Research Article

Applying the Concept of Peptide Uniqueness to Anti-Polio Vaccination

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Background. Although rare, adverse events may associate with anti-poliovirus vaccination thus possibly hampering global polio eradication worldwide. Objective. To design peptide-based anti-polio vaccines exempt from potential cross-reactivity risks and possibly able to reduce rare potential adverse events such as the postvaccine paralytic poliomyelitis due to the tendency of the poliovirus genome to mutate. Methods. Proteins from poliovirus type 1, strain Mahoney, were analyzed for amino acid sequence identity to the human proteome at the pentapeptide level, searching for sequences that (1) have zero percent of identity to human proteins, (2) are potentially endowed with an immunologic potential, and (3) are highly conserved among poliovirus strains. Results. Sequence analyses produced a set of consensus epitopic peptides potentially able to generate specific anti-polio immune responses exempt from cross-reactivity with the human host. Conclusion. Peptide sequences unique to poliovirus proteins and conserved among polio strains might help formulate a specific and universal anti-polio vaccine able to react with multiple viral strains and exempt from the burden of possible cross-reactions with human proteins. As an additional advantage, using a peptide-based vaccine instead of current anti-polio DNA vaccines would eliminate the rare post-polio poliomyelitis cases and other disabling symptoms that may appear following vaccination.

1. Introduction

Vaccine-associated paralytic poliomyelitis (VAPP) [1] is the consequence of the replication of vaccine-derived polioviruses (VDPVs) that originate by genetic mutations from the strain contained in the oral polio vaccine (OPV). In fact, the poliovirus (PV) genetic mutability [2] appears to be the molecular basis of VAPP, which throws a shadow over the great success represented by vaccination in fighting PV infection [3, 4].

Currently, two new monovalent OPVs [5] and the change of the schedule from OPV to the exclusive use of inactivated polio vaccine (IPV) [6] represent options to interrupt PV transmission. However, it has been observed that reduction of exposure to a live attenuated virus such as that contained in OPV will inevitably lead to a decrease in herd immunity to a live microorganism and to natural boosters [7]. Such considerations, along with recent PV infection outbreaks, further complicate the issue of polio eradication [8].

In this scenario, new vaccine formulations and renewed research efforts might help to specifically fight PV infection. Recently, we analyzed the peptide overlap between PV1, strain Mahoney, and the human proteome and described a high extent of peptide sharing involving human proteins linked to fundamental cellular functions [9]. These data appeared to be of interest also because PV has been studied mainly at the nucleotide level [10], and, in general, there is a lack of knowledge of the interaction(s) between PV and the human host at the peptide/protein level. Actually, phenetic analyses of PV might help define peptide-based therapeutic approaches against PV infection, given that, for example,

(i) one single amino acid (aa) change, that is, His to Tyr at aa position 142 of virion protein 2 (VP2) or Val to
Ile at aa position 160 of virion protein 1 (VP1), on the capsid surface of PV1 Sabin allows the establishment of persistent infections in HEp-2c cell cultures [11]; (ii) replacement of the Ala residue with Asp at aa position 3 is linked to 50% loss of virion protein 4 (VP4) precursor myristoylation and severe reduction in specific infectivity [12]; (iii) the 25th aa, Ile, of PV 2C protein interacts with human reticulon 3, a protein involved in viral replication and/or pathogenesis [13].

Moreover, since the 1980s we have known that small aa groupings can play a critical role in neutralizing PV. For example, the discontinuous $E^\text{143}T^\text{144}S^\text{145}E^\text{146}S^\text{147}$ tetrapeptide, which is present in PV VP1, is crucial for neutralizing PV3 by anti-PV3 25-1-14 monoclonal antibody (mAb) [14]. Additional examples of short immune determinants in the immune response against PV are the following ones:

(i) A linear heptapeptide (DNNQTSP) is an Ab-binding site mapped to aa residues 164–170 of VP2 [15].

(ii) Short synthetic peptides (e.g., DNTVRET, RSRSES, RSRSESSIESF, and STTNKD) from PV VP1 prime the immune system of rabbits for a long-lasting, virus-neutralizing IgG Ab response following a single inoculation of intact virus [16].

(iii) The Immune Epitope Database (IEDB; http://www.immuneepitope.org) [17] includes the continuous linear pentapeptide STTNK (IEDB ID: 61944) [18, 19] and the discontinuous pentapeptide $T^\text{41}E^\text{42}S^\text{43}E^\text{44}S^\text{45}P^\text{46}$ (IEDB ID: 91064) [20] as PV-derived epitopes.

