

Characterization of formaldehyde-inactivated poliovirus preparations made from live-attenuated strains

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Formaldehyde-inactivated virus samples from type 1 poliovirus live-attenuated strains were prepared in the laboratory. The effect of treatment with formaldehyde on virus infectivity and immunogenicity in mice was investigated and the results compared with those from Mahoney wild-type poliovirus strain, the common type 1 component in commercial inactivated polio vaccines (IPV). Differences in the potency and specificity between these experimental vaccines were identified in both normal mice and transgenic mice expressing the human poliovirus receptor. The possible advantages/disadvantages of using live-attenuated strains for IPV production are discussed in the context of the global polio eradication initiative. A novel transgenic mouse model to study *in vivo* the immune protection induced by IPV preparations is described.

INTRODUCTION

The use of oral live-attenuated (OPV) and formaldehyde-inactivated (IPV) poliovirus vaccines has dramatically reduced the incidence of paralytic poliomyelitis in the last 30 years (Dowdle *et al.*, 1999). The enormous success of the Program for Global Eradication of Polio inspired by WHO indicates that eradication of the disease and wild-type virus circulation could be possible in the near future. These new circumstances open a debate about strategies on how and when to stop polio vaccination once eradication has been achieved and highlight safety concerns regarding laboratory work with poliovirus and the safe containment of all wild polioviruses and potentially infectious materials (Dowdle *et al.*, 2002).

Live-attenuated strains developed by Sabin replicate in the human gut and give rise to viral strains of increased neurovirulence that on very rare occasions, ~1 case per 2·5 million doses, cause vaccine-associated paralytic poliomyelitis (VAPP) (Melnick, 1994; Strebel *et al.*, 1992). However, it has recently become clear that, in populations with low vaccine coverage and poor surveillance, vaccine-derived strains can 'silently' circulate for long periods of time, leading to poliomyelitis outbreaks. Sabin-derived type 2 strains circulated in Egypt during 1982 to 1993 and were responsible for at least 32 poliomyelitis cases (Centers for Disease Control and Prevention, 2001). More recently, two unrelated outbreaks of type 1 poliomyelitis in the island of Hispaniola (Kew *et al.*, 2002) and in the Philippines (Anon., 2001) and an outbreak of type 2 poliomyelitis in Madagascar

(F. Delpeyroux, personal communication) have been linked to circulating vaccine-derived poliovirus (cVDPV) strains.

Inactivated poliovaccines (IPV), on the other hand, are 'killed' vaccines in which viral infectivity has been disabled by treatment of purified virus preparations with formaldehyde (Salk, 1994). Consequently, the use of IPV does not involve the risks of VAPP or cVDPV outbreaks. Incubation with formaldehyde partially modifies the antigenic structure of poliovirus (Ferguson *et al.*, 1993) but inactivated vaccines have been shown to protect efficiently against the disease and have been the only vaccine used to control and eliminate poliomyelitis in several countries (Murdin *et al.*, 1996). With global eradication of polio in prospect, IPV could therefore play an increasingly important role during the next few years, which may include its use for routine immunizations in countries that still use OPV but where wild polio eradication has long been completed and/or the global use of IPV for some interim period after global eradication is achieved and vaccination with live-attenuated strains interrupted (Hovi, 2001). However, since IPV was first developed by Salk in the 1950s, wild-type pathogenic poliovirus strains have been used for IPV production; most commonly Mahoney, MEF-I and Saukett strains of types 1, 2 and 3, respectively. In the context of a polio-free world and in the light of the above remarks it would be more appropriate to produce IPV from live-attenuated strains instead. Scientific considerations such as the effect of formaldehyde inactivation on the antigenic and immunogenic properties of prospective candidate strains as well as economic matters such as the cost-effectiveness of large-scale vaccine production as compared to the current IPV production have to be carefully assessed.

