STUDIES ON TYPE 1 POLIOVIRUS ISOLATED IN BRAZIL BY
ELECTROPHORESIS AND MONOCLONAL ANALYSIS

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In spite of the drastic decrease of paralytic poliomyelitis infections in Brazil following
nationwide campaigns of vaccination carried out since 1980, the incidence of this disease still
remain high. mainly in the northeastern region of the country as described by Risi (1984, Rev.
infec. Dis., 6: 5400-5403). Even in developed countries specially where oral vaccines are used,
a low level of cases appear every year as observed by Melnick (1982, Medical Virology, Else-
vier Biomedical, 262-299).

The persistence of the disease in many countries, brought new interest to the study of
poliomyelitis. This interest is due mainly to the incomplete immunity of population reached
by the oral vaccines especially in tropical areas and to the report of clinical cases with
55). This last aspect is directly related to the high rate of viral mutation during its replication
in the intestinal tract. For the above reasons we believe that the virus strains isolated from epidemiologically relevant cases should
be thoroughly characterized.

Field isolates of poliovirus included in this study are listed in Table I. All were isolated from
stools of patients with clinical symptons of poliomyelitis. The strains were sent from different parts of the country for character-
ization at our National Reference Center for Enteroviruses. After isolation the viruses were
cultivated for not more than three passages in GMK<sub>2</sub> cells. P1 Mahoney (reference strain)
was supplied by the Institut Merieux (Lyon, France) and did not show genetic variation when
analyzed by RNA fingerprinting (not shown). Poliovirus type 1 vaccine strain was supplied by the National Institute of Health (Japan). Virus titers were assayed by the microtiter method in GMK<sub>2</sub> cells.

Radiolabeled cytoplasmic extracts were obtained in the following way: monolayer cul-
tures of GMK<sub>2</sub> cells in 35mm petridishes were infected with 150-200 viral particles/cell and
allowed to adsorb for 30 min. at 35°C. The inoculum was withdrawn and the cells main-
tained for 3 hrs. in 1,5ml Dulbecco's MEM, lacking methionine. After this period, the
medium was replaced by 0,5ml of the same medium but containing 10uCi [<sup>35</sup>S] methio-
nine (supplied by Dr. Moacyr A. Rebello of the Instituto de Biofísica - Universidade Federal
do Rio de Janeiro). The period of pulse was 3 hrs. after which the labeling medium was re-
moved and the cells solubilized in 250ul of a buffer consisting of 10mM tris-HCl pH 7,2,
100mM NaCl; 2mM EDTA; 1% NP40; 0,5mM Phenylmethyl sulfonyl fluoride. All the exper-
iments were conducted at 35°C.

Electrophoresis was performed as described by Laemmli (1970, Nature, 227:680-685)
slightly modified as follows: separating gels contained 12% acrylamide, 0,3% bxs-acrylamide and
the stacking gel contained 4% acrylamide 0,1% bxs-acrylamide. Both stacking and separa-
tion gels contained 0,1% SDS and 0,5M urea. The tris-glycine upper reservoir contained
0,5M urea. Slab gels were 1,0mm thick, 16cm wide and 14cm high. Samples were boiled with
equal parts of sample-Buffer (10M urea; 10% 2-mercaptoethanol, 0,3% SDS) and electro-
phoresed at 150v for 5hrs. at room temperature. Gels were fixed in 10% trichloroacetic acid
(w/v) 10% acetic acid and 30% methanol for 30 min, stained in 0,06% coomassie blue, 10% acetic acid and 30% methanol and destained
in this same solution without coomassie blue. The gels were stained to visualize the structural proteins of the purified Mahoney strain which
was not labeled. After drying, gels were exposed to Sakura x-ray film for 4 days.

The monoclonal antibodies used in this work (kindly supplied by Dr. F. Horaud of
Institute Pasteur, Paris) were designated Io which reconizes only wild type polio 1 and Io
which recognizes vaccine-related strains of poliovirus type 1. All the strains under study
were tested against the two classes of antibodies by neutralization methods as described by
Crainic et al. (1982, Develop. biol. Standard, 50:229-234) and classified as Sabin-like or non
Sabin-like (Table I).