Following the mathematical quantification of pentapeptide sharing between PV1 and human proteins, we reported that 2,040 out of the 2,204 pentamers composing the PV1 proteome are shared with the human proteome for a total of 18,223 matches, including multiple occurrences [9]. In general, a vast peptide sharing with human proteins is a characteristic of viral proteomes [21]. This peptide commonality suggests the existence of common evolutionary links between entities widely different as viruses and Homo sapiens and, in addition, indicates that potential cross-reactivity may affect antivirus vaccine formulations [22] and serological analyses [23]. Hence, it is reasonable to postulate that vaccines based on peptides unique to a virus and absent in the host proteome would guarantee high specificity and, at the same time, eliminate potential cross-reactivity.

Pursuing the objectives of overcoming the difficulties posed by the PV tendency to mutate and eliminating the viral reactivation-related VAPP in order to contribute to the global eradication of poliomyelitis, here we examine PV1 Mahoney polyprotein primary sequence and describe a set of pentapeptides uniquely owned by PV1, endowed with immunologic potential, and conserved among 43 PV strains. We propose that this set of pentapeptides might be used in preclinical and in vivo protocols with the ultimate aim of formulating effective, safe, and universal anti-PV vaccines.

2. Methods

The primary aa sequence of human PV1 polyproteins, strain Mahoney (NCBI taxonomic identifier: 12081; Swiss-Prot/UniProtKB entry: P03300), consisting of 11 viral proteins, 2,209 aa long [25] (further details at http://www.uniprot.org/uniprot/P03300), was analyzed for aa sequence similarity to the human proteome at the pentapeptide level. In brief, the viral polyprotein was dissected into 5-mers sequentially overlapped by four residues: MGAQV, GAQVS, AQVSS, QVSSQ, VSSQK, and so forth; then, each viral pentapeptide was used as a probe to scan the human proteome for instances of the same pentapeptide, as already described [9]. The similarity analysis used the Protein Information Resource (PIR) peptide match program (http://pir.georgetown.edu/) [26].

PV-derived epitopes were retrieved from the Immune Epitope Database (IEDB; http://www.immuneepitope.org) [17]. Only PV epitopes that had been experimentally validated in the human host were considered in this study.

Consensus peptide sequences were defined by ClustalW multialignment analysis (http://www.uniprot.org/program/?query=clustalw&sort=score) [24] of sequences from 43 PV strains retrieved from UniProt database (http://www.uniprot.org/) on the basis of the following characteristics: (1) derived in scientific literature; (2) corresponding to the entire PV polyprotein; (3) derived from PV1 and PV3; (4) derived from PV variants isolated from VAPP or acute flaccid paralysis (AFP) patients or from immunocompromised patients with residual paralysis. Description and references of the 43 PV sequences used for multialignment analysis are reported in detail at http://www.uniprot.org/; the relative Swiss-Prot/UniProtKB entries are P03300, P03301, P03302, P06209, Q9Q281, Q9Q280, Q71AZ9, Q5TLH5, DIYS19, DIYS1, DIYS2, DIYS3, DIYS4, DIYS5, DIYS6, DIYS7, DIYS9, DIYS11, DIYS12, DIYS13, DIYS14, DIYS15, DIYS16, DIYS17, DIYS19, DIYS23, DIYS25, DIYS3, DIYS4, DIYS5, DIYS6, DIYS7, DIYS8, DIYS9, DIYS10, DIYS11, DIYS12, DIYS13, DIYS14, DIYS15, D2X673, D8L541, B4YUL3, B4YUL4, Q84792, C5HJY2, C5HJY3, D1GE40, D2E679, D1GE41, and D2XUS9.

3. Results and Discussion

3.1. Identification of Pentapeptides Unique to PV Type 1, Strain Mahoney. Using the procedure described under Section 2, we searched the human PV1, strain Mahoney, primary sequence for pentapeptides not shared with human proteins. We used pentapeptides as probes since a pentapeptide is a minimal functional unit in immunology [27], thus representing an appropriate length unit in measuring the qualitative/quantitative parameters of immunological phenomena [28].