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For this study, we prepared small-scale samples of formaldehyde-inactivated type 1 poliovirus using three different live-attenuated strains, Sabin 1, CHAT and Cox. All three strains are genetically related, all independently derived from the wild-type parental poliovirus Mahoney strain by successive passages in various *in vitro* and *in vivo* cell substrates (Martin & Minor, 2002). Sabin 1, CHAT and Cox strains were selected, on the basis of their lack of neurovirulence in monkeys, to be used as vaccines in humans during the 1950s. The Sabin 1 strain, together with Sabin attenuated versions of poliovirus serotypes 2 and 3, was eventually selected for licensing and has been used almost universally since then. The process of virus inactivation with formaldehyde and its effect on the immunogenicity in mice of the three type 1 viral preparations were analysed and the results compared with those obtained with equivalent preparations of inactivated Mahoney virus, which is the common type 1 strain used for IPV production.

METHODS

Cells and viruses. HEp-2C cells were used in the different assays shown in this paper and were grown in culture as described before (Minor, 1980). Poliovirus type 1 strains Mahoney, Sabin 1, CHAT and Cox were used. The Cox strain (Pool 7-1231-114), also named Lederle SM strain, was developed at the Viral and Rickettsia Research, Lederle Laboratory Division, American Cyanamid Comp., Pearl River, New York (Cabasso *et al.*, 1959). The CHAT vaccine was originally prepared by Hilary Koprowski at the Wistar Institute of Anatomy and Biology, Philadelphia, USA, from a precursor of the Cox strain, the SM N-90 strain (Koprowski, 1958). Sabin 1 and Mahoney virus stocks were derived from full-length cDNA clones available in the laboratory (McGoldrick *et al.*, 1995).

Virus purification. Virus stocks of Mahoney, CHAT, Cox and Sabin 1 strains were prepared by infecting HEp-2c cells at high m.o.i. (> 10 p.f.u. ml^{-1}) at 35 °C in MEM without foetal calf serum; 1–2 days later supernatants were collected and virus purified by ultracentrifugation through 15–45% sucrose gradients as previously described (Minor, 1980). Fractions containing the virus were pooled and concentrated by ultracentrifugation. The virus concentrates were then resuspended in 1–2 ml of Inactivation Medium (Pasteur Merieux propriety product; formula undisclosed) and stored at –70 °C until use. The protein concentration in purified viral preparations was determined using the Bio-Rad protein assay kit. The viral samples were analysed by SDS-PAGE followed by Coomassie blue staining. The virus content was determined by titration of virus infectious particles (plaque-forming units) using standard plaque assays in HEp-2c cells.

Neutralization of poliovirus strains with monoclonal antibodies. A standard microneutralization assay was employed (Minor *et al.*, 1986). One-hundred 50% cell culture infectious doses (CCID₅₀) of virus per well were used in the test. Viruses were incubated with Sabin 1-specific monoclonal antibodies (diluted at 1:50) corresponding to antigenic sites 1 to 4 for 4 h at 35 °C in 96-well plates; 10⁵ HEp-2c cells were then added to each well and 5 days later cell survival was monitored by staining the plates with buffered medium containing 0.1% Naphthalene Black.

Virus inactivation with formaldehyde. Sucrose gradient-purified virus preparations were used for these experiments. The virus preparations were resuspended in Inactivation Medium (Pasteur Merieux) to a final concentration of 10⁹ p.f.u. ml^{-1} (~7.5 µg viral

protein ml^{-1}). Prior to formaldehyde inactivation, virus pools were filtered through a 0.2 µm filter (Schleicher & Schuell) to remove viral aggregates and facilitate formaldehyde access to all virus particles. Formaldehyde (37/40% formaldehyde; Fisher Scientific) was added to the purified virus solutions to give a final formaldehyde dilution of 1/4000 of the concentrated stock. Inactivation was carried out for 12 days at 37 °C in a constant-temperature water-bath. Viruses were again filtered through a 0.2-µm filter at day 6. Aliquots (50 µl) were taken at regular intervals and free formaldehyde in the samples was neutralized by addition of 1 vol. of a 1:8 dilution of a 35% (w/v) aqueous solution of sodium bisulfite (Sigma) to 100 vols of sample. The presence of infectious virus in inactivated samples and sequential aliquots was measured by plaque assays in HEp-2c cells and by addition and passage of treated virus samples on HEp-2c cell monolayers for periods of up to 3 weeks.

