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Clinical Development of a New Inactivated Hepatitis A Vaccine

Introduction

The first identification of hepatitis A virus (HAV) in stool specimens occurred in 1973 [1]. Successful isolation and propagation of HAV in cell culture [2a, b] made the development of an HAV vaccine possible by 1979. Primate models permitted tests of immunization against HAV [3, 4], and both attenuated and inactivated vaccines were developed [5–8]. Various viral variants were used for live attenuated virus vaccines tested in animals [9–12] and humans [12, 13]. The safety of these attenuated HAV variants remain to be established in man. Advanced work has proceeded with inactivated vaccines derived from different viral strains: CR-326 [14, 15], GBM [16], HM-175 [17, 18], LSH/S [19], and RG-SB [20].

Inactivated hepatitis A vaccines are already licensed in many countries [21, 22], supported by extensive clinical experience. Efficacy trials of two inactivated hepatitis A vaccines in two prospective field experiments [23, 24] showed them to be protective. In addition, intervention during hepatitis A outbreaks has been successful [25].

This article reviews data on a new inactivated hepatitis A vaccine (AVAXIM®) developed by Pasteur Mérieux Sérums & Vaccins. The HAV strain used in the vaccine (GBM) was isolated in 1975 from patients during an outbreak in Gomaringen, Germany [26] by *B. Flehmig* at Tübingen University. The virus was isolated, adapted and propagated on a primary human kidney cell culture, followed by adaptation in human diploid fibroblast cells and human diploid cells (MRC5) [27–29]. Nucleotide sequencing of this cell culture-adapted virus showed some mutations [30, 31], correlating with attenuation when inoculated into chimpanzees [11]. Experimental GBM strain-derived vaccines tested in guinea pigs, goats, mice [32] and humans [16, 33, 34] were immunogenic and safe.

Materials and Methods

Preparation of the vaccine: Nine batches of AVAXIM® were produced in July 1991 for pre-clinical tests, phase I and II dose-range trials. After the final industrial process was defined, seven consecutive batches were produced for the remaining phase II and III trials. After formaldehyde inactivation, final products were prepared with purified HAV adjusted for antigen content according to previous results from the experimental vaccines, and according to results provided by an in-house ELISA assay in comparison with in-house reference materials, as there was no international reference antigen preparation available at that time. Different lots were prepared with HAV antigen doses ranging from 20 to 320 ELISA antigen units per dose. The antigen was adsorbed to aluminium hydroxide and 2-phenoxyethanol was added as preservative in a total injection volume of 0.5 ml diluted in aqueous medium 199. The volume injected in the phase I study was 1 ml.

Objectives of the clinical development: In addition to phase I (study 1), six clinical studies were conducted to answer one specific medical question each about the immunogenicity or safety of the vaccine: what is the optimal dose (study 2: randomized, dose-escalating trial on four different dosages), what is the best route of administration (study 3: randomized, controlled trial of intramuscular route, subcutaneous route and jet injection), how does its immunogenicity and safety compare with the only licensed vaccine available on the market at that time (HAVRIX®, 720 ELISA antigen unit dose, 0–1–6 month schedule, SmithKline Beecham) (study 4: European, randomized, controlled trial), how consistent is the product immunogenicity between batches (study 5: randomized trial of three lots), does immune globulin (IG) administration interfere with vaccination (study 6: randomized, controlled trial), and what is the product's safety in a large group of vaccinated subjects (study 7: non-controlled trial)?

Study populations and design: Subjects enrolled in studies were healthy adults, who had all given informed consent. All studies were conducted in accordance with European Good Clinical Practices and were approved by Ethics Committees. Both female and male subjects were included in studies except in the dose range study (study 2), where only male subjects were vaccinated, as it was known from unpublished results that males are lower responders to inactivated hepatitis A vaccines than females. The age range was from 16 to 63 years and the effect of sex and age on immune responses was analysed in study 4. All volunteers were screened for the presence of serum antibodies against HAV before enrollment. In studies 2, 4, 5 and 7, subjects were vaccinated regardless of serological status against HAV, and safety of the vaccine was analysed according to their HAV serological status at enrollment. Clinical reactogenicity was assessed using the same methods in all trials, using standardised check lists covering a 7 to 14-day period after each vaccination.

Serological tests: Vaccine-induced antibodies were determined by radioimmunoassay (RIA) (HAVAB, Abbott Laboratories) during the phase I trial using an unmodified technique (detection cut-off at 90 mIU/ml). In all subsequent studies a modified procedure (mRIA) was used, as previously described [35] for clinical trials of other inactivated hepatitis A vaccines. This modification increased test sensitivity (detection cut-off around 7 mIU/ml) and enabled the detection of anti-HAV as early as 2 weeks after vaccination. A titre of 20 mIU/ml was considered the threshold for seropositivity and as a protective level.