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TABLE I
Type I Poliovirus isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Year of isolation</th>
<th>Place of isolation</th>
<th>Reaction by monoclonal antibodies</th>
</tr>
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<tbody>
<tr>
<td>14165</td>
<td>1980</td>
<td>Rio de Janeiro</td>
<td>SL</td>
</tr>
<tr>
<td>14485</td>
<td>1980</td>
<td>Rio de Janeiro</td>
<td>SL</td>
</tr>
<tr>
<td>14548</td>
<td>1980</td>
<td>Rio de Janeiro</td>
<td>SL</td>
</tr>
<tr>
<td>3827</td>
<td>1981</td>
<td>São Paulo</td>
<td>NSL</td>
</tr>
<tr>
<td>18058</td>
<td>1981</td>
<td>Pernambuco</td>
<td>NSL</td>
</tr>
<tr>
<td>18062</td>
<td>1981</td>
<td>Pernambuco</td>
<td>NSL</td>
</tr>
<tr>
<td>20294</td>
<td>1981</td>
<td>Pernambuco</td>
<td>NSL</td>
</tr>
<tr>
<td>20300</td>
<td>1982</td>
<td>Pernambuco</td>
<td>NSL</td>
</tr>
<tr>
<td>21517</td>
<td>1982</td>
<td>Pernambuco</td>
<td>NSL</td>
</tr>
<tr>
<td>25232</td>
<td>1984</td>
<td>Pernambuco</td>
<td>NSL</td>
</tr>
<tr>
<td>25236</td>
<td>1984</td>
<td>Sergipe</td>
<td>NSL</td>
</tr>
<tr>
<td>25243</td>
<td>1984</td>
<td>Alagoas</td>
<td>NSL</td>
</tr>
<tr>
<td>25245</td>
<td>1984</td>
<td>Rio Grande do Norte</td>
<td>SL</td>
</tr>
<tr>
<td>25815</td>
<td>1985</td>
<td>Pernambuco</td>
<td>NSL</td>
</tr>
<tr>
<td>25817</td>
<td>1985</td>
<td>Rio Grande do Norte</td>
<td>NSL</td>
</tr>
<tr>
<td>25820</td>
<td>1985</td>
<td>Pernambuco</td>
<td>NSL</td>
</tr>
<tr>
<td>26072</td>
<td>1985</td>
<td>Bahia</td>
<td>NSL</td>
</tr>
</tbody>
</table>

SL = Sabin-like; NSL = Non Sabin-like.

Fig. 1 A – Autoradiogram of an SDS-PAGE of [35S] methionine-labeled proteins of the seventeen isolates of type 1 poliovirus. B – Autoradiogram of an SDS-PAGE of [35S] methionine-labeled proteins of Mahoney strain, Sabin strain, 25243, 25817 and uninfected cells (control). Arrows indicate the unusual polypeptide found. Markers sizes are shown in kilodaltons (KD). Nomenclature of polioviral proteins follows that of Nottay et al. (1981).

As it can be seen in Fig. 1, the electrophoretic migration of VP1 was slightly slower in strains 3827, 18058 and 18062. VP2 showed an electrophoretic pattern relatively constant among the wild strains. However, the lower mobility of VP2 and its vicinity to the polypeptide 6B was the main characteristic which distinguished among wild strains and the Sabin-related strains (14165, 14485, 14548).

Strain 25245 (a Sabin-like strain as classified by monoclonal antibodies) displayed an intermediate electrophoretic patterns with respect to VP2. This findings has been considered as a possible reversion to virulence from an originally attenuated vaccine-strain.
With regard to VP3, strain 3827 shows a slower migration pattern when compared to the other strains.

A high correlation was found between the results of monoclonal analysis and the electrophoretic pattern in the determination of the origin (vaccine or non vaccine) of the isolates.

Among the non-structural polypeptides, differences were also noted mainly in polypeptide 2 but not in its cleavage product 4. Some differences were also noted in polypeptide 9A.

The most striking feature among all viruses studied was shown by strains 25243 and 25817 that displayed an extra polypeptide with MW around 40,000, localized between 6A and x (note the arrows) and not found in the uninfected cells nor in Mahoney or Sabin reference strains (Fig. 1B). At the moment the meaning of this finding is not understood. It is possible that with the use of more specific and sensitive techniques (fingerprinting analysis are under way), this matter will be elucidated.

In spite of being a simple method, the SDS-PAGE was sensitive enough to demonstrate close relationship among virus strains and clearly distinguished the origin (vaccine or non vaccine) of field isolates of poliovirus, as has been shown previously by Minor (1980, J. Virol., 34: 73-84) and Nottay et al. (1981, Virology, 108:405-423).

The present results show heterogeneity among poliovirus strains isolated on Brazil. This can be partially explained by (i) the wide dissemination of vaccinal viruses in the community, where in the last five years in a single day each year more than 20 million children have been vaccinated during the two national vaccination days (ii) the selective pressure of antibodies and (iii) the high rate of mutation of the poliovirus during its replication in the intestinal tract.

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