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Evaluation of the use of various rat strains for immunogenic potency tests of Sabin-derived inactivated polio vaccines

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ABSTRACT

Slc:Wistar rats have been the only strain used in Japan for purpose of evaluating a national reference vaccine for the Sabin-derived inactivated polio vaccine (sIPV) and the immunogenicity of sIPV-containing products. However, following the discovery that the Slc:Wistar strain was genetically related to the Fischer 344 strain, other "real" Wistar strains, such as Crlj:WI, that are available worldwide were tested in terms of their usefulness in evaluating the immunogenicity of the past and current lots of a national reference vaccine. The response of the Crlj:WI rats against the serotype 1 of sIPV was comparable to that of the Slc:Wistar rats, while the Crlj:WI rats exhibited a higher level of response against the serotypes 2 and 3. The immunogenic potency units of a national reference vaccine determined using the Slc:Wistar rats were reproduced on tests using the Crlj:WI rats. These results indicate that a titer of the neutralizing antibody obtained in response to a given dose of sIPV cannot be directly compared between these two rat strains, but that, more importantly, the potency units are almost equivalent for the two rat strains.

1. Introduction

Tetracomponent DTaP-sIPV vaccines containing inactivated polio vaccines made from Sabin attenuated poliovirus strains (sIPV) have been used in the routine immunization schedule in Japan since 2012 [1]. Also in 2012, a single component Salk IPV (cIPV) was introduced in Japan, resulting in coexistence of the two kinds of IPVs [1]. At the National Institute of Infectious Diseases, we are responsible for the official testing of both DTaP-sIPVs and cIPVs. We use *in vivo* rat immunogenicity tests to assess the potency of DTaP-sIPVs according to the recommendations by the WHO [2,3], and *in vitro* D-antigen detection ELISA to test the potency of cIPVs.

Although the WHO recommendations do not specify the rat strain to be used in the immunogenicity assay of IPVs, the Wistar rats are generally used at most of the vaccine manufacturers and at the National Control Laboratories [4]. In line with this, the Wistar rats available from Japan SLC (Slc:Wistar) have been principally used for immunogenicity assays of DTaP-sIPVs as well as to evaluate the national reference vaccine for sIPVs [5]. However, a recent study indicates that the Slc:Wistar rat is genetically related to the Fischer 344 rat, and thus is different from the globally distributed Wistar strain [6]. This report raises concerns or interesting question – namely, whether or not our immunogenicity data accumulated over the last dozen years can be compared on an equal footing with the massive amounts of data collected at other laboratories worldwide. In this study, therefore, we evaluated the immunogenicity of sIPV in the globally distributed Wistar rats. In addition, we confirmed that the immunogenic potency unit of a current national reference vaccine for sIPV in Japan, Lot 12A, determined using the Slc:Wistar rats could be applied to the potency assays using the real Wistar rats.

Abbreviations: cIPV, conventional inactivated polio vaccine; DTaP, diphtheria–tetanus–acellular pertussis combination vaccine; DU, D-antigen unit; GM, geometric mean; IPV, inactivated polio vaccine; JPRI, Japan Poliomyelitis Research Institute; NIID, National Institute of Infectious Diseases, Japan; OPV, oral polio vaccine; sIPV, Sabin-derived inactivated polio vaccine; WHO, World Health Organization

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Table 1
Immunogenic responses of various rat strains against a national reference vaccine for sIPV, Lot 12A.

Rat strains	Test number	Serotype 1	Serotype 2	Serotype 3
F344/NSlc	Test 1	0.97 (0.67–1.41)	1.38 (0.77–2.71)	0.82 (0.45–1.42)
Slc:SD	Test 1	0.47 (0.30–0.68)	2.03 (1.29–3.65)	2.15 (1.13–5.69)
Crlj:WI	Test 2	0.79 (0.59–1.04)	3.75 (2.07–9.73)	2.03 (1.15–4.47)
	Test 3	0.61 (0.40–0.89)	5.25 (2.98–12.8)	1.64 (1.08–2.68)
Crl:WI(Han)	Test 1	0.76 (0.49–1.15)	4.63 (2.44–15.0)	3.48 (1.81–10.6)
	Test 2	0.95 (0.64–1.39)	10.8 (5.10–48.5)	6.22 (3.22–21.5)
Crl:CD(SD)	Test 1	0.36 (0.22–0.54)	4.41 (2.71–9.20)	2.92 (1.66–6.86)
	Test 2	0.61 (0.41–0.88)	3.96 (2.25–9.25)	2.76 (1.60–6.10)

Immunogenic responses of several rat strains against sIPV were compared with that of the Slc:Wistar rats. Neutralizing antibody titers of sera from different rat strains are indicated as relative values to that from the Slc:Wistar rats when titers for the Slc:Wistar rat are set to 1 for each poliovirus serotype. Values in parentheses are the 95% confidence intervals. The Crlj:WI, Crl:WI(Han) and Crl:CD(SD) rats were compared with the Slc:Wistar rat in two independent tests. Results from Tests 1, 2 and 3 (for details, see Supplementary Table 1) are summarized.