Poliovirus RNA extraction and RT-PCR reactions. Poliovirus RNA was purified from live or inactivated samples and used for reverse transcription (RT) and PCR following standard procedures. Synthetic oligonucleotides PCR_F (nucleotides 29–41 of the poliovirus genome, 5'-CCAGAGGCCACGTTGGCGGCTAG-3') and PCR_R (nucleotides 784–800, 5'-GAGCGCTACTTTTGGGATGATAC-3') were used as primers. Nucleotide numbering is as in Toyoda *et al.* (1984). The limit of detection of poliovirus RNA in our RT-PCR assay is that equivalent to the viral RNA content in 10² p.f.u. of live virus.

Transfection of poliovirus RNA. Infectious virus was recovered by transfection of HEp-2c cells in culture using the DEAE-dextran method: 80% confluent HEp-2c cells grown in six-well plates were washed twice with PBS and incubated with 1 ml of pre-transfection mix containing 0.1 mg gelatin (Sigma) ml^{-1} , 0.5% DMSO (Sigma) and 300 µg DEAE-dextran (Pharmacia) ml^{-1} in PBS for 25 min at room temperature. Purified poliovirus RNA (250 µl) in PBS with 0.1 mg gelatin ml^{-1} was then added to the cells and incubated for 1 h at room temperature. After this, the inoculum was removed and 2 ml of MEM with 2% foetal calf serum was added to each well. Cells were monitored for cytopathic effect for up to 5 days.

Immunization of mice with inactivated virus samples. Female, 5–7-week-old ICR mice were immunized by the intraperitoneal route with 8, 40 or 200 ng of native or inactivated virus solutions, which corresponded to 0.02, 0.1 or 0.5 equivalents, respectively, of the regular human dose of 40 D-antigen units as estimated by an ELISA method specifically designed for the detection of poliovirus antigen in inactivated poliovaccines (Singer *et al.*, 1989). The samples were dissolved in 0.5 mg aluminium hydroxide (Reheis, Ireland) ml^{-1} buffered in normal physiological saline. Nine mice were inoculated with each virus dose in groups of three mice per dose. Sera from each group of three mice were pooled for analysis. Each immunization involved two inoculations given 2 weeks apart. The mice were bled out at day 28.

Immunization/challenge experiments in transgenic mice. Tg21-Bx transgenic mice (Martin & Minor, 2002) expressing the human poliovirus receptor and therefore susceptible to polio infection were used for these experiments. Mice, 6–8-week-old of both sexes, were immunized by the intraperitoneal route with the equivalent of 0.25 human vaccine doses of the inactivated samples and, after a boost at day 14, challenged with paralyzing doses of live poliovirus at day 28. Ten mice per inactivated virus sample were immunized. One-hundred 50% paralytic doses (PD₅₀) of live poliovirus were used to challenge the immunized mice. The mice were then monitored for any sign of paralysis for 14 days. A trivalent commercial IPV preparation (NIBSC Ref. 90/716) was used in preliminary experiments to validate the transgenic mouse model.

Titration of mice sera for poliovirus-neutralizing antibodies. Sera from immunized mice were tested for the presence of

antibodies against type 1 poliovirus by the established neutralization test recommended by WHO (1997). Serial twofold dilutions of serum samples were mixed with 100 CCID₅₀ of each of the four different type 1 poliovirus strains and the neutralization antibody titre was considered to be that of the highest dilution of serum that protected 50% of the cultures. Antibody titres were expressed as reciprocals of that dilution in log₂ values.

RESULTS

Antigenic structure of type 1 poliovirus strains

The antigenic structures of the four type 1 poliovirus strains were analysed by studying their reactivity with a panel of Sabin 1 monoclonal antibodies of known specificity (Minor, 1990) in a microneutralization assay. As shown in Table 1, neither Mahoney, CHAT or Cox virus strains were

Table 1. Reactivity of CHAT and Cox strains with anti-Sabin 1 monoclonal antibodies

+, Neutralization; -, no neutralization.

