TRABAJO DE REVISION/
REVIEW ARTICLE

THE HEPATITIS B VIRUS (HBV) INFECTION AND ITS PREVENTION BY A RECOMBINANT-DNA VIRAL SURFACE ANTIGEN (rec-HBsAg) VACCINE

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SUMMARY

This paper contains a summarized review of the main aspects related to the development, physical-chemical and biological characterization of a yeast-derived rec-HBsAg, which was directly followed by the scaled-up production as well as the quality assurance and control of the corresponding vaccine, under Good Manufacturing Practices (GMP) and Good Laboratory Practices (GLP) standard procedures. Pre-clinical experiences were carried out in animal models (including the HBV-challenging of immunized and control chimpanzees) and clinical trials were performed by (or achieved in agreement with) health authorities in Cuba and several countries. Double-blind controlled three phase protocols according to strict ethical and methodological guidelines for clinical trials, demonstrated at least a similar and often a better performance of the Cuban vaccine than parallel immunizations with other commercially available rec-HBsAg vaccines. These results enabled the patent application and sanitary registration followed by commercialization of millions of doses of the product in more than 15 countries. The first vaccination program in America including the complete populations of newborn and children up to one year old, primary and secondary school children and high infection risk groups, is currently ongoing in Cuba using this vaccine.

RESUMEN

Este trabajo contiene una revisión de los principales aspectos relacionados con el desarrollo y la caracterización físico-química y biológica del antígeno rec-HBsAg derivado de levadura, seguidos directamente por el escalado de producción, así como el aseguramiento y control de la calidad de la vacuna correspondiente, bajo condiciones de procedimientos normados conforme a las BPF (Buenas Prácticas de Fabricación) y BPL (Buenas Prácticas de Laboratorio). Se efectuaron experiencias pre-clínicas en modelos animales (incluyendo el reto de chimpancés inmunizados y controles), de acuerdo con las autoridades de salud en Cuba y en varios países. Los protocolos controlados de tres fases a doble ciegas se realizaron conforme a estrictas normaciones éticas y metodológicas para pruebas clínicas. Sus resultados demostraron un comportamiento al menos similar y a menudo superior de la vacuna cubana con relación a inmunizaciones en paralelo con vacunas rec-HBsAg disponibles comercialmente. Estos resultados pemitieron la solicitud de patente y registro sanitario seguidas por la comercialización de millones de dosis del producto en más de 15 países. El primer programa de vacunación en América que incluye la población completa de recién nacidos y niños hasta 1 año de edad, así como los escolares de primaria y secundaria y grupos de alto riesgo, está actualmente en desarrollo en Cuba con el empleo de esta vácuna.

INTRODUCTION

Hepatitis B is a serious health problem and a matter of growing concern for all public health organizations at both the national and international levels.

In recent years, vaccines produced using the HBsAg, purified from plasma of HBV carriers [1] or derived by recombinant (rec)-DNA techniques [2-4] have been proven to be sufficiently safe and effective [5] to be licensed by health authorities in several countries, for distribution and application to human populations.

However, the fact that high-yield recombinant microrganisms, improved purification procedures and different types of rec-HBsAg vaccines are already or will soon be available, in no way means that the millions of people who require this vaccine will necessarily have access to it. Unfortunately, the current price per dose continues to be beyond the reach of the health services of poor and even medium level countries for the implementation of mass immunization programs. So, the idea of developing an owned vaccine under competitive quality standards, although challenging from the scientific and technological points of view, seemed worthy and rewarding for the Cuban scientists and decision making authorities.

In this paper we present an overall review on the background knowledge of the HBV infection and its prevention by a recombinant anti-hepatitis B viral surface antigen vaccine obtained and produced in a developing country (Cuba).