Randomly selected sera from subjects enrolled in the comparative study were tested for the presence of IgM and seroneutralizing antibody. A double sandwich ELISA was developed for IgM

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detection as follows. Microtitre plates were coated overnight at 5°C with monoclonal anti-HAV murine IgG (Clonatec Laboratory) diluted to 1/1,000 in PBS. After washing with 0.05% PBS-tween 20, the plates were allowed to saturate with 1% albumin for 1h30 min at 37°C. The plates were again washed and antigen (purified inactivated harvest or vaccine bulk) diluted to 1/10 in 0.05% PBS-tween 20 – 0.5% albumin was incubated for 2h at 37°C. After washing, serum samples diluted in 0.05% PBS-tween 20 were added in eight two-fold dilutions and the plates were incubated for 2h at 37°C. After washing, 100 µl of 1/5,000 diluted conjugate (human anti-IgM coupled to alkaline phosphatase, Immunotech Laboratory) were added and the plates were incubated for 1h 30 min at 37°C. After washing, phosphatase activity was measured by the addition of 100 µl of substrate (1 mg/ml paranitrophenylphosphate in diethanolamine buffer, pH 9.8). The colour was allowed to develop for 30 min at room temperature. The absorbances were read at 405 nm. Anti-HAV titres were calculated using the four-parameter method and expressed in arbitrary units by comparing them to the activity of a pool of sera collected at weeks 2 and 4 after vaccination. A titre above 15 mU/ml was considered positive.

A simple HAV antigen-reduction neutralization assay was developed for neutralizing antibodies on the basis of routine techniques used for a polio virus antibodies neutralizing assay [36] and for HAV [36–38]. Briefly, serial dilutions of sera (1/5 to 1/10,240) were incubated with 10^2 $TCID_{50}$ of HAV GBM strain (4h at 37°C and one night at 5°C). Samples were then incubated on MRC_5 cells in 96-well plates for 21 days at 37°C in a 5% CO_2 atmosphere. HAV measurement was done by ELISA on lysed cells (freezing/thawing) with appropriate controls. Neutralizing antibody titre was defined as the reciprocal of the highest serum dilution yielding an HAV growth inhibition in 50% of the wells. WHO reference globulin [39] was used as a positive control and its neutralization titre was from 1/74,000 to 1/280,000. Results were expressed in mIU/ml by comparison with this reference and by convention, titres below the detection cut-off (between 10 to 20 according to the series) were considered equal to 10 mIU/ml for GMC calculation.

Definition of the protective antibody level: For many years, protection against HAV disease depended on the use of IG [40, 41]. The lowest effective dose of IG is 0.02 ml per kg body weight (ml/kg), and the duration of protection is dose-related, ranging from 3 months with the 0.02 ml/kg-dose to 4 to 6 months with the 0.05 ml/kg-dose [42, 43]. Data from pre-exposure prophylaxis and post-exposure administration have shown an efficacy rate between 87% and 95%, depending on the dosage used and the length of follow-up. Whether IG prevents HAV infection or only limits the clinical manifestations of virus replication has not been clearly demonstrated [44].

The identity of serological correlates of protection is a point of considerable debate [45]. HAV antibody titres vary among IG batches and are decreasing in batches obtained from pooled serum donors in developed countries, leading to outbreaks of clinical cases despite IG treatment [46].

Efforts to detect the antibody levels obtained by IG treatment have used different techniques (ELISA, RIA, or neutralization assay) standardised against the WHO international reference preparation of IG [39]. In general, antibodies cannot be detected with a standard RIA (HAVAB, Abbott Laboratories) [47], but are detectable by a modified RIA (mRIA), ELISA or neutralization assay. Detectable antibodies are found as early as 5 days after IG administration and may remain for 2 months. Nevertheless, the level of HAV antibody that is protective remains unknown. As the low levels of antibody transferred by IG confer up to 90% protective efficacy, it would appear that the low serum antibody levels detected in IG recipients are sufficient for protection. In a study comparing inactivated hepatitis A vaccine with IG administration, anti-HAV geometric mean concentrations (GMC) measured by mRIA in subjects receiving only immunoglobulin were 96 mIU/ml on day 5, 58 mIU/ml at week 4, and less than 10 mIU/ml by week 20 [48]. Based on these mRIA data and in a conservative approach, the threshold for protection was established at 20 mIU/ml.

Results

Safety and Immunogenicity Validation

The phase I study of 20 HAV seronegative, healthy, young adult volunteers used a 0–1–2–6-month vaccination schedule with a 1 ml-vaccine containing 320 ELISA antigen units and 0.6 mg of aluminium. The antigen content was derived from clinical data with experimental vaccines previously tested in humans [16, 33, 34, 49]. The vaccination schedule tested hypersensitivity after repeated subcutaneous (s.c.) injections, a condition known to amplify local reactions.