Antigenic site	Antibody	Reactivity with strain:				
		Sabin 1	CHAT	Cox	Mahoney	Sabin 2
Site 1	955	+	-	-	-	-
Site 2	237	+	+	+	+	-
	428	+	+	+	+	-
	432	+	+	+	+	-
	426	+	+	+	+	-
	429	+	+	+	+	-
	430	+	-	+	+	-
	431	+	-	+	+	-
	427	+	+	+	+	-
Site 3	423	+	-	-	-	-
	424	+	-	-	-	-
Site 4	234	+	+	+	+	-
	236	+	+	+	+	-
	1585	+	+	+	+	-

neutralized by antibodies against sites 1 and 3, whereas CHAT strain alone did not react against antibodies 430 and 431 specific for antigenic site 2a. All strains reacted with site 4 monoclonal antibodies. The results were consistent with the known amino acid changes at the predefined antigenic sites summarized in Table 2.

Inactivation of virus samples with formaldehyde

Typically, infection of 10⁸ HEp-2c cells generated 10¹⁰ p.f.u. for Mahoney, CHAT and Cox strains. In repeated experiments we consistently found that, in the same conditions, Sabin 1 generated 2–3 times less virus than cultures infected with any of the other three strains. However, the ratio between protein and infectivity was maintained for Sabin 1. A decrease in virus yields correlated with a proportional decrease in protein content. Viral solutions containing 10⁹ p.f.u. ml⁻¹ (~7.5 µg viral protein ml⁻¹) of each of the purified virus strains were inactivated by incubation with formaldehyde at 37 °C as described in Methods. The destruction of viral infectivity during the inactivation process was monitored by determining the virus titre in aliquots taken at different times of incubation with formaldehyde. As illustrated in Fig. 1(A), all four strains showed similar kinetics of inactivation. In all cases, no infectious particles were detected at or after 60 h of incubation with formaldehyde as judged by the plaque assay method. 25 µl aliquots of the virus solutions were used for these assays, so a 0% virus titre value actually means that at least 2.5 × 10⁷ p.f.u. of virus infectivity had been destroyed during the inactivation process. The possible presence of residual infectivity in inactivated samples not detected by plaque assay was ruled out after incubation of the inactivated samples in successive HEp-2c cell cultures for periods of up to 3 weeks.

The integrity of viral RNA during the process of inactivation was analysed by RT-PCR using poliovirus-specific primers. Samples from Mahoney wild-type strain were used for this experiment. Poliovirus RNA was not detected from phenol/SDS extracts of virus samples after 60 h of incubation with formaldehyde (Fig. 1B). The biological activity of viral RNA

Table 2. Comparison of deduced amino acid sequences at antigenic sites between CHAT, Cox, Mahoney and Sabin 1 strains

Virus strain	Antigenic site				
	Site 1	Site 2a	Site 2b	Site 3	Site 4
	VP1 88–102	VP1 219–226	VP2 166–172	VP3 58–60,71,73	VP2 72,VP3 76
Mahoney*	TIMTVDNPASTTNKD	DQSAALGD	NQTSPAR	SAT R S	T P
CHAT†	-----S-----G	--P-----	-----	--- --	--
Cox‡	-----S-----G	-----	-----	--- --	--
Sabin 1§	A-I----S---K---	-----	-----	--K --	--

*EMBL accession no. V01148.

†EMBL accession no. AJ416942.

‡EMBL accession no. AJ430385.

§EMBL accession no. V01150.