The molecular biology of HBV

HBV is a small (42 nm) partially double stranded circular DNA virus of the Hepadnaviridae family with a 3 200 nucleotides genome (the shortest known animal virus genome), having its plus strand 50-80% shorter than the minus strand and containing the genes s-c-p-x in an overlapping compact form, which also includes control sequences in different reading frames [6]. The upstream region of the HBV genome contains the pre **s1** and pre **s2** segments which together with the **s** gene codify for three envelope proteins (surface antigens), according to: pre **s2** + **s** for the medium protein, pre **s1** + pre **s2** + **s**, for the large protein and **s** for the small protein (24 kDa) and each one of them can be in its glycosylated or non glycosylated forms [7]. Some 100 subunits of these proteins get together to form highly immunogenic polymeric structures (2 400 kDa), which can be seen by electron microscopy as 22 nm particles and in other associations. The envelope or surface antigens are the basic components of today's vaccines and define the serological subtypes of HBV by combination of the epitopes a-d-r-y-w, identified by monoclonal antibodies and useful as epidemiological tracers [8]. The pre **s1** segment plays an important role in the entrance of the virus into the hepatocyte and the pre **s2** region has been claimed to confer better immunogenic characteristics to the medium protein [9]. The **c** gene [10] and its preceding region (pre **c**) codify for the capsid (core) protein or **c** antigen and the **e** antigen. The **p** gene [11], which is very long and includes segments of the other genes, codify for proteins of the viral replicative cycle and the **x** gene appears to be an ubiquitious transcriptional trans-activator [12]. The demonstration of the presence of the HBsAg or antibodies against the **c** antigen (anti-HBcAg) in blood denotes infection by HBV and that of the **e** antigen implies replicative activity of the virus, while anti-HBsAg antibodies beyond a certain conventional limit of 10 IU/L (international units per liter) indicate protective seroconvertion by previous infection or vaccination. The serological transition from HBeAg to anti-HBeAg is considered a signal of good prognosis [13] and is understood as a positive evolutive trend to healing.

Hepadnavirus, including HBV, replicates reversely as compared to retroviruses (RNA-DNA), as far as they have a DNA genome and make use of an intermediary RNA (DNA-RNA) for replication. After the enzymatic extension of the short DNA strand of the viral genome it migrates to the hepatocyte nucleus to be copied into a 3.5 Kb RNA strand or intermediary pregenome which once packed into a newly synthesized capsid, is copied into DNA by polymerases giving place to a long strand identical to that of the original genome which serve as template for the polymerase driven reconstruction of the short complementary strand and the pregenome disintegrates [14]. Transcriptional enhancer type elements activate the preferential expression of viral genes in hepatic cells.

The human infection by HBV

At the present time there should be around 300 million HBV infected virus carriers the World over (approximately 5% of its population) [15], 3/4 of them living in Asia. Although in 90% of these persons the infection does not develop as actual disease, these "healthy" or subclinical chronic carriers with minimal or non existing hepatic damage and no functional deficiency, constitute the main reservoir of the virus, considered the second known human carcinogen after tobacco [16]. One to 1.5 million deaths yearly (at least ten times more than AIDS), are estimated to be due to HBV infection, its consequences and sequels (i.e., liver cirrhosis and primary hepatocarcinoma).

Hepatitis B may become a severe disease with an hyper-acute, acute or chronic (persistent or active infections) course that may lead to liver failure, cirrhosis or hepato-carcinoma. Asymptomatic HBV carriers as well as those suffering from the different forms of hepatitis B, have a 100 fold increased risk for developing a primary hepatocellular carcinoma [18] with an estimated delay time of 30-50 years, although it has also been observed in children.

The risk for developping the carrier state is greatest in early life and diminishes with increasing age. Up to 90% of babies born to carrier mothers may become carriers themselves [19].

HBV is distributed all over the World though with a relatively low prevalence (lower than 2%) in North America (except eskymos and other minorities with higher rates), Western Europe and Australia and a much marked infection level (higher than 5% and oftenly 5-20%) in Asia, especially the South East, Sub-Saharan Africa and Amazonic

South America, while intermediate figures (usually between 2-5%) prevails in most of Latin-America and the Caribbean, the Middle East, South and Eastern Europe and North Africa [20].

Person-to-person HBV transmission occurs through contact of the even slightly damaged skin or mucosae (e.g. hypodermic needle shot), with infected body fluids (i.e., blood [21], but probably also semen [22] and saliva [23]). Therefore, the higher risk groups include patients who have received blood transfusions, health services workers, drug addicts (percutaneous/parenteral transmission) [24], new-born children from infected mothers (vertical transmission) [25], homo or bisexual men and promiscuous heterosexual persons (sexual transmission) [26], a distribution pattern similar to that of AIDS but with a higher degree of contagiousness.