Reactogenicity of the vaccine was similar to that of other adsorbed adult vaccines, such as hepatitis B and inactivated poliomyelitis vaccine. Local pain, redness, hematoma, or induration occurred after 22.5% of the injections, and malaise, fatigue, headache, insomnia, or gastro-intestinal tract disorders were reported after 22.5% of the injections. AST and ALT serum levels and complete blood cell

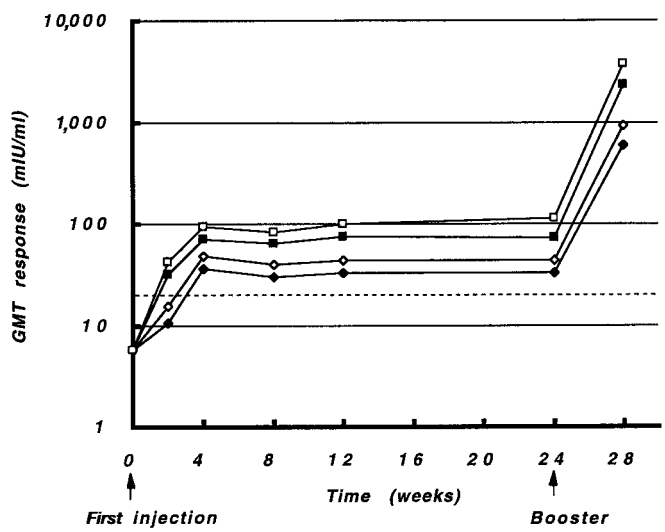

Figure 1: Geometric mean titer (GMT) responses (mIU/ml) by modified radioimmunoassay (mRIA) in volunteers vaccinated with one of four doses of AVAXIM® (Pasteur Mérieux Sérums & Vaccins) inactivated hepatitis A vaccine at weeks 0 and 24 (□ 160 ELISA antigen units, ■ 80 ELISA antigen units, ◇ 40 ELISA antigen units, ◆ 20 ELISA antigen units). The dotted line represents the 20 mIU/ml level.

counts performed before each vaccine administration and for the three days after it evidenced no changes in the parameters tested.

Immunogenicity results showed a 100% seroconversion rate at 1 month after the first dose, with a GMC of 337 mIU/ml. One month after the second dose, GMC was 1,538 mIU/ml, and it rose to 2,872 mIU/ml after the third dose. At the 6-month booster dose, GMC was 2,460 mIU/ml, and it increased to 8,131 mIU/ml 1 month later.

Dose-Range Effect

A range of doses were evaluated in 195 healthy, young, adult, male volunteers [50]. Based on the previous immunogenicity results, the vaccine was administered with a simplified 0-6-month vaccination schedule, and the antigen content reduced. Furthermore, the vaccine volume was reduced to 0.5 ml, and the amount of aluminium decreased to 0.3 mg per dose, to optimize the safety profile of the product. Doses ranging from 1/2 (160 ELISA antigen units) to 1/16 (20 ELISA antigen units) of the dose used in the phase I study were given intramuscularly (i.m.) in the deltoid region by two injections over a 6-month period.

Multivariate regression analysis showed a significant dose effect ($p < 0.0001$) on GMC achieved after the first injection (Figure 1). Two weeks after the first 160 ELISA antigen unit dose, 78.1% of the subjects seroconverted with a GMC of 43 mIU/ml. After 1 month, the seroconversion rate was 100%, with a GMC of 95 mIU/ml. Six months after the first injection, the GMC in the 160 ELISA antigen unit group was 113 mIU/ml and rose to 3,828 mIU/ml 4 weeks after the booster.

Reactogenicity of the vaccine was similar in the four dose groups: 6.1% of subjects reported immediate local or systemic reactions after vaccination (feeling sick, local pain, headache), 8.8% had reactions at the site of injection (spontaneous pain, hematoma, regional adenopathy) and 13.5% had systemic reactions ("flu-like" syndrome, gastrointestinal tract disorders, fatigue, headache). Following the booster dose, incidences of immediate, local, and systemic reactions were 1.6%, 4.7% and 4.7%, respectively. Mean serum levels of ALT and AST did not rise in the four groups 14 days after the first injection. Although six subjects developed abnormal AST or ALT levels after vaccination, the values remained under twice the upper limit of normal, and were not considered as indicators of liver toxicity.

Because of its high seroconversion rate within 2 weeks of the first dose, the 160 ELISA antigen unit per dose was chosen for clinical development as a vaccine with a 0-and-6-month schedule, and was used in further studies.