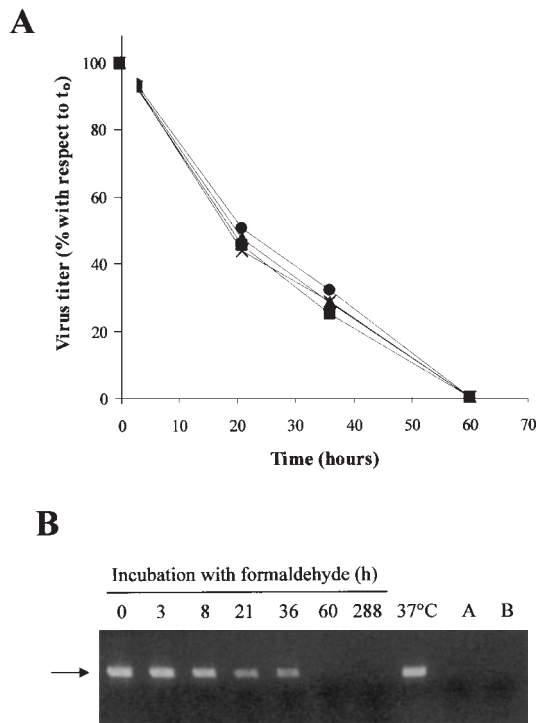


Fig. 1. Kinetics of virus inactivation with formaldehyde. Determination of virus titre of Mahoney (■), CHAT (▲), Cox (●) or Sabin 1 (x) samples by the plaque assay method (A) and detection of poliovirus RNA by RT-PCR (B) from sequential virus samples taken after different times of incubation with formaldehyde. Aliquots of 25 μ l (~1% of the original sample) were used for both experiments. Samples from Mahoney wild-type poliovirus were chosen for the RT-PCR experiment. Commercial vaccines 90/716 (lane A) and 91/574 (lane B) were used as controls. The lane labelled 37°C indicates the RT-PCR product of an equivalent aliquot of the Mahoney virus taken from a virus solution incubated at 37°C for 288 h in the absence of formaldehyde.

isolated from partially inactivated samples was confirmed by its ability to produce infectious virus after transfection into HEP-2c cells (data not shown). Again, no infectious virus was recovered in this manner from samples taken after 60 h of incubation with formaldehyde. When equivalent virus samples were incubated at 37°C in the absence of formaldehyde, viral RNA was readily detected by RT-PCR (Fig. 1B) and transfection experiments. No poliovirus RNA was detected in two commercial IPV preparations (NIBSC Refs. 90/716 and 91/574) by either of the two methods.

Immunogenicity of inactivated virus samples in normal mice

The purpose of these experiments was to investigate the extent to which formaldehyde inactivation affected the viral immunogenicity of each of the four type 1 strains. The D-antigen form of poliovirus, expressed in the native infectious virus, as opposed to the C-antigen form, present

on non-infectious empty particles, is thought to be responsible for the protective immune response induced by poliovirus vaccines or natural infection. For this reason the D-antigen content is regarded as critical for the effectiveness of IPV and is used to measure and compare the potency of different IPV preparations. The D-antigen content of inactivated vaccines can be related to plaque-forming units in live virus for each virus strain and therefore solutions containing identical concentration of native virus or the equivalent amounts of inactivated virus were used to immunize mice and directly compare the immunogenicity of Mahoney, CHAT, Cox and Sabin 1 before and after formaldehyde inactivation. The use of D-antigen-specific monoclonal antibodies normally used to measure the potency of commercial IPV was considered less appropriate because D-antigen units have been arbitrarily defined for the Mahoney strain used in current IPV preparations. The type 1 monoclonal antibody used in this test at NIBSC reacted differently with the different strains in the standard D-antigen ELISA assay (data not shown) and therefore comparisons would be valid between different preparations of the same strain but not among different virus strains.

Groups of ICR mice were immunized twice with 0.5 ml of 1 : 10, 1 : 50 or 1 : 250 dilutions of either native or inactivated virus stocks of each of the four strains. These dilutions correspond to 5×10^7 , 1×10^7 or 0.2×10^7 p.f.u. of virus equivalent to 0.5, 0.1 or 0.02 human doses of type 1 IPV as estimated by comparing the D-antigen content of purified Mahoney virus preparations with that of a commercial IPV of known potency using the standard ELISA method.

Blood specimens taken after 5 weeks were tested *in vitro* for the presence of neutralizing antibodies against Mahoney, CHAT, Cox and Sabin 1 virus strains. The results are presented in Fig. 2. There was some degree of reduction in the neutralization titres induced by the inactivated form as compared to those induced by intact virus for all four viral strains. Interestingly, this effect was less significant in the case of Sabin 1 strain. Sabin 1 preparations induced levels of immunogenicity similar to those induced by Mahoney preparations although immunization with Mahoney samples generated a slightly better immune response at the lowest dilution of immunogen. Both native and inactivated CHAT preparations induced poor immune responses in general, whereas Cox samples showed intermediate values. Sera from mice immunized with CHAT samples exhibited a degree of neutralizing specificity against the homologous strain, CHAT. In contrast, Mahoney and Sabin 1 induced a more uniform neutralization response against the four virus strains. None of the mice sera was able to neutralize Sabin 2 poliovirus infectivity (data not shown).