Indications of the anti-hepatitis B vaccination

Anti-hepatitis B vaccination is intended for the active immunization against infection by HBV and prevention of its potential consequences such as acute or chronic hepatitis, liver failure, cirrhosis and primary hepatocarcinoma. It is specially recommended for the following high-risk population groups:

Health workers in direct contact with patients (physicians, surgeons, dentists, nurses, hospital first-aid, ambulance and cleaning personnel, etc.). Morgue, funeral parlor and forensic services staffs.

Students in medical, dental and nursing schools and related technical schools in contact with patients.

People who work with blood and blood derivatives.

Travelers going to (or coming from) high-risk countries or regions.

Household contacts and sexual partners of infected persons.

Handicapped persons receiving social services; persons living in institutions and community homes, and the staff of these institutions.

Patients receiving or expected to receive blood transfusions, haemodyalisis or plasmapheresis such as those affected by oncological disorders, nephropathy or cirrhosis, among others.

New-born children of infected mothers or all new-born children in high- or medium-risk countries or regions.

Patients who will undergo elective surgery with sufficient time for seroconversion.

Receptors of transplanted organs.

Hemophiliacs and other systematic blood receivers.

Soldiers and other military personnel on active duty.

Prisoners, prison guards and other prison employees.

Persons at risk of sexual contamination (e.g., promiscuous persons, male homosexuals, prostitutes and venereal disease patients), parenteral drug and tattoing addicts.

Apparently healthy HBV infected persons constitute the main reservoirs of the virus in the population and for this reason universal undiscriminated vaccination campaigns may be required to stop the transmission of the infection.

The hepatic infection produced by the delta virus which is considered dependent on the co-infection with the HBV [27], is therefore expected to be prevented by this vaccine. Hepatitis A, parenteral non A non B hepatitis (i.e., hepatitis C), hepatitis E and other viral hepatic infections, however, will not be prevented.

Anti-hep B vaccines currently in use. A developing country experience.

In 1981, Cuba attained its first interferon (IFN) production at the Center for Biological Research (CIB) using leukocyte concentrates, purified and prepared for clinical applications. Later, alpha-2 type IFN was cloned and expressed in *E. coli* [28] and purified using monoclonal antibodies.

Once a certain amount of these IFNs became available and after running through the required pre-clinical and clinical tests, they began to be used successfully in apparently healthy HBV carriers, (i.e., between 85 and 90 percent of all infected persons, since the remaining 10 to 15% exhibit clinical symptoms [17]).

Due to the still persisting difficulties related to the replication of HBV in infected cell cultures or in animal models, the first effective anti-hep B vaccine was derived from HBsAg purified from plasma of asymptomatic or subclinical HBV carrier blood donors [1].

In 1983, a research team of the Institute of Basic and Preclinical Medical Sciences of Havana (ICBP) managed to purify the HBsAg from HBV carriers and a preparation was obtained that proved to be immunogenic in the animal models tested. They also developed an ELISA-type technique now widely used in the country by research teams and at blood banks [29].

During 1984 and 1985, a series of alternatives were explored to obtain the HBsAg through recombinant DNA techniques, using a range of host cells such as mammalian cells (CHO) [30], a vaccinia virus vector [31], transgenic mice [32] and several yeast strains. Although all these experiments concluded successfully, factors such as high levels of expression, feasibility of scaled-up production, product acceptability and the existence of other commercial vaccines of the same type, recommended the adoption of yeast as the most convenient acceptor cells [33].

Anti-hepatitis B vaccines derived from the virus present in the plasma of infected persons, are products of a technological generation which has been surpassed some years ago by the recombinant vaccines to overcome fears related to their source (blood derivative obtained from a cancerogenic infective virus), which might be infected with other virus such as HIV. The strict measures neccesary to keep these risks under control will always be subjected to eventual failures, human faults or mismanagements.

By the other hand, it must be taken into consideration that the population of blood donors which provide the virus for the preparation of the plasma vaccine coincide exactly with the population at risk for HIV and other infective agents. The process for the isolation of the plasma HBV, besides risky, is self-limiting on the long-term because it is used for the production of a vaccine intended for the erradication of its own source.

Background of the present rec-HBsAg vaccine. Initial genetic constructions

In 1986, the Food and Drug Administration of the United States of America (FDA) granted the first license to market a rec-HBsAg vaccine cloned and expressed in yeast, once it was demonstrated to meet all the necessary safety and effectiveness requirements [34]. This approval was the first of its kind granted to a recombinant DNA product to be used as a vaccine in human beings and is a milestone in the history of medical biotechnology.

