passive immunoprophylaxis following orthotopic liver transplantation. There is some fear that the S-gene mutant viruses will replace wild-type HBV and pose a serious threat to international vaccination programmes that utilize recombinant or plasma-derived vaccines against wild-type virus. It is unlikely that currently recognized S-gene mutants will pose major public health problems, but more epidemiologic information is necessary before a final evaluation can be made. However, it is more important not to let

Table 3. Currently available treatment modalities for hepatitis B

Antivirals	Immunomodulators
Interferons	Corticosteroids
Adenosine arabinoside	Interleukin-2
Zidovudine	'Pulse' prednisolone
Suramin	Gamma interferon (IFN)
Ribavirin	Combination therapy
Ganciclovir	IFN/Ara-A
Quinacrine	IFN/Ara-AMP
	Alpha IFN/Gamma IFN
(+)-cyanidanol-3	Prednisolone/Ara-A
Thymosin	Prednisolone/Ara-AMP
Dideoxy-nucleosides/3Tc	Prednisolone/Ara-IFN
FIAV	Acyclovir/IFN
	Deoxyacyclovir/IFN
	Prednisolone/acyclovir

the potential risk of future vaccination problems stand in the way of vigorously pursuing current vaccination programmes against the very real risk of acute and chronic hepatitis B throughout the world. Modifications of hepatitis B vaccines can be achieved if it proves necessary, but the impetus of the current worldwide vaccination programmes must not be lost.

(c) *Therapy in acute and chronic hepatitis B.* Regardless of vaccination programmes, nearly 370 million chronic carriers of HBV exist in the world at present and there is little to offer them in terms of therapy. The currently available treatment modalities are listed in Table 3. Of these, only alpha interferon offers some hope in clearing 10–20% HBsAg carriage and bringing about 30–40% HBeAg seroconversion. However, its prohibitive cost and profound toxicity restricts its wider use. Traditional systems of medicine have come up with several treatment approaches. The only medicinal plant scientifically analysed in this regard is *Phyllanthus amarus*. It has been consistently shown to clear HBsAg in 25% of HBV carriers and to bring about HBeAg seroconversion to a tune of 50% besides proving to be absolutely safe without any observable side-effects[22].

There is a pressing need for effective therapeutic regimens for the treatment of HBV. It is unclear at this time whether major advances will come from the biochemistry of nucleoside analogues and oligonucleotides, from the immunology of the host's B-cell and T-cell responses, from a better understanding of the molecular biology of viral replication, or from an as yet unrecognized discipline.

Conclusion

The Daedalus myth is a metaphor for all aspects of a scientific process. Daedalus, a legendary figure of the classical world, was a craftsman, inventor, architect and artist. He was also a generator and solver of problems. Every time a question was answered it raised several more and these in turn led to other questions; his search was endless. Daedalus repeatedly learned that even the most ingenious solutions were neither flawless nor definitive.

The medical scientist will recognize a familiar pattern in the career of Daedalus. When a hypothesis is tested it generates others and when tested these yield still more. Scientific research has an infinite quality; the more we know, the more we know about what we do not know, and this unknown must in turn be understood. Each time a medical treatment, diagnostic technique, or public health measure is introduced, no matter how effective it may be, it nearly always raises other problems. All drugs have undesirable side-effects; diagnostic procedures are rarely, if ever, entirely specific or infinitely sensitive; and public health measures often have medical, social and even political consequences that are difficult to foresee.

Research on the hepatitis B virus is an example!

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S. P. Thyagarajan is in the Department of Microbiology, Dr ALM Postgraduate Institute of Basic Medical Sciences, University of Madras, Taramani, Madras 600 113, India