Overall Analysis of Immunogenicity

Table 1 lists results obtained from the five other studies. Of over 600 adult subjects vaccinated by the i.m. route and monitored for anti-HAV response, seroconversion rates at week 2 ranged from 78% to 98%, depending on the study. Nearly all subjects had seroconverted by week 4 to 8, and they remained protected at the week 24 booster injection. Maximum GMCs were seen as early as week 4 after the first dose, and no significant variation in antibody titres was observed between week 4 and week 24. In studies 4 and 5, male and older subjects, respectively, produced lower antibody titres, which confirmed previous observations with other inactivated hepatitis A vaccines [14, 51]. In a comparative study with the SB vaccine [52], a statistically significant difference ($p < 0.01$) in seroconversion rate and GMC was observed in favor of the Pasteur Mérieux Sérums & Vaccins vaccine at week 2 after the first injection, but there was no difference at week 8. Nevertheless, one must take into account that different vaccination schedules were used: one dose for primary immunisation with the Pasteur Mérieux vaccine compared to two doses with the SmithKline Beecham vaccine. However, since these results were obtained, a new formulation of the SmithKline Beecham vaccine has been licensed containing 1440 E.U.A., administered for primary immunisation with only one injection.

An anamnestic response to the booster dose of vaccine, given i.m. 6 months after the first, was found in all studies (Table 1), with at least a fifteen-fold increase of antibody titres 1 month after the booster (range, 15.0 to 33.8). Route of administration (study 3) made no difference in the booster effect. A similar anamnestic response occurred among all subjects, regardless of the initial levels of antibody at the time of booster. Absence of a specific IgM anti-HAV response to the booster also attested to the priming of the immune system by the first dose (Table 2).

Vaccination of HAV Seropositive Subjects

Subjects were recruited for studies 2, 4 and 5 without prior determination of serological status against HAV. Thus some received their first vaccine while being HAV seropositive. Baseline antibody titres in these subjects ranged from 2,000 to over 20,000 mIU/ml. Regardless of the level of antibodies, antibody titres increased slightly after vaccination, indicating that vaccination could further stimulate an immune response (Table 3).

Interference with IG

To compare the immunogenicity of the vaccine given alone or in combination with IG, 40 subjects received two doses of vaccine at week 0 and 24 together with 2 ml of IG given simultaneously with the first vaccine injection, and 40 subjects received vaccine alone (study 6). Whereas seroconversion rates 4 weeks after the first dose were 100% in both vaccinees receiving vaccine with IG and vaccine alone, GMCs were 307 mIU/ml in the vaccine with IG group compared to 589 mIU/ml in the vaccine alone group (Table 1). After the booster, GMCs increased about 13–15-fold in both groups, reaching highly protective levels (3,350 mIU/ml and 5,842 mIU/ml, respectively).

Table 1: Summary of immunogenicity of AVAXIM® (Pasteur Mérieux Sérums & Vaccins).

Study	Route	no.	Week 2		Week 4		Week 8		Week 24		Week 28	
			% positive*	GMT (mIU/ml)	% positive	GMT (mIU/ml)	% positive	GMT (mIU/ml)	% positive	GMT (mIU/ml)	% positive	GMT (mIU/ml)
Dose range (Study 2)	i. m.	41	78.1%	43	100%	95	100%	84	100%	113	100%	3828
Different administration routes (Study 3)	i. m.	46	nd**	nd	100%	210	nd	nd	97.8%	157	100%	3,152
	s. c.	46	nd	nd	97.5%	165	nd	nd	100%	152	100%	2,082
	Jet	46	nd	nd	100%	305	nd	nd	100%	251	100%	3,727
AVAXIM® Comparison with SB (Study 4)	i. m.	308	93.4%	59	nd	nd	99.3%	138	99.7%	279	100%	4,189
SB	i. m.	312	76.1%	30	nd	nd	100%	161	99.0%	244	100%	3,163
Batch consistency (Study 5)	i. m.	75	93.3%	73	nd	nd	nd	nd	100%	216	100%	5,062
	i. m.	72	91.7%	68	nd	nd	nd	nd	100%	176	100%	4,026
	i. m.	63	98.4%	82	nd	nd	nd	nd	100%	197	100%	5,636
	Total	210	94.3%	74	nd	nd	nd	nd	100%	195	100%	4,830
Interference with IG (Study 6) Vaccine + IG	i. m.	40	nd	nd	100%	307	100%	154	97.4%	220	100%	3,350
Vaccine only	i. m.	40	nd	nd	100%	589	100%	332	100%	446	100%	5,842

* > 20 mIU/ml; ** not done; SB = HAVRIX® vaccine (SmithKline Beecham); GMT = geometric mean titer.

Batch Consistency

Six batches of vaccine of the final formulation were used in five clinical studies in adults. In most studies, only one vaccine batch was tested, although more than one batch was included in two studies (studies 5 and 7). The anti-HAV responses in these five studies were similar in seroconversion rates after either the first or the booster vaccine doses, with some variability of GMCs between studies

Table 2: IgM and neutralizing antibodies after vaccination with AVAXIM® (Pasteur Mérieux Sérums & Vaccins) inactivated hepatitis A vaccine (160 ELISA antigen units given at weeks 0 and 24) or HAVRIX® (SmithKline Beecham) inactivated hepatitis A vaccine (720 ELISA antigen units given at weeks 0, 4 and 24).