In 1987, CIGB produced the first version of its own vaccine through the cloning of the HBV genome fragment coding for HBsAg under a high expression promotor in yeast. To produce this vaccine, a small group of researchers subcloned the TaqI-HpaI fragment containing the HBsAg gene taken from plasmids in which the complete adw2-type hepatitis B viral genome had been inserted, in the *E. Coli* pBR 322 plasmid at the EcoRI restriction site.

An intermediate construction was derived from the first through the insertion of the EcoRI restriction fragment in a second plasmid within a BamHI-BamHI expression cassette, between yeast GAP dehydrogenase 5' promotor (short GAP) and its 3'-end region.

The final construction at this stage was obtained through the introduction of the BamHI expression cassette, already containing the HBsAg gene, within a shuttle vector containing the 2-micron yeast plasmid fragment, ura3 and leu2 selection markers and the ampicillin-resistance gene. Several yeast strains were transformed with the selected clones, obtaining recombinant microorganisms with expression levels slightly under 1 percent of the total protein content.

HBsAg cloning and expression in yeast. Cell banks and their control

Genetic construction of the current production strain

An integration vector containing the HBsAg gene under the *P. pastoris* alcohol oxidase I enzyme gene promotor (pAOX1) control was constructed in several steps [35]. This plasmid also contained the non-codifying 5' and 3' homology regions of the AOX1 gene which are needed for homologous recombination with the yeast genome, the transcription termination signal (terminator) of the *S. cerevisiae* enzyme glyceraldehyde 3 phosphate dehydrogenase (GAP) gene and the *S. cerevisiae* his 3 gene used as marker for the recombinant yeast selection.

The cloning procedures were done according to Maniatis *et al.* [36].

Transformation of the P. pastoris host strain

The integration cassette used for the transformation of the host strain was located between the restriction sites ClaI and SalI of this plasmid and its size is 6 678 bp approximately, it did not include antibiotic resistance genes or other markers. Electroporation was used as the transformation method.

The *P. pastoris* yeast strain with the histidine synthetic pathway blocked [38] used for the transformation was obtained at CIGB by mutagenesis. Total DNA from the transformed his^+ colonies was extracted to perform hybridization analysis by Southern-blot [39] using the HBsAg gene as a probe. To demonstrate the presence of the HBsAg gene integrated to the transformed yeast genome, the polymerase chain reaction (PCR) according to Saiki *et al.* [37] was employed.

Table 1
Composition of the Cuban anti-hepatitis B rec-DNA viral surface antigen (rec-HBsAg) vaccine.

Description	Composition per dose 0.5 mL	Composition per dose 1 mL	Function	Standards
HBV rec-DNA surface antigen	10 μg	20 μg	Active antigenic ingredient ≥ 97% purity	WHO Standard
Aluminum hydroxide gel	0.9 mg	1.8 mg	Adjuvant	Quality according to specification
Thiomersal	0.025 mg	0.050 mg	Antimicro bial growth preserva tive	USP and BP
Sodium chloride	3.9 mg	7.9 mg	To maintain pH and/or ionic strength	USP and BP
Dibasic sodium phosphate mono-hydrate	0.63 mg	1.27 mg		
Monobasic sodium phosphate mono-hydrate	0.55 mg	1.10 mg		
Water for injection up to	0.5 mL	1.0 mL	Diluent	USP and BP

USP =United States Pharmacopoieia XXII BP =British Pharmacopoieia 88

Table 2
Summary of quality controls of the non-adsorbed active ingredient (active raw material)

Type of control	Acceptance limits	Method
Organoleptic characteristics	Clear, transparent, slightly yellowish liquid. No precipitate or particles in suspension.	Visual
Specific activity	≥90%	ELISA, Lowry
Contaminant nucleic acids	≤10 pg/20 μg dose	Dot/blot hybridization
Carbohydrates	≤3 μg/20 μg dose	Spectrophotometry
Mouse immunoglobulins	≤100 ppm	ELISA
Lipids	≤25 μg/20 μg dose	Spectrophotometry
Sterility	To comply	WHO 530 (973)
Thiocyanate ion	≤1 μg/20 μg dose	Spectrophotometry
Pyrogens	To comply	USP XXII
Contaminant yeast proteins	To pass the test	Western-blot
Purity (97%)	24 and 46 kDa (dimer) bands Characteristic elution pattern	SDS-PAGE (laser densitometry) Molecular exclusion HPLC
Subunits aggregation	Major particles size 22 nm	Electron microscopy

Cell banks and their control

Master and working seed banks (MSB and WSB) were constructed according to established procedures [40] and controlled by the assessment of the microbial purity and viability, conservation of the selection marker, stability studies by determination of the HBsAg gene by Southern-blot and PCR techniques in the Master Seed Bank, Working Seed Bank and after the fermentation processes selected for controls.