Table 1 reports the pentapeptide platform that characterizes PV1 Mahoney polyprotein when compared to the Homo sapiens proteome. We find that 164 pentapeptides are unique to the viral polyprotein and absent in human proteins. In a few instances, viral 5-mers consecutively overlap (Table 1, pentapeptides in bold), thus forming 6-, 7-, and 8-mer stretches (e.g., PV1 $163–169$ QNMYYHY, PV1 $446–453$ NYYTHWAG,
Table 1: Pentapeptides unique to PV type 1, strain Mahoney, and absent in the human proteome.

| Pos^a | Sequence^bc | Pos^a | Sequence^bc | Pos^a | Sequence^bc | Pos^a | Sequence^bc | Pos^a | Sequence^bc | Pos^a | Sequence^bc |
|-------|-------------|-------|-------------|-------|-------------|-------|-------------|-------|-------------|-------|-------------|
| 11    | GAHEN       | 393   | MIPLN       | 746   | PAKWD       | 969   | YYPAR       | 1399  | CHQPA       | 1750  | EIQWM       |
| 29    | TINYY       | 403   | KNTMD       | 748   | KWDDY       | 974   | YQSHI       | 1403  | ANFKR       | 1751  | IQWMR       |
| 72    | NIEAC       | 404   | NTDM        | 749   | WDDYT       | 996   | CHHGV       | 1413  | CGKAI       | 1752  | QWMRP       |
| 73    | IEACG       | 408   | MYRVPQ      | 752   | YTWTQ       | 1014  | FADIR       | 1418  | QLMDK       | 1762  | YPIIN       |
| 104   | YGRWP       | 414   | NDNP        | 763   | FYTYG       | 1021  | YAYEE       | 1437  | IVNFR       | 1810  | YVGNNK      |
| 106   | RWPEY       | 436   | LSHTM       | 783   | AYSHF       | 1084  | ITRNY       | 1458  | PIQKY       | 1840  | TEQMC       |
| 107   | WPEYL       | 446   | NYYTH       | 784   | YSHFY       | 1105  | VSPWQ       | 1475  | ECIND       | 1849  | MYGTD       |
| 130   | CRFYT       | 447   | YTHW        | 785   | SHFYD       | 1136  | TEACN       | 1489  | VRNYC       | 1933  | VAMRM       |
| 145   | RGWWW       | 448   | YTHWA       | 835   | KIRVY       | 1139  | CNAAK       | 1490  | RNYCE       | 1935  | MRMAF       |
| 146   | GWWWK       | 449   | THWAG       | 841   | KPFHI       | 1188  | STHQ        | 1496  | KGWIV       | 1946  | FHKNP       |
| 148   | WVKLP       | 464   | SMMAT       | 847   | VWCP        | 1190  | IHQSC       | 1497  | GWIVN       | 1947  | HKNPG       |
| 149   | WKLKD       | 465   | MMATG       | 857   | AYYGP       | 1204  | FNNVR       | 1498  | WIVNI       | 1966  | WSKIP       |
| 163   | QNMYY       | 494   | HVIWD       | 863   | VYKKD       | 1205  | NNVRW       | 1513  | NRAMT       | 1979  | AFYTD       |
| 164   | NMYMYH      | 495   | VIWDI       | 880   | TGYFG       | 1209  | WLSIQ       | 1532  | VVYMY       | 1983  | TGYDA       |
| 165   | MYHYH       | 497   | WDIGL       | 881   | YGFGH       | 1231  | LEHTI       | 1534  | VMYKL       | 1993  | WFEAL       |
| 179   | VQCNQ       | 507   | MYPWPV      | 883   | FHGHQN      | 1291  | FDGYK       | 1536  | YKLEA       | 2028  | YVCVK       |
| 199   | MCLAG       | 510   | PWISN       | 884   | GHQNK       | 1311  | GADMK       | 1588  | GEFTM       | 2070  | KMIAY       |
| 242   | RFCPV       | 518   | RQTNN       | 894   | GYKIC       | 1314  | MKLFC       | 1592  | MLGIH       | 2073  | AYGGD       |
| 244   | CPVDY       | 534   | FYQTR       | 895   | YKICN       | 1326  | FIPPM       | 1594  | GHIDN       | 2115  | TWENV       |
| 271   | TNNCA       | 556   | SACND       | 897   | ICNYH       | 1340  | FTSNY       | 1595  | HIDNV       | 2131  | KYPFL       |
| 292   | KHNNN       | 557   | ACNF        | 912   | VSTMW       | 1341  | TSNYV       | 1596  | HDNVA       | 2148  | SIRWT       |
| 293   | HNNWG       | 558   | CNDFS       | 914   | TMWDW       | 1368  | RFAFD       | 1660  | TETND       | 2158  | TQDHV       |
| 295   | NWGIA       | 568   | DTHII       | 934   | ARNCN       | 1369  | FAADM       | 1677  | MFVVP       | 2168  | LAWHN       |
| 323   | PMCC        | 569   | TTHIG       | 942   | VYYCE       | 1371  | FMDDI       | 1677  | RTELMY      | 2201  | LYRRW       |
| 324   | PMCCE       | 588   | MIDNT       | 948   | RRKYY       | 1388  | DMTMA       | 1700  | TLMYN       | 2308  | LYMNP       |
| 326   | CCEFN       | 665   | CVSSI       | 959   | PFTQY       | 1389  | MTMAT       | 1701  | LMYNF       | 2313  | MYNF       |
| 327   | CEFNG       | 708   | RDFME       | 961   | FQYME       | 1394  | EMCKN       | 1702  | MYNF       |
| 391   | DTMIP       | 735   | QIMYV       | 962   | QYMEA       | 1398  | NCHQP       | 1742  | SYFTQ       |