Protection of transgenic mice immunized with inactivated virus samples against challenge with live virus

Transgenic mice that express the human poliovirus receptor were used to test the capability of the

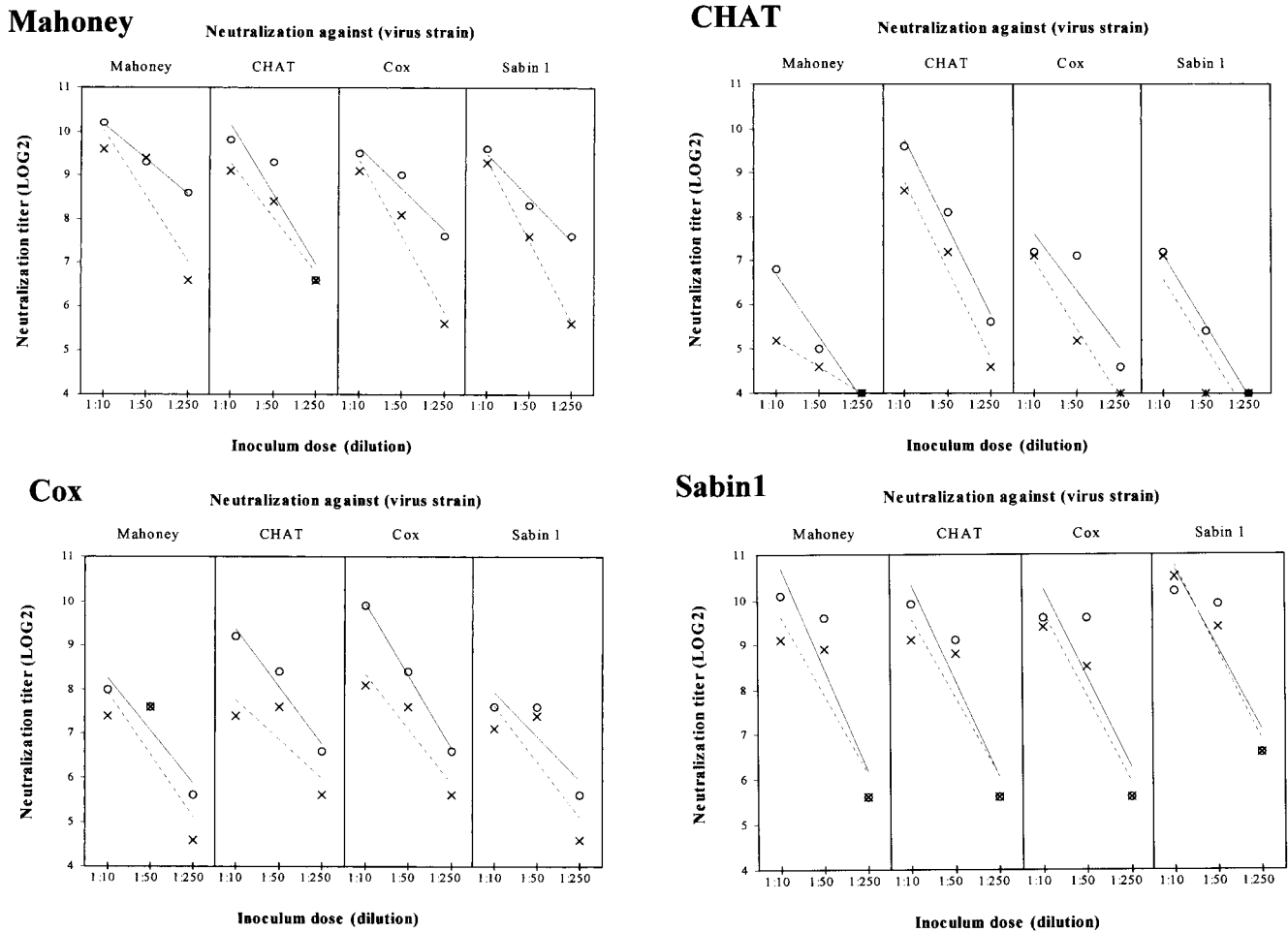


Fig. 2. Immunogenicity of inactivated virus samples in normal mice. Mice were immunized with serial dilutions of purified virus samples before (○) or after (x) inactivation with formaldehyde. The antibody neutralization titres in blood from immunized mice are shown. The virus strain used for immunization is shown on the top-left corner of each box. The neutralization titres are expressed as the reciprocals of the serum dilutions in \log_2 values. For details see text.