2. Materials and methods

2.1. Rat strains

In addition to the Slc:Wistar rats, we purchased rats of the SD strain (Slc:SD) and the Fischer 344 strain (F344/NSlc) from Japan SLC (Hamamatsu, Japan). The Slc:Wistar and Slc:SD rats are maintained as closed colonies, while the F344/NSlc rats are an inbred strain. We purchased the Wistar rats (Crlj:WI) from Charles River Laboratories Japan (Yokohama, Japan) and used them as the "real" Wistar rats. The Wistar Hannover strain (Crl:WI(Han)) and the SD strain (Crl:CD(SD)) purchased from Charles River Laboratories Japan were also tested. These three strains from Charles River Laboratories Japan were maintained as closed colonies. The Crl:WI(Han) strain is a derived from the Wistar strain. All rats used in Test 1 and the Crlj:WI rats used in Test 2 (Supplementary Table 1) were generous gifts from the respective animal suppliers.

2.2. Vaccines

A current national reference vaccine of sIPV, Lot 12A, that was manufactured by the Japan Poliomyelitis Research Institute (JPRI; currently BIKEN Poliomyelitis Research Institute, Tokyo, Japan) was used [5]. The former lots of this vaccine, 05J and 09A, were also used. IMOVAX POLIO (Sanofi K. K., Tokyo, Japan) was purchased from ZENKOKU Vaccine Co., Ltd. (Tokyo, Japan).

2.3. Rat immunogenicity tests

All animal procedures were approved by the Committees on Biosafety and Animal Handling Regulations of NIID. Animal research was undertaken in compliance with the "Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions under the Jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology" issued by the Ministry of Health, Labour and Welfare, Japan. All studies with animals also adhered to the principles stated in the guidelines. The immunogenic potency of the sIPV preparations was assessed essentially according to the methods described in the WHO Recommendations [2,3].

Immunization of rats with test vaccines and neutralization assays were performed as described previously [5]. All the tests performed in this study are summarized in Supplementary Table 1. Test vaccines were twofold diluted except in the case of vaccines for the Crlj:WI rats in Tests 10 and 11, which were threefold diluted. Four to 6 dilutions were prepared for each test vaccine. Ten rats were used for each dilution of the respective test vaccines. Rats were intramuscularly injected with 0.5 mL of each dilution of test vaccines, and sera from each rat were prepared after 3 weeks of inoculation. Neutralizing antibody titers of sera were measured using the Sabin strains (serotypes 1, 2 and 3) and HEp-2C cells as challenge viruses and indicator cells, respectively, and expressed as $\log_2$. The immunogenic potency of test vaccines was calculated with a 95% confidence interval by the parallel line method with help from an Excel sheet created by the Department of Bacterial Pathogenesis and Infection Control (currently the Department of Quality Assurance and Radiological Protection) in NIID [5]. Statistical analysis of the data was performed using functions embedded in the Excel software.

3. Results

3.1. Differences in the immunogenic response against sIPV among various strains of rats

In order to assess the difference in the immunogenic responses against sIPV, various strains of rats were immunized with a national reference vaccine of sIPV, Lot 12A. Four twofold dilutions of Lot 12A, which started from the undiluted, were prepared and 10 rats were used for immunization with each dilution. Their sera were subjected to neutralizing assays (Tests 1 to 3 in Supplementary Table 1). The relative potency of sera from the immunized Slc:SD, F344/NSlc, Crlj:WI, Crl:WI (Han) and Crl:CD(SD) rats was compared by the parallel line method with the neutralizing potency of sera from the immunized Slc:Wistar rats. In spite of limited numbers of experiments, as judged from the relative potency shown in Table 1, it is apparent that the Crlj:WI rats and also Crl:WI(Han) rats exhibit immunogenic responses different from the Slc:Wistar rats. The responses of both the Crlj:WI and Crl:WI (Han) rats against serotype 1 of sIPV were similar to or slightly less than that of the Slc:Wistar rats, whereas both the Crlj:WI and Crl:WI(Han) rats were highly reactive against serotypes 2 and 3. The relative response of the F344/NSlc rats against each serotype was around 1, indicating that the Slc:Wistar rat rather had the characteristics similar to those of the F344/NSlc rats in terms of the immunogenic response to sIPV. This appears to be consistent with the report that the Slc:Wistar rat is genetically related to the Fischer 344 strain [6]. The two SD rat strains (Slc:SD and Crl:CD(SD)) exhibited a lower response against serotype 1 and much higher responses against serotypes 2 and 3.

3.2. A unit for the immunogenic potency of national reference vaccines of sIPV in Crlj:WI rats

The immunogenic potency of national reference vaccines used in Japan has been monitored using Slc:Wistar rats, and units expressing the immunogenicity have been assigned [5]. A report by Nakanishi et al. [6] raised a question as to whether the immunogenic potency units given for national reference vaccines are also applicable to immunogenicity tests using other rat strains, especially the "real" Wistar strain, since Wistar rats have been the most frequently used animal for