Production process

The production process [35] was designed taking into account the preservation of the characteristics of the antigen based on the laboratory approach outlined above. Its description and the quality assurance /control facilities and procedures currently in use, which have been worked out for several years to produce millions of doses of the vaccine under GMP and GLP standard conditions, can not be even briefly reviewed and should be the matter of an independent paper. Nevertheless, the main features of the active antigenic ingredient rec-HBsAg and

Table 3
Controls for releasing the final product (summary)

Type of control	Range of values		Determination method
	10 μg rec-HBsAg per dose of 0.5 mL	20 μg rec-HBsAg per dose of 1.0 mL	
Aspect	White-gray slightly opaque particle free suspension which separates in two phases		Visual
pH	6.4 - 7.4		pH meter
Volumen	To pass the test		USP XXII
Al^{3+} concentration	0.35 - 0.65 mg / 20 μg dose		Complexometric
Thiomersal content	0.03-0.07 mg / 20 μg dose		Spectrophoto-metric
Sterility	To pass the test		USP XXII
Innocuosness in mice and guinea pigs	To pass the test		BP 88
Potency in mice relative to standard	≥0.5		WHO procedure and international standard Series 1487 (1986)
Pyrogenicity in rabbits	To pass the test		USP XXII

Table 4.
Summary of concluded clinical studies

Investigation (reference)	Mean age	No. of cases	Route	Dose (μg)	Schedule (months)	SRC (%)	GMT (IU/L)	Side effects
Workers of the Center of Genetic Engineering and Biotechnology CIGB [43]	20-34	18	IM	20	0-1-2	100	231.6	**
		17		10		100	113.1	*
		13	SC	20		100	250.7	****
		13		10		100	353.9	***
		16	IM	SK20		87.5	27.1	*
		11	SC			90.9	31.6	**
Blood Bank Workers [43]	38	30	SC	20	0-1-2	100	402.9	***
		30		10		100	153.4	
		35		SK20		93.5	23.5	**
Gastroenter. Inst. workers (Galban & Bravo) [43]	37	19	IM	20	0-1-2	100	316.3	**
		19		10		94.7	89.6	*
		19		SK20		88.9	53.0	
Handicapped Children Hospital workers (Galban & Bravo) [43]	46	17	IM	20	0-1-2	100	524.4	**
		16		10		94.1	283.9	
		17		SK20		87.5	50.0	*
Navy Hosp.workers (Galban & Bravo) [43]	31	35	IM	20	0-1-2	97.4	134.1	***
		34		10		100	98.5	**
		34		SK20		96.2	30.4	*
Young Soldiers (Galban & Bravo) [43]	19	13	IM	20	0-1-2	100	497.5	**
		14		10		100	692.9	
		14		SK20		100	88.8	*
Nefrol. Inst. (Galban & Bravo) [43]	n.a.(adults)	6	IM	20	0-1-2	100	n.a.	**
		9		SK20		100		
Trop.Medic.Inst.(Galban & Bravo) [43]	38	15	IM	20	0-1-6	100	11883	***
		20		10		100	1912	**
		17		SK20		100	1468	***
Newborn Children(Galban & Bravo [43]	0	83	IM	10	0-1-2	96.4	441.7	*
		23		SK10		89.5	53.5	*
CIGB Workers 2nd study (Bravo et al.) [43]	20-34	55	IM	20	0-1-2	94.4	109.3	*
Medicine Students Venezuela (Torres,J.) [44]	23	126	IM	20	0-1-2	98	72[a]	*
			ID	2			36[a]	*