		Week 0 (dose 1)		Week 2		Week 8 after dose 2		Week 24 (booster)		Week 28	
		P. M. sv.	SB	P. M. sv.	SB	P.M. sv.	SB	P. M. sv.	SB	P. M. sv.	SB
IgM	No.	30	29	30	29	30	29	30	29	30	29
	% ≥ 15mU/ml	0%	0%	100%	75.8%	100%	96.5%	23.3%	13.7%	0%	0%
	GMC (mU/ml)	5	5	227*	42	136	90	8	7	5	5
	Min.–Max.	5–13	4–5	17–1,886	5–339	26–1,144	5–1,876	5–34	5–20	5	5
Neutralizing antibodies	No.	60	60	60	60	60	60	60	60	60	60
	GMC (mIU/ml)	9	9	41*	14	67	54	125	83	3,552	2,309
	Min.–Max.	10–80	10–125	10–1,280	10–80	10–640	10–480	20–7,680	10–1,920	240–61,440	160–61,440

* statistically significant difference versus SB, $p < 0.01$, Student's t-test; P. M. sv. = AVAXIM®; SB = HAVRIX®; GMC = geometric mean concentration.

Table 3: Geometric mean concentrations after vaccination with AVAXIM® (Pasteur Mérieux Sérums & Vaccins) inactivated hepatitis A vaccine (160 ELISA antigen units) in initially HAV seropositive subjects.

Study		Week 0	Week 2	Week 8
2	No. = 9 GMC (mIU/ml) 95% CI	9,991 (2,731–36,539)	13,714 (3,437–54,725)	14,860 (3,221–68,555)
4	No. = 78 GMC (mIU/ml) 95% CI	16,656 (13,226–20,976)	25,612 (21,577–30,401)	44,406 (37,559–52,502)
5	No. = 24 GMC (mIU/ml) 95% CI	26,687 (17,751–40,121)	35,426 (25,471–49,272)	ND

ND = not done; GMC = geometric mean concentration.

(Table 1). This variation has been also observed with the SmithKline Beecham vaccine [17, 53]. Study 5 confirmed the consistent immunogenicity of three consecutive batches.

Route of Administration

Administration route can influence vaccine immunogenicity. For example, studies with the hepatitis B vaccine have shown that the s.c. route impairs immunogenicity, and that the intradermal (i.d.) route induces lower levels than the i.m. route [54]. During development of our vaccine, different modes of administration were used. In study 1 (phase I), the s.c. route was used to document vaccine safety under the worst conditions because adsorbed vaccines are often better tolerated by the i.m. route than the s.c. route. Immunogenicity results were good and it should be noted that the vaccine used during this trial contained 320 ELISA antigen units. When the final formulation (160 ELISA antigen units) was given subcutaneously (study 3), immunogenicity was quite satisfactory, although lower than by the i.m. route. This indicated that the s.c. route can be used, if necessary, but that the i.m. route is preferable. The jet injector (PM-3C™ Injector, Pasteur Mérieux Sérums & Vaccins) [55] used in study 3 showed better immunogenicity with this mode of administration (probably combining i.d., s.c., and i.m. routes) than with the i.m. route (Table 1). This confirmed previous observations with other Pasteur Mérieux Sérums & Vaccins vaccines [56] and the Merck hepatitis A vaccine [57] but not with the SmithKline Beecham vaccine given i.d. [58].

Analysis of Vaccine-Induced Response in Humans

In study 4 comparing the final adult formulation of the Pasteur Mérieux vaccine (160 ELISA antigen units) to the SmithKline Beecham vaccine (720 ELISA antigen units),

Table 4: Summary of reactogenicity to AVAXIM® (Pasteur Mérieux Sérums & Vaccins) inactivated hepatitis A vaccine (160 ELISA antigen units given at weeks 0 and 24). Compilation of studies 3, 4, 5, 6 and 7.

Type of reaction	% of subjects with reaction	
	After first dose (no. = 2,038)	After booster (no. = 1,880)
Local:	13.1	9.5
Pain	11.7	8.9
Redness > 3 cm	0.5	0.5
Hematoma > 3 cm	1.0	0.5
Other (pruritus, ...)	0.2	< 0.1
Systemic:	27.3	13.7
Feverish feeling	5.2	2.1
Fatigue	13.5	6.8
Headache	9.7	4.7
Myalgia/arthralgia	10.3	5.5
Gastrointestinal tract disorders	6.1	2.5
Other (dizziness, discomfort, ...)	0.8	0.2

IgM responses and neutralizing antibodies were evaluated in selected subjects. Two weeks after the first dose, the Pasteur Mérieux vaccine induced a greater IgM response that did the SmithKline Beecham vaccine (Table 3), as exemplified by a higher GMC of HAV IgM antibody ($p < 0.05$, Student's t-test) but this difference did not persist at week 8. Neutralizing antibodies were also detected for both vaccines within 2 weeks of the first dose, and neutralizing antibody titres were statistically higher ($p < 0.01$) with the Pasteur Mérieux vaccine (Table 2). Sensitivity and specificity of this test was confirmed by analysis of sera from immune non-vaccinated subjects and a good correlation ($r = 0.85$, $p < 0.001$, data unpublished) was shown between the Pasteur Mérieux vaccine's mRIA and this assay, confirming previous results obtained in other laboratories [59].