^a^ aa position along the human PV type 1, strain Mahoney, primary sequence.
^b^ aa sequences given in one-letter code.
^c^ Consecutively overlapping pentapeptides forming 7- and 8-mer stretches unique to PV are given in bold.

3.2. Analysis of the Immunologic Potential of Peptides Unique to PV. Next, the pentapeptides described in Table 1 were analyzed for their immunologic potential as follows. PV-derived epitopes were retrieved from IEDB, and the epitopes that had been experimentally validated in the human host were analyzed for the presence of pentapeptides unique to PV1 (see Table 1).

As reported in Table 2, the search through IEDB produced a final list of 78 viral epitopes derived from PV1 Mahoney, PV3 Sabin, and PV3 (P3/LEON/37 and P3/LEON 12A (1B)). Epitopes derived from PV2 strains were not considered since only data from immunoassays in mice, rats, and/or rabbits were available in IEDB for PV2 strains at the time of this study. Following sequence analysis, it was found that 20 of the 78 epitopes (i.e., IEDB IDs: 31814, 48785, 58511, 59797, 71769, 79272, 79480, 99910, 100138, 100244, 100349, 100382, 100536, 100576, 100631, 100667, 100672, 146181, 146248, and 146390; see IEDB IDs in bold in Table 2) have a total of 12 viral pentapeptides that are absent in the human proteome (pentapeptides in capital letters in Table 2). That is, a first conclusion from Table 2 is that 12 out of the 164 pentapeptides unique to PV1, strain Mahoney, are part of 20 PV epitopic sequences endowed with an immunologic potential in the human host.

Moreover, it can be seen that the 12 unique PV1 pentapeptides are not distributed at random among the PV-derived epitopes. For example, three unique pentapeptides (KWDDY, WDDYT, and YTWQT) overlap each other in the epitope IEDB ID 48785, sequence ppgapvpeKWDDYTWQTSnp (with unique overlapping pentapeptides in capital); the heptapeptide AYSHFYD, formed by three overlapping unique...
Table 2: Twelve pentapeptides unique to PV1, Mahoney strain, and absent in the human proteome are distributed among twenty PV-derived epitopes recognized by human sera and/or T cells.