Workers of the Nat. Inst. of Health. Colombia(Juliao,O.)[45]	n.a (adults)	63	IM	20	0-1-2	100	51[a]	**
		59		SK20		87	11[a]	
		63		20	0-1-6	100	64[a]	
		59		SK20		96	48[aa]	
Russian Young Soldiers & Children(Pavlova,L.) [47]	18-21	73	IM	20	0-1-3	95	95[a]	*
	5-10	30		10	0-1-2	92.3	76[a]	
Young Soldiers Batch1014, Batch 1019 (Bravo & Herrera) [43]	19	20	IM	20	0-1-2	95	1014	**
		21				100	1321	
		16		SK20		93.3	721.4	
Handicaped Children(Diaz & Pedroso) [43]	11	47	IM	10	0-1-6	100	86.4[a]	*
		38		5		100	70.8[a]	
Old Persons Caring Center (Diaz & Pedroso) [43]	81	52	IM	20	0-1-6	100	149.7	*
Nursery (Diaz & Bravo) [43]	3	43	IM	10	0-1-6	100	12569	**
Primary School Children [43] (Diaz & Bravo)	9	51	IM	10	0-1-6	100	17498	**
Secondary School Children (Diaz & Bravo) [43]	12	49	IM	10	0-1-6	100	5366	**
Minimal dose Study Children	6-9	117	IM	2.5	0-1-6	100	364	*
				5		97.2	2052	
				10		100	2380	
HIV infected persons	n.a. (adults)	121	IM	20	0-1-2	77	34[a]	*
		17		40		41	14[a]	
African Students B+D coinfection outbreak	18	249	IM	20	0-1-2	99	98	n.a.

SRC = Seroconversion. GMT = Geometric mean of titers in international units per liter. IM, SC, ID = Intramuscular, subcutaneous, intradermal.
SK(10,20) = Commercial control vaccine 10 and 20 micrograms per dose. ****40% with symptoms. ***20-40% with symptoms.
**10-20% with symptoms *<10% with symptoms. n.a.: not available. [a]:Hyper-response percent (titers > 99.9 IU/liter)

the batch releasing criteria for the final product according to WHO requirements for the vaccine [41] are summarized in tables 1, 2 and 3.

Preclinical studies of the rec-HBsAg vaccine in animal models. HBV-challenged chimpanzee trial.

Safety and immunogenicity tests in chimpanzees

Protection trials against HBV infection of chimpanzees vaccinated with the rec-HBsAg vaccine were performed after inoculating 3 doses of 20 μg with one month intervals between them, using placebo (aluminum hydroxide) inoculated monkeys as controls. The challenge was done with $10^{3.5}$ infective chimpanzee doses (ICD) of the ayw subtype kindly provided by Dr. Huub Schellekens (TNO, The Primate Research Center. Rijwick, The Neetherlands), by intravenous route [42]

The main hepatic infection markers and routine histology and hematology were studied. Specific techniques for the detection of the HBV were applied to serum and hepatic tissue samples from the monkeys, which included ELISA test for detection of HBsAg, anti-HBsAg, HBeAg and anti-HBcAg and dot-blot nucleic acid hybridization as well as electron immunomicroscopy to detect the presence of the HBsAg.

The vaccinated monkeys produced immune responses against HBsAg after the first dose of the vaccine higher than the level observed afterwards in human volunteers. The presence of the HBV as evidenced by these markers was not detected in vaccinated chimpanzees while control monkeys developed the infection 6 weeks after the challenge [42] (figures 1, 2).

It was concluded that the rec-HBsAg vaccine prepared at CIGB is able to produce an adequate protective immune response in vaccinated chimpanzees against a challenge infection with HBV.

Clinical studies

Once accomplished satisfactorily the laboratory tests and the corresponding farmacobiological, toxicological and preclinical trials in the recomended animal models (i.e., chimpanzee monkeys), the vaccine was authorized for controlled clinical trials designed according to strict ethical and methodological guidelines and organized in three succesive phases, intended to demonstrate its reactogenicity, immunogenicity and effectiveness.

The protocols were applied to randomized representative and controlled population samples of the adequate size, according to the objective of the study and defined inclusion and exclusion criteria. Many of these studies were double blind and compared the Cuban vaccine at different doses and schedules against a similar known and stablished commercial vaccine (table 4). At least a similar and often a significantly better performance of the Cuban vs the commercial vaccine was demonstrated in most studies.

In Cuba the vaccine was evaluated by an Expert Committee stablished by the Ministry of Public Health for this purpose, which performed several studies during two years before delivering a final report reccomending its sanitary registration and its inclusion in the national vaccination system.