formaldehyde-inactivated virus samples to induce immune protection *in vivo* against challenge with paralyzing doses of live poliovirus. A commercial IPV preparation (NIBSC Ref. 90/716) was used to evaluate this model in preliminary studies for which transgenic mice were immunized with fourfold serial dilutions of 90/716 and challenged with high doses of Mahoney wild-type 1 poliovirus. A good dose-response effect was observed in several independent experiments when mice were inoculated twice in a 2-week interval and challenged by intramuscular inoculation of 100 50% paralytic doses (PD_{50}) of Mahoney virus at day 28 after the first immunization (Table 3). A similar schedule but using a fixed amount of immunogen was followed to test the laboratory inactivated samples. The results, shown in Table 4 and Fig. 3, closely resembled those obtained in the *in vitro* neutralization assays. Transgenic mice immunized with inactivated CHAT samples were poorly protected against challenge with the Mahoney strain but showed good levels of protection when challenged with paralyzing doses of the

CHAT strain (CHAT strain can paralyse mice at high doses). Inactivated Sabin 1 induced complete protection, in these conditions, against challenge with both Mahoney and CHAT viruses and results with inactivated Cox were somewhere in the middle. The fact that the immune protection induced by 90/716 vaccine seemed slightly less efficient than that induced by the Mahoney laboratory inactivated sample at equivalent doses could be due to immune interference by the serotype 2 and 3 poliovirus components also present in the 90/716 trivalent vaccine. Neutralizing antibody titres in blood samples taken from selected animals before the challenge with live virus showed good correlation with the immune protection *in vivo* results described above (data not shown).

DISCUSSION

The possibility of using live-attenuated strains for the production of inactivated poliovirus vaccines (IPV) seems like an ideal option in the context of the current effort for the

Table 3. Immunization/challenge experiments in transgenic mice using a commercial IPV preparation (Ref. 90/716)

Results of three independent experiments are shown.

Vaccine inoculum		Clinical results (no. protected/no. challenged)		
Dilution of 90/716 IPV	Type 1 dose (human doses)	Expt 1	Expt 2	Expt 3
1:4	0.25	7/11	8/10	9/10
1:16	0.062	4/9	6/10	6/10
1:64	0.016	2/11	5/10	6/9
1:256	0.004	0/10	4/10	2/10
Diluent	–	0/10	0/10	0/9

Table 4. Immunization/challenge experiments in transgenic mice using laboratory inactivated virus samples

Immunogen	Dose (human doses)	Virus challenge	No. of mice protected/total no. of mice
Experiment 1			
Mahoney	0.25	Mahoney	10/10
CHAT	0.25	Mahoney	2/10
Cox	0.25	Mahoney	8/10
Sabin 1	0.25	Mahoney	10/10
Diluent	–	Mahoney	0/10
CHAT	0.25	CHAT	9/10
Sabin 1	0.25	CHAT	10/10
Diluent	–	CHAT	0/10
Experiment 2			
Mahoney	0.25	Mahoney	9/10
CHAT	0.25	Mahoney	5/10
Cox	0.25	Mahoney	5/5
Sabin 1	0.25	Mahoney	10/10
Diluent	–	Mahoney	0/10

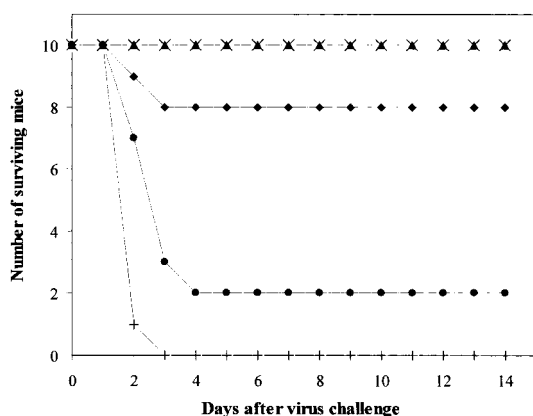


Fig. 3. Protection of IPV-immunized transgenic mice against challenge with live poliovirus. Mice were immunized twice with the equivalents of 0.25 human doses of Mahoney (▲), CHAT (●), Cox (◆) or Sabin 1 (x) laboratory inactivated virus samples and 28 days later challenged with 100 PD₅₀ doses of Mahoney live poliovirus. (+) indicates mice immunized with diluent alone. The numbers of mice surviving per day are shown.