| IEDB ID<sup>a</sup> | Epitope sequence<sup>bc</sup> | PV antigen | PV strain | Immune context |
|---------------------|-----------------------------|-------------|-----------|----------------|
| 30661               | kevpaltavetgat              | VPI         | PV3 Sabin | B              |
| 31814               | klefftyRFDMEhftvtn          | VPI         | PV1 Mahoney | T              |
| 46859               | paltavetgatnpl              | VPI         | PV1 Mahoney | B-T            |
| 48785               | pgapvpveKWDDYTWQIlsnnp      | VPI         | PV1 Mahoney | T              |
| 55952               | rsrssessief                 | VPI         | PV1 Mahoney | B              |
| 58511               | siFYTYTGateparisvpyvgi      | VPI         | PV1 Mahoney | T              |
| 59797               | snAYSHFYDgskvplkdsqs       | VPI         | PV1 Mahoney | T              |
| 66978               | tivnsasttnkdklfavwk         | Polyprotein | PV1 Mahoney | T              |
| 71769               | vvnhdhptkvtksKIRVYlkp       | Polyprotein | PV1 Mahoney | T              |
| 79155               | altlspkqtdalpdkta           | Polyprotein | PV3 (P3/LEON/37 and P3/LEON12A (1)B) | T              |
| 79160               | atnplapsdtvqrthvq           | Polyprotein | PV3 (P3/LEON/37 and P3/LEON12A (1)B) | T              |
| 79186               | dncgtptraqklfam             | Polyprotein | PV3 (P3/LEON/37 and P3/LEON12A (1)B) | T              |
| 79269               | kevpaltavetgatnpl           | Polyprotein | PV3 (P3/LEON/37 and P3/LEON12A (1)B) | T              |
| 79272               | khvrVWCPRprraprpyyg         | Polyprotein | PV3 (P3/LEON/37 and P3/LEON12A (1)B) | T              |
| 79318               | nghalnqvyqmyppga            | Polyprotein | PV3 (P3/LEON/37 and P3/LEON12A (1)B) | T              |
| 79350               | qkflamwritykdtv             | Polyprotein | PV3 (P3/LEON/37 and P3/LEON12A (1)B) | T              |
| 79354               | qpttraqklfamwri             | Polyprotein | PV3 (P3/LEON/37 and P3/LEON12A (1)B) | T              |
| 79433               | traqklfamwrityk             | Polyprotein | PV3 (P3/LEON/37 and P3/LEON12A (1)B) | T              |
| 79434               | traqklfamwritykdtv          | Polyprotein | PV3 (P3/LEON/37 and P3/LEON12A (1)B) | T              |
| 79435               | trhvqrrsressiesf            | Polyprotein | PV3 (P3/LEON/37 and P3/LEON12A (1)B) | T              |
| 79436               | tskvrimkpkhrrvw             | Polyprotein | PV3 (P3/LEON/37 and P3/LEON12A (1)B) | T              |
| 79443               | vaievdneqtptraqkl           | Polyprotein | PV3 (P3/LEON/37 and P3/LEON12A (1)B) | T              |
| 79461               | vrvnhdhptkvtksvkri          | Polyprotein | PV3 (P3/LEON/37 and P3/LEON12A (1)B) | T              |
| 79480               | yippgaptksWDDYTtwq           | Polyprotein | PV3 (P3/LEON/37 and P3/LEON12A (1)B) | T              |
| 80446               | argacvtimtdnva              | VPI         | PV1 Mahoney | B              |
| 81394               | easgpthskieipalt            | VPI         | PV1 Mahoney | B              |
| 82831               | gpthskieipaltave            | VPI         | PV1 Mahoney | B              |
| 83234               | hskeipaltavetta             | VPI         | PV1 Mahoney | B              |
| 88446               | sdtvqrthvqhrs               | VPI         | PV1 Mahoney | B              |
| 88495               | sessiesfiargacv             | VPI         | PV1 Mahoney | B              |
| 99863               | aaparisvpyvga               | Polyprotein | PV3 Sabin | B              |
| 99886               | ahskeipalvatav              | Polyprotein | PV3 Sabin | B              |
| 99901               | arisvpygianay               | Polyprotein | PV3 Sabin | B              |
| 99910               | AYSHFYDgfkvpl               | Polyprotein | PV3 Sabin | B              |
| 99933               | dfgvlarvvrndnh              | Polyprotein | PV3 Sabin | B              |
| 99963               | dtvqrkrlleffty              | Polyprotein | PV3 Sabin | B              |
| 100029              | famwritykdtvq               | Polyprotein | PV3 Sabin | B              |
| 100101              | hffygagyvpltd               | Polyprotein | PV3 Sabin | B              |
| 100117              | hnpktvskvrrim               | Polyprotein | PV3 Sabin | B              |
| 100138              | iFYFYGaapariv                | Polyprotein | PV3 Sabin | B              |
| 100244              | lanAYSHFYDgfk                | Polyprotein | PV3 Sabin | B              |
| 100349              | npsiffyTYGaapar              | Polyprotein | PV3 Sabin | B              |
| 100382              | pnhvVWCPRpppra               | Polyprotein | PV3 Sabin | B              |
| 100391              | pdsdtvqrthvqrr               | Polyprotein | PV3 Sabin | B              |
| 100425              | qkflamwritykdt               | Polyprotein | PV3 Sabin | B              |
| 100430              | qlrklefftyrfs               | Polyprotein | PV3 Sabin | B              |
| 100439              | qpttraqklfamw                | Polyprotein | PV3 Sabin | B              |
| 100482              | rppravpyygpvvd               | Polyprotein | PV3 Sabin | B              |
| 100492              | samtvddsfyalvr               | Polyprotein | PV3 Sabin | B              |
Table 2: Continued.