By the end of 1990 the vaccine was registered in Cuba [43] but the controlled studies continued in the country and abroad to accumulate information on its performance in different groups according to their professions, life styles, ethnical origin, cultural and socio-economical status. From 1992 a massive vaccination program started in Cuba, for the first time in America, including all newborn children and children up to one year old (more than 175 000).

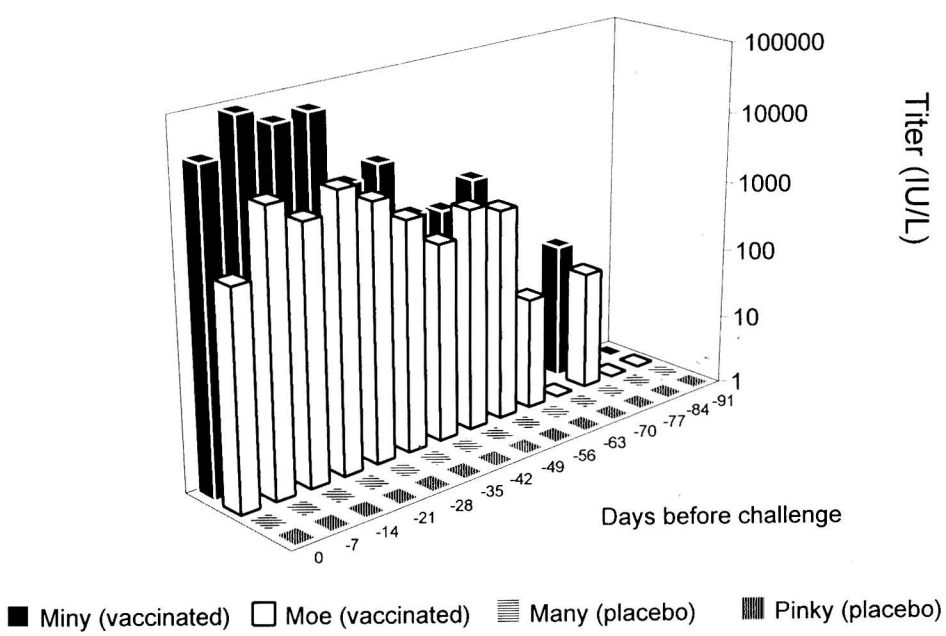

Fig. 1 Anti-HBsAg antibody titers in chimpanzees

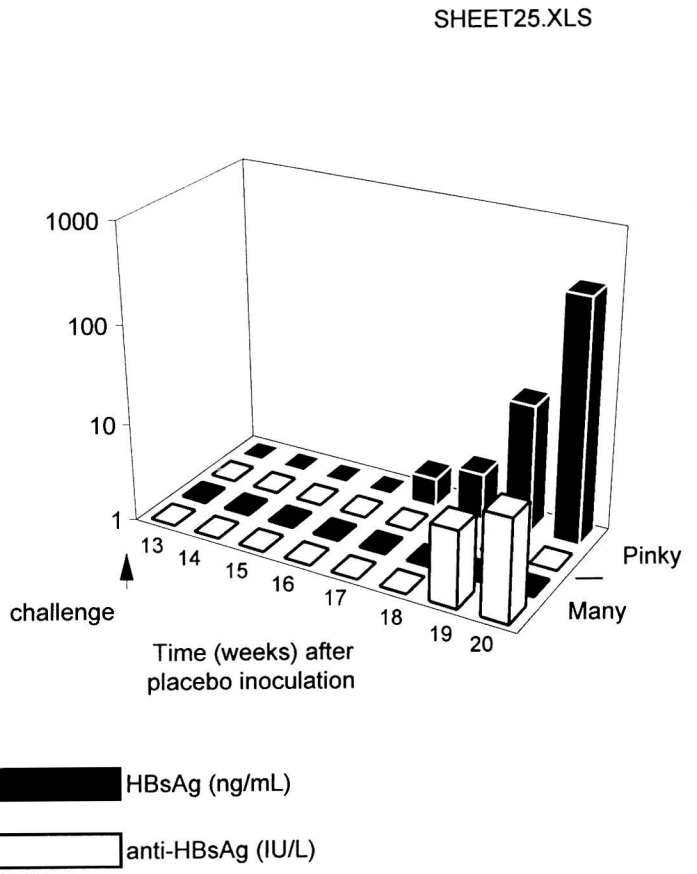

Fig. 2 HBV infection markers in non vaccinated chimpanzees

Studies in foreign countries which include Latin American such as Venezuela by Dr. Jaime Torres [44] of the Tropical Medicine Institute, Colombia by Dr. O. Juliao [45, 46] from the National Health Institute and others in course (Brazil, Perú, Bolivia), Eastern European countries such as Russia by Prof. Bektimirov [47] from the Tarasievich Institute, African students in Cuba and studies near to start in India and Iran, support the representativeness of the trials. More than 2 000 persons in several countries participated in the concluded studies and more than twice that quantity are included in the ongoing trials.

Moreover, the preparation was tested on children delivered by pregnant women carriers of the HBV to demonstrate the protection efficiency of the vaccine in this cohort.

Since May 1992 a national immunization program is carried out intended to control perinatal hepatitis B infection by vaccinating newborn children by HBV infected women. More than 1 000 children delivered from pregnant women infected with HBV were vaccinated using the the 0-1-2 +12 months schedule.

In June 1992 the program envisaged to control hep B infection of public health care workers and other risk groups. Workers of the nephrology services and patients submitted to haemodialysis, to peritoneal dialysis or suffering from renal failure were vaccinated. This schedule was spread to other health care workers depending on their level of risk.

In October 1992 the vaccination program was extended to cover yearly the whole Cuban population of newborn children, included in a nation wide immunization program. In this moment Cuba is the first country of America fulfilling the request of Manaos and Yaoundé resolution [48] to include anti-hepatitis B vaccination in the expanded program of immunizations. In connection with this, more than 600 000 vaccine doses have already been administered to newborn children.

It is remarkable that the Cuban recombinant vaccine against HBV was applied to control an outbreak of fulminant hepatitis B + D (delta) coinfection among 629 students from Guinea Bissau (a country with a large infection prevalence by hepatitis B virus) under fellowship grants in Cuba. After a vaccination carried out in April

1992, the sanitary authorities have not found new cases of symptomatic hepatitis B disease or detected new individuals positive for the HBsAg.