global eradication of wild polio. Indeed, some attempts have been carried out in this regard or are currently in progress (Doi *et al.*, 2001; Kersten *et al.*, 1999; Murph *et al.*, 1988; Taffs *et al.*, 1997). Here we describe the properties of inactivated poliovirus preparations made with type 1 live-attenuated strains (Sabin 1, CHAT and Cox) in comparison to those of inactivated Mahoney, the wild-type 1 strain commonly used in commercial IPV.

Our experiments showed that the kinetics of inactivation of virus infectivity with formaldehyde was very similar for the four poliovirus strains. This observation contrasts with the fact that attenuated strains have clearly shown differences in some physical–chemical properties with respect to their wild-type parental virus, such as a decreased thermostability at 45 °C or a marked sensitivity to replicate in cell culture at high temperatures or low concentrations of bicarbonate (Lu *et al.*, 1994; Melnick, 1994).

During the course of our studies we observed that incubation of purified poliovirus with formaldehyde had an effect on the ability to detect viral RNA by RT-PCR. This effect closely paralleled that of the loss of infectivity

discussed above. After 60 h incubation with formaldehyde, viral RNA was no longer detected by RT-PCR and nor was infectious poliovirus recovered from nucleic acid extracts transfected into susceptible cells. These results are most likely due to the fact that viral RNA cannot be extracted from formaldehyde-inactivated virus particles by standard phenol/SDS methods because it becomes cross-linked with the capsid proteins (Twomey *et al.*, 1995). The question whether the viral RNA is irreversibly degraded during this process remains unclear. A new molecular assay based on this observation, designed to monitor the effectiveness of the inactivation process, is a potentially useful development that could be of interest to vaccine manufacturers.

Treatment with formaldehyde had a slight effect on the immunogenicity in mice of the viral preparations tested in this study. However, the observed reduction in potency was not greater for any of the three type 1 poliovirus live-attenuated strains than that observed for the wild-type Mahoney virus. This effect could be due to the partial destruction of specific D-antigenic epitopes known to occur during the inactivation process (Ferguson *et al.*, 1993) and/or to non-specific loss of potency during the preparation of inactivated stocks caused, for example, by the adsorption of part of the immunogen to the filters (Twomey *et al.*, 1995). Studies to evaluate the extent of antigenic modification in inactivated virus samples using monoclonal antibodies to all type 1 poliovirus antigenic sites are planned.

Differences in potency and specificity were identified among the immune responses induced by the formaldehyde-inactivated versions of the four related type 1 strains in both normal and transgenic mice expressing the human poliovirus receptor. The immune responses induced by CHAT preparations were particularly poor and highly specific. Immunization with inactivated CHAT samples failed to protect transgenic mice against challenge with Mahoney virus whereas CHAT-immunized mice in the same conditions were well protected against paralyzing doses of the CHAT strain. The reasons for these differences are not clear at present. Remarkably, the CHAT strain contains sequence differences at only four capsid amino acid residues with respect to the other three strains, at VP1-43, VP1-138, VP1-221 and VP3-192 (Martin & Minor, 2002). Mutations at VP1-221, which is part of antigenic site 2a, and at VP3-192, which has been identified as a possible T-cell epitope, appeared to be unstable in humans and were shown to revert to the wild-type sequence in isolates from patients with vaccine-associated paralytic poliomyelitis and healthy vaccinees (Martin & Minor, 2002).

The results reported here indicate that changing to the use of novel strains for IPV production is not trivial and would require careful planning and detailed clinical trials to assess the immune response in humans. These studies need to be extended to the other two poliovirus serotypes in order to identify suitable strains and optimal heterotypic combinations for a prospective attenuated-inactivated poliovaccine. The transgenic mouse immunization/challenge models for

IPV described here and elsewhere (Taffs *et al.*, 1997) could have potential applications for vaccine standardization and control.

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