| IEDB ID | Epitope sequence<sub>a,b,c</sub> | PV antigen | PV strain | Immune context |
|---------|----------------------------------|------------|-----------|----------------|
| 100504  | sevaqalislplpk                   | Polyprotein | PV3 Sabin | B              |
| 100536  | svpyvglanAYSHF                   | Polyprotein | PV3 Sabin | B              |
| 100559  | tkvtskriympkpk                   | Polyprotein | PV3 Sabin | B              |
| 100573  | traqklamwritry                   | Polyprotein | PV3 Sabin | B              |
| 100575  | tskvriympkphkrv                  | Polyprotein | PV3 Sabin | B              |
| 100576  | tssnspsfYTTYGaa                  | Polyprotein | PV3 Sabin | B              |
| 100580  | tvddfglavrvvnn                   | Polyprotein | PV3 Sabin | B              |
| 100583  | tvqtrhvqrrrs                    | Polyprotein | PV3 Sabin | B              |
| 100585  | twqttssnspsifyty                 | Polyprotein | PV3 Sabin | B              |
| 100586  | tygaaparisvpyyv                 | Polyprotein | PV3 Sabin | B              |
| 100587  | tykdtvqlrkref                    | Polyprotein | PV3 Sabin | B              |
| 100613  | vlavvvnvdnpktk                  | Polyprotein | PV3 Sabin | B              |
| 100619  | vndhnpktvktswkv                  | Polyprotein | PV3 Sabin | B              |
| 100628  | vriymkpkhvrvwc                   | Polyprotein | PV3 Sabin | B              |
| 100630  | vrvvndhpntktvs                   | Polyprotein | PV3 Sabin | B              |
| 100631  | vrvVWCPpprapvpy                  | Polyprotein | PV3 Sabin | B              |
| 100638  | wcprrprapvyyggy                 | Polyprotein | PV3 Sabin | B              |
| 100644  | writykdtvqlrrk                   | Polyprotein | PV3 Sabin | B              |
| 100667  | ymkpkhvrvWCPRp                   | Polyprotein | PV3 Sabin | B              |
| 100672  | yvglanAYSHFYDg                   | Polyprotein | PV3 Sabin | B              |
| 146178  | ayappgaqptprsk                   | Polyprotein | PV3 Sabin | B              |
| 146181  | cgSMMATGkilvay                   | Polyprotein | PV3 Sabin | B              |
| 146248  | fllgcSMMATGkild                   | Polyprotein | PV3 Sabin | B              |
| 146311  | hqgalgfaifepec                   | Polyprotein | PV3 Sabin | B              |
| 146333  | ilvayappgaqptpt                   | Polyprotein | PV3 Sabin | B              |
| 146390  | kftllgcSMMATG                  | Polyprotein | PV3 Sabin | B              |
| 146494  | phqjinlrntnsat                   | Polyprotein | PV3 Sabin | B              |
| 146496  | ppqaqpptsrkeam                   | Polyprotein | PV3 Sabin | B              |
| 146516  | ravyppyyggyvdyn                 | Polyprotein | PV3 Sabin | B              |

<sup>a</sup>PV-derived epitopes are listed according to increasing IEDB ID number. Further details and reference(s) on each IEDB ID are reported at http://www.immuneepitope.org<sup>[17]</sup>.

<sup>b</sup>Only PV-derived epitopes that had been experimentally validated in the human host are reported.

<sup>c</sup>The twenty PV-derived epitopes (and related IDs) containing PV-pentapeptide(s) absent in the human proteome are in bold, with the pentapeptide(s) absent in the human proteome given in capital.

pentapeptides shifted by one residue, characterizes four PV-derived epitopes (IEDB IDs: 59797, 99910, 100244, and 100672); the hexapeptide SMMATG formed by two overlapped 5-mers is present in three epitopes (IEDB IDs: 146181, 146248, and 146390).