Safety

Over 3,000 subjects were vaccinated during the clinical development of the vaccine, and nearly 6,000 doses of vaccine were given. Reactogenicity was assessed using the same methodology in all trials, making it possible to consolidate the results. Reactogenicity in adults to whom the vaccine containing the final formulation was administered by the i.m. route was satisfactory and quite comparable to that to other aluminium salt-adsorbed vaccines (e.g., hepatitis B vaccine, inactivated poliomyelitis vaccine) (Table 4). Consistent reactogenicity was observed between different batches (studies 5 and 7). Local reactions were experienced by 13.1% of vaccinees after the first dose and by 9.5% after the booster dose. As expected, pain was the most common symptom, and a nodule at injection site was rarely observed (less than 0.1%). Regional lymphadenopathy (axillary) was observed after 2.7% of the vaccinations (data compiled from studies 3, 5 and 7), but this sign is difficult to interpret because the baseline prevalence of axillary adenopathy was 7.6% (3,890 injections) on the day of vaccination. General symptoms, such as fatigue, fever, headache, myalgia/arthralgia, or gastrointestinal tract disorders, were reported in 27.3% of vaccinees after the first dose, and in 13.7% after the booster dose. Fatigue was the most common symptom (13.5% after the first dose and 6.8% after the booster dose). In study 4, the Pasteur Mérieux vaccine was compared to SmithKline Beecham vaccine, and no differences in reaction rates were found. In all studies, fewer reactions were reported for the booster dose compared to the first dose, and a statistically significant decrease was observed in study 7 when the data were analysed by logistic regression. Subjects were recruited for studies 2, 4, 5 and 7 regardless of their HAV serological status. As expected, some subjects already seropositive against the hepatitis A virus were vaccinated. Reaction rates were comparable between the initially seronegative and initially seropositive groups. No statistically significant differences were observed in study 7, where data were analysed by logistic regression. Local and systemic reactions after the first dose were observed in 10.3% versus 9.9% and in 26.2% versus 23.8% of initially HAV seronegative and seropositive vaccinees, respectively.

In the complete clinical experience, twelve adverse events were reported to be vaccine-related by the investigators. Causality analysis was based on chronological (onset of the reaction, outcome after the second administration) and clinical criteria. Ten adverse reactions (cutaneous rash, regional lymphadenopathy, headache, vascular purpura, zoster, hepatic enzyme increase) were identified for nearly 6,000 doses administered (17/10,000).

Discussion

These studies have consistently demonstrated the immunogenicity of the Pasteur Mérieux Sérums & Vaccins inactivated hepatitis A vaccine. The lower immunogenicity observed in the dose-range study (study 2) was probably attributable to the fact that the final manufacturing processes and release tests were not fully defined (the later batches were formulated using the final manufacturing process). In the other studies, over 90% of the subjects were immune within 14 days of the first dose, as defined by mRIA titres greater than 20 mIU/ml. One month after primary injection, 100% of subjects achieved a protective level.

Two serological assays (commercial or in-house ELISA versus RIA) have been used to measure vaccine-induced antibody response to inactivated hepatitis A vaccines [8, 60, 61]. These provide different results [62], even though there are correlations between the two techniques [63]. The RIA method uses enzyme-labelled hepatitis A antibodies supplied with the kit that compete with sample antibodies for the HAV antigen binding site, so the affinity of sample antibodies strongly influences the final result. As antibodies produced early after antigenic stimulation are generally of lower affinity than those produced later, titres measured by RIA soon after vaccination are lower than those measured by ELISA, which is less influenced by antibody affinity. This could explain differences in GMC observed between studies that use the same vaccine in the same type of population [48]. The SmithKline Beecham vaccine studies generally used an in-house ELISA method [51, 64–67]. Merck vaccine studies [68–70] used mRIA (HAVAB, Abbott) [35], and the Berna vaccine studies [62, 71–74] used an automated microparticle commercial enzyme immunoassay test (IMX HAVAB, Abbott) or commercial ELISA (ENZYMUN, Boehringer). Moreover, the minimum titre taken to indicate seropositivity (10 or 20 mIU/ml) obviously influences seroconversion rates [75]. Thus, anti-HAV titres depend on the antibody test system used [62], documenting the necessity of using the same assay during clinical trials.