The Public Health Ministry of Colombia recently acquired more than two million doses of the Cuban vaccine, other large deliveries are under discussions with other countries, while small and medium size deliveries have been supplied to several countries

Strategy to increase the credibility of the product

The results of the clinical trials should be considered an overall assessment of all the work previously done from the production, physical-chemical and biological characterization of the immunogen through the production process of the vaccine, its batch-to-batch reproducibility and consistency up to the release of the final product, according to predefined international standards. The validation of these results is supported by the use of internationally recognized [41] laboratory procedures, materials and reagents, standards and control samples, as well as up-to-date techniques for collection, storage and data processing.

The adequate selection, experience and proficiency of the professional, technical, administrative and auxiliary staff dealing with the production, quality assurance and control, clinical trials and commercialization of the product are of utmost importance to guarantee a competitive quality.

The transparency of the production process and the quality assurance/control measures, the willingness to show the facilities for operation and control and the verifiable character of the producer's claims, including the laboratory tests and the field trials, contribute to increase the confidence of the users.

To struggle against prejudice and lack of tradition and image the producer encourage the field trials of the product with the participation (or in agreement with) local health authorities and specialists under the particular conditions of each country or region. For this purpose the product is supplied freely for the controlled studies agreed, provided that the protocols are officially approved and meet the internationally recognized ethical and methodological guidelines for "good clinical practices" regarding controlled field trials.

Another strategy directed towards increasing the knowledge, credibility and prestige of the vaccine suppose an open invitation to health authorities, specialists, opinion leaders and trade dealers aswell asto international healthorganizations officers to visit in any moment the production facilities and to receive the corresponding explanations and demonstrations.

CONCLUSIONS

Hundred thousands of peoples of different ages, sexes and sexual behaviours, geographic areas and nationalities, ethnical groups and occupations, life styles and risk of exposition, social and economical levels, received the Cuban rec-HBsAg vaccine, including controlled studies and vaccination campaigns in Cuba and abroad.

The Cuban rec-HBsAg vaccine, is commercially available since 1990, millions of doses have been distributed in several countries where it has been registered and some results on its performance in Latin-America and other countries started to be published in journals [43-47] or presented in congresses. Controlled trials and mass vaccination programs are ongoing in Cuba and other countries.

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