Theoretically, vaccines based on such PV epitopic peptides (i.e., KWDDYWQ, AYSHFYD, and SMMATG) might evoke highly specific anti-PV immune responses exempt of possible collateral cross-reactions in the human host.

3.3. Identification of Consensus Pentapeptides Unique to PVs and Endowed with Immunologic Potential. We reasoned that using epitopic peptides unique to the virus and conserved among PV strains might help develop a global anti-PV peptide-based vaccination protocol. Such an approach would be of special importance in providing an effective and wide coverage to the human population worldwide, thus allowing reaching the goal of PV eradication [29, 30]. To this aim, we searched for unique conserved sequences by analysing a set of 43 PV polyproteins selected as described under Section 2 and comprehending also PV variants isolated from faeces of VAPP or AFP or from immunocompromised patient(s) with residual paralysis. The 43 PV polyprotein sequences were aligned using multialignment ClustalW program [24] and the peptide sequences present in PV-derived epitopes (see Table 2) and absent in the human proteome were localized.

Table 3 shows that seven potentially immunogenic peptides (in the order PV<sub>465</sub>–<sub>469</sub>MMAVT, PV<sub>708</sub>–<sub>712</sub>RFDME, PV<sub>752</sub>–<sub>756</sub>DYTQWQT, PV<sub>763</sub>–<sub>767</sub>FYTyG, PV<sub>783</sub>–<sub>789</sub>AYSHFYD, PV<sub>835</sub>–<sub>839</sub>KIRVY, and PV<sub>847</sub>–<sub>851</sub>VWCPR), derived from epitopes corresponding to (or formed of) pentapeptides unique
### Table 3: Conservativeness of epitopic PV peptide regions among 43 PV strains and variants.

| PV strain | UniProt/Swiss-Prot entry | aa Pos 464 | aa Pos 708 | aa Pos 748 | aa Pos 763 | aa Pos 783 | aa Pos 835 | aa Pos 847 |
|-----------|--------------------------|------------|------------|------------|------------|------------|------------|------------|
| PV1 Mahoney | P03300                   | FMMATG     | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV1 Sabin  | P03301                   | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV3 P3/Leon/37 | P03302         | SMATG      | RDFME      | sWDDYTWQT  | FYTYG      | AYSHFVYD  | KvRiY      | VWCPR     |
| PV3 23127 | P06209                   | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KvRvY      | VWCPR     |
| PV1 isolated | Q9Q281               | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV1 isolated | Q9Q280               | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV1 isolated | Q71AZ9               | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV1-HAI01008C2 | D1YSJ9            | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV1-HAI01009 | D1YSJ1              | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV1-HAI01008 | D1YSJ2              | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV1-HAI01002 | D1YSJ3              | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV1-HAI01001 | D1YSJ4              | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV1-DOR01012 | D1YSJ5             | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV1-DOR01002C | D1YSJ6            | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV1-DOR01002 | D1YSJ7             | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV1-DOR01001C1 | D1YSJ9         | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV1-DOR00042C2 | D1YSK1          | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV1-DOR00042C3 | D1YSK2          | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV1-DOR00042 | D1YSK3             | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV1-DOR00028C | D1YSK7             | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV1-DOR000028 | D1YSK8             | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV1-DOR00023C | D1YSK9             | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV1-DOR00025 | D1YSL0              | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV1-DOR24    | D1YSL1              | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV1-DOR00015 | D1YSL2              | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV1-DOR00016 | D1YSL3              | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV1-HAI01013a1l | D1YSL4         | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV1-HAI01015 | D1YSL5              | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV1-S302     | D2X673              | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| CHN8184/GZ/CHN/2004 | D8L541        | SMATG      | RDFME      | KWDYTWQT   | FYTYG      | AYSHFVYD  | KIRVY      | VWCPR     |
| PV3 isolated | B4YUL3              | SMATG      | RDFME      | sWDDYTWQT  | FYTYG      | AYSHFVYD  | KvRvY      | VWCPR     |
| PV3 isolated | B4YUL4              | SMATG      | RDFME      | sWDDYTWQT  | FYTYG      | AYSHFVYD  | KvRvY      | VWCPR     |
| PV3 (vacc.StrainSabin 3 (Leon 12alb)) | Q84792       | SMATG      | RDFME      | sWDDYTWQT  | FYTYG      | AYSHFVYD  | KvRvY      | VWCPR     |
| PV3-33239    | C5HJY2              | SMATG      | RDFME      | sWDDYTWQT  | FYTYG      | AYSHFVYD  | KvRvY      | VWCPR     |
| PV3-33974    | C5HJY3              | SMATG      | RDFME      | sWDDYTWQT  | FYTYG      | AYSHFVYD  | KvRvY      | VWCPR     |
| PV3-FIN84-60212 | D1GE40         | SMATG      | RDFME      | sWDDYTWQT  | FYTYG      | AYSHFVYD  | KvRvY      | VWCPR     |
| PV3-SW10947  | D2E679              | SMATG      | RDFME      | sWDDYTWQT  | FYTYG      | AYSHFVYD  | KvRvY      | VWCPR     |
| PV3-FIN84-2493 | D1GE4i       | SMATG      | RDFME      | sWDDYTWQT  | FYTYG      | AYSHFVYD  | KvRvY      | VWCPR     |
| PV3-P3/finan/1/09 | D2UX59       | SMATG      | RDFME      | sWDDYTWQT  | FYTYG      | AYSHFVYD  | KvRvY      | VWCPR     |