The mRIA assay (HAVAB) has been validated through studies of IG-induced [48] or vaccine-induced protection [35, 48, 69]. Conventional RIA was not sensitive enough to detect low titre of IG-acquired HAV antibodies [45]. By contrast, the modified assay we used detects antibodies in

the serum after IG administration, and was used to measure protective antibodies in vaccinees. By testing the same sera in both Pasteur Mérieux Sérums & Vaccins and Merck laboratories, correlation between mRIA assays was validated (correlation coefficient obtained from 135 paired sera was 0.95, unpublished data). Similar correlation was observed when the Boehringer commercial ELISA used in some vaccine trials [62] was compared to the Pasteur Mérieux vaccine's mRIA (correlation coefficient obtained from 99 paired sera was 0.94, unpublished data). The comparative study we performed was the first comparison between two inactivated hepatitis A vaccines, although preliminary data have been discussed [76, 77]. Results should be analysed by taking into account the fact that vaccines were administered by different schedules. Another difficulty comes from the fact that the antigen content in the vaccines cannot be directly compared as the ELISA assays used for vaccine antigen titration are not standardized and use different reagents. As there was no international reference HAV antigen preparation available and as no direct antigenic titration could be performed on the final vaccines due to antigen adsorption onto aluminium, the clinical trial was the only way to document the relative immunogenicity of the two vaccines.

Data have been collected on the IgM response to inactivated hepatitis A vaccination [14, 51, 68, 69, 78]. In all studies, except one (where IgM were undetectable in parotid fluid and saliva [78]), IgM antibodies were found in a high proportion of vaccinees, and they peaked between 2 and 4 weeks after the first injection. Results with the Pasteur Mérieux vaccine confirmed these observations with a peak IgM response between week 2 and 4 after the first dose.

The effect of concomitant administration of IG on the immunogenicity of inactivated hepatitis A vaccine has been previously studied. A two-fold reduction in GMC was observed after simultaneous active and passive immunization, as compared to active immunization alone [48, 62, 79–81] and this was confirmed with the Pasteur Mérieux vaccine. The decrease in antibody response was observed whatever the assay used (in-house ELISA, commercial ELISA or mRIA); seroconversion rates, however, were not affected. Therefore, inhibition of antibody production does not appear to affect the overall protection afforded by the vaccine.

A duration of protection lasting 10 years after booster vaccination has been estimated for the SmithKline Beecham vaccine [82]. GMCs observed 2 and 3 years after initial tests of the first vaccination with this vaccine [83–85] are congruent with this estimation. Study 4 showed that antibody levels induced by the Pasteur Mérieux vaccine after booster injection were at least equal to those achieved with the SmithKline Beecham vaccine, implying that a similar long-term protection can be expected. Nevertheless, this estimate must be confirmed by appropriately designed studies with a yearly evaluation of antibody titres after the booster vaccination.

Two efficacy trials of inactivated hepatitis A vaccine have been made [23, 24]. The first used the SmithKline Beecham vaccine in Thailand in a double-blind, placebo-controlled, cross-over, randomized design. Over 40,000 children (1–16 years of age) received either the Smith Kline Beecham vaccine (360 ELISA antigen units/dose, 0-1-12-month schedule) or Engerix B. The primary endpoint was occurrence of hepatitis A (clinical symptoms, ALT > 45U/L, and IgM against HAV). During the follow-up period (days 138 to 532), there were 40 reported cases of hepatitis A, of which 38 were in the control group. Observed protective efficacy was 95% (95% confidence interval, 82% to 99%). Levels of HAV antibody after vaccination were determined by in-house ELISA in over 400 randomly selected children who lacked antibody at entry to the study. Seroconversion rates (titre > 20 mIU/ml) at 8, 12 and 17 months after the first vaccine dose were 94%, 94% and 99%, respectively. The GMCs in these children declined slightly between 8 and 12 months to about 200 mIU/ml, and rose to over 2,000 mIU/ml 5 months after the booster dose.

The second study used the Merck product and was conducted in the USA as a double-blind, placebo-controlled, randomized trial. Among 1,037 seronegative children (2 to 16 years of age), 519 received the Merck vaccine and 518 the placebo. The primary endpoint was the occurrence of hepatitis A (clinical symptoms, ALT > twice the upper normal limit, and IgM against HAV). During the follow-up period (days 50 to 137), there were 25 cases of hepatitis A, all in the control group. Observed protective efficacy was 100%, with a 95% confidence interval of 87.3% to 100%. HAV antibody levels after vaccination in randomly selected children were determined by mRIA [35]. The seroconversion rate (titre > 10 mIU/ml) 1 month after vaccination was 99.6% (304/305), and GMC was 42 mIU/ml.

No efficacy trial has been conducted with the Pasteur Mérieux vaccine. However, there are several arguments in favor of its protective efficacy. The Pasteur Mérieux product derives from the GBM hepatitis A virus strain. Although it differs from the strains used in the other vaccines, no antigenic variation between wild or cell-culture adapted HAV strains has been demonstrated [86–88]. At least three inactivated hepatitis A vaccines are produced on the same MRC5 cell line, purified using similar techniques, formaldehyde-inactivated, and adsorbed onto aluminium hydroxide. The mRIA antibody levels observed after vaccination with the Pasteur Mérieux vaccine are better than those observed after IG administration, which has been shown to be protective [89, 90]. The mRIA antibody levels produced by vaccination with the Pasteur Mérieux vaccine are equal to those observed after vaccination with the SmithKline Beecham vaccine (study 4) or the Merck vaccine [24]. The anti-HAV GMC, measured by mRIA, seen at week 4 after vaccination with the Merck inactivated hepatitis A vaccine was 42 mIU/ml [24]. This level is comparable to that observed with the Pasteur Mérieux vaccine (Table 1). Two weeks after the first vaccination, biological activity (neutralizing antibodies) de-

tected in sera from subjects who received the Pasteur Mérieux vaccine was higher than that observed in SmithKline Beecham vaccinees.

The safety profile of this vaccine was consistent with other adsorbed vaccines used in adults (e.g., hepatitis B, inactivated poliomyelitis vaccine). In clinical trials where injections were given i.m. in the deltoid region, adverse reactions were mostly mild, confined to the first few days after vaccination, and followed by uneventful recovery. The most common local reaction was mild pain at the injection site, occasionally associated with redness. General symptoms, such as mild fever, fatigue, headache, myalgia/arthralgia, or gastrointestinal tract disorders, were also reported. Reactions were less frequently reported after the booster dose than after the first dose. The Pasteur Mérieux vaccine was as well tolerated by HAV seropositive subjects as by seronegative ones. The rate of clinically significant adverse reactions was similar to the rate of 13 adverse reactions for 10,000 vaccinations described in a UK report of post-marketing safety surveillance of the SmithKline Beecham vaccine [91] which included one probable post-vaccination encephalopathy [92].

Groups recommended for active immunization against HAV disease include travellers from developed countries visiting endemic areas [93, 94] and military personnel [95, 96]. Vaccination is more controversial for other groups presumed to be at risk, such as children [97]. Travel in endemic areas can be an indication for vaccination in children [98], but the situation of children who are institutionalized or who attend day-care centers is more difficult to analyse. Such children are at high risk for HAV infection [99–102], and the cost/benefit ratio associated with prevention in this population probably justifies vaccination. Whether or not health care workers are at high risk for HAV infection is debated [103–105]. Working in pediatric units or in day-care centers carries a possible risk [106–108], and health care workers have been identified as HAV vectors [109] and the principal source of clinical cases during outbreaks in health-care units [99, 100, 110]. Hepatitis A represents an occupational hazard for sewage workers according to some studies, suggesting that this population is also at risk [111–116], but negative data have also been published [117–119]. Vaccination of food handlers in combination with administration of IG has been proposed to interrupt transmission during outbreaks when this population is implicated [120, 121]. Only a small proportion of the reported cases of hepatitis A have been related to foodborne outbreaks [122–124], and the cost/benefit ratio of vaccination in food handlers versus implementation of hygienic practices in handling food had not been compared. Finally, it has been proposed that certain groups such as homosexual men [125, 126], primate handlers and laboratory workers should be vaccinated [127, 128]. Parenteral HAV infection after blood transfusion [110] or treatment with factor VIII [129–134] has been described in hemophiliacs. HAV contamination of purified factor VIII concentrate by the plasma or water used in production has been suspected in such outbreaks [135, 136], but not confirmed [137, 138]. In many European countries, hemophiliacs are routinely vaccinated against hepatitis A [139].

The prevalence of HAV antibodies varies among the general population according to geographical area and age group, so it has been suggested that individuals be screened before vaccination [140–143]. Screening before hepatitis A vaccination could make it more cost-effective [144–147]. A saliva test that can detect antibodies [148–150] should reinforce the strategy of pre-exposure vaccination, supplemented by vaccination of susceptible subjects during outbreaks [74].

Apart from the vaccination schedule, in which the first booster injection can be given between 6 and 12 months after the primary injection [17], published studies suggest other factors that effect vaccine immunogenicity. Females usually respond with higher GMC of HAV antibodies than males (unpublished data, Merck and SmithKline Beecham) which is reminiscent of the experience with hepatitis B vaccine. It has also been suggested that older or overweight subjects are poor vaccine responders [14, 51]. Human immunodeficiency virus-infected people respond less well than non-infected persons [151, 152].

In conclusion, the public health impact of hepatitis A vaccination seems promising [153] and will become evident with the growing use of available vaccines such as this new inactivated hepatitis A vaccine (AVAXIM®).

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