The 43 PV sequences were aligned using ClustalW multialignment program (http://www.uniprot.org/align/) [24]. The analyzed PV sequences and related references are described at http://www.uniprot.org/ and reported by Swiss-Prot/UniProtKB accession number. Peptide sequences present in PV-derived epitopes (see Table 2) and absent in the human proteome were localized along the 43 aligned PV sequences and analyzed for conservativeness. Peptide sequences are indicated by their position along the PV polyprotein. Mutated aa residues are in lower case.
to PV1 (see Tables 1 and 2), have 100% conservation among the 43 PV strains/variants under analysis. The seven potentially immunogenic unique peptide sequences had the same level of conservativeness in PV2 derived strains (data not shown).

4. Conclusion

Anti-PV immunization has been one of the major public health measures of the last century. International campaigns to eliminate polio reduced the incidence of this disease in the world. However, problematic issues remain, such as the tendency of the PV genome to mutate, the potential risk to develop vaccine-related paralytic poliomyelitis, and the difficulty to completely eradicate PV infection in the world. In fact, according to the World Health Organization, in 2012, still three countries in the world remain polio-endemic: Nigeria, Pakistan, and Afghanistan [31].

The present data propose the concept of sequence uniqueness as a tool to define specific immunotherapies exempt of collateral effects [22] and describe a methodology to identify PV peptides that have zero percent identity to human proteins, are endowed with an immunologic potential, and are highly conserved among PV strains (e.g., PV2_465–469, MMTATG, PV708–712, RFDM, PV752–756, DYTQST, PV763–767, FYTYG, PV783–789, AYSHFYD, PV835–839, KIRVY, and PV847–851 VWCPR; see Table 3). Importantly, polio peptide sequences alternate through the human proteome with a frequency versus rarity pattern that characterizes other pathogens too [32–35].

Theoretically, such viral consensus epitope peptides appear to be ideal tools to generate anti-PV immune responses promising of high specificity, thus avoiding serological cross-reactivity between human polyomaviruses [23], as well as possible cross-reactions with the human host [22]. As an example, a construct composed of the coding frames corresponding to the immunogenic consensus sequences described above (Table 2) might determine a specific anti-PV immune response and, at the same time, by being based on peptides, might eliminate the issues inherent to the tendency of the PV genome to mutate (i.e., VAPP). Such viral peptide sequences might also be used in passive anti-PV immunotherapies, that is, to produce specific antibodies capable of reacting with intact viral protein antigens.

Actually, the present report is intended to represent a first approach to preclinical and animal studies. As a matter of fact, the solidity of a large body of theoretical and in silico data is the mandatory basis to design in vivo experimentation and validation protocols especially when considering that (i) although monkeys can be experimentally infected, humans are the only known natural hosts of poliovirus; (ii) small animal models for testing polio pathogenesis mainly relate to transgenic mice to express a human receptor to poliovirus [36]; and, moreover, (iii) current laws on in vivo experimentation are increasingly restrictive.

Conflict of Interests

The authors declare that this paper is based on research that was not funded entirely or partially by an outside source.

Authors’ Contribution

Darja Kanduc proposed the original idea, supervised the work, interpreted the data, and wrote the paper. All authors contributed to the computational analyses; Giovanni Capone also contributed to the project definition. All authors discussed and approved the paper.

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