[SV-4]

Development of Safe and Effective rec-OPV Using Poliovirus Sabin 1-derived Mucosal Vaccine Vector

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Abstract

This work was initiated to develope a recombinant oral poliovaccine (OPV), which is highly advanced in safety (minimizing VAPP) by introducing Type 2, 3 poliovirus epitopes into our RPS-Vax system. We have introduced several potential vaccine epitopes of poliovirus Type 2, and 3 into RPS-Vax system, resulting in production of recombinant polioviruses. Any of these chimeric viruses, however, were not detected for their foreign gene expression by serotype-specific mouse antiserum. We have designed several folding units to stabilize the introduced vaccine protein and attached short epitope-concatamer or epitope-multimer to them, followed by production of chimeric viruses. Only those who have an HIV-1 Tat-mediated folding unit were nicely detected for the introduced foreign proteins by anti-Tat antiserum and type-specific peptide-induced antisera. Nevertheless, introduced epitopes were not detected in Western blot experiment with each serotype-specific antiserum. None of the mice inoculated with these chimeric viruses showed preventative immunity when challenged with Lansing and Leon wildtype 2 and 3 poliovirus, and the antiserum did not show neutralizing capacity *in vitro*. Conformational epitope covering B/C loop region of type 2 and 3 were newly designed by computer modeling, and introduced into the RPS-Vax vector system, followed by production of chimeric viruses. Introduced epitope regions were nicely detected by anti-Tag23 mAb or peptide antibody, but still not detected by poliovirus antiserum. Nevertheless, neutralizing antibody was detected in the Tg-PVR mice even when inoculated once with these chimeric viruses. Also, the immunized mice showed perfect preventative immunity against the wild Type poliovirus Lancing or Leon. When boosted appropriately, those chimeric virus-inoculated Tg-PVR mice produced equivalent amounts of neutralizing antibody to those in Sabin 2/3-immunized mice. These data strongly suggest that our recombinant poliovirus (RPS-PV2 and RPS-PV3) can be used as a safe and effective rec-OPV instead of any preexisting poliovaccine.

Results and Discussion

Poliovirus is a causative agent of paralytic polyomyelitis, which was one of the most serious fatal diseases in the early half of the 20th Century in the world. However, the incidence of polio has been markedly reduced during the later half century by successful vaccine programs. According to the WHO reports, the incidence was down to the 3500 cases in the year of 2000 all over the world. However, these official case reports have

been assumed to be 1/10th of the real incidence in the world. The incidence in India has been increasing recently by 25% an year.

Four kinds of polio vaccines have been widely used up to now. By the time of Salk vaccine became available in 1953, there were about 200,000 cases per year of paralytic poliomyelitis in the USA. But the number of cases has decresed to 1000/yr in 1962 on behalf of the development of formalin inactivated Salk vaccine. After that Sabin and his colleagues developed three attenuated much more immunogenic poliovirus vaccines. We call Sabin 1, 2 , and 3 oral polio vaccines (OPV) which were generated from the three virulent type of poliovirus by passages in cell culture, which maintains the replication capacity in enteric organ, but looses neuro-tropism. OPV was first approved in the USA in 1961, and into general use by 1963. Since then, the number of cases was reduced to 5-8/yr at present, and most of the recent cases are vaccine-associated polio. OPV has been used as one of the most successful vaccine programs against polio during the last 40 yrs. Recently, however, it has been reported that, even though in a very rare cases, OPV causes VAPP to the vaccinee. That's the reason why many developed countries preferred eIPV to OPV for the reasons of safety. However eIPV has also its own drawbacks such as inefficiency for mucosal immunogenicity and high titer of virus required for single immunizations. That's why the eIPV is not recommended in the high endemic area. IPV/OPV combination vaccine program has also limitation such as preexisting immunity induced by IPV may attenuate the OPV-mediated mucosal immunogenicity instead of boosting effects.

Our work was initiated to develop a safe and effective rec-OPV, whose immunogenicity is equivalent to that of the OPV but is completely free from the risk of VAPP caused by traditional OPV. Most of the VAPP were caused by back mutations of Sabin 2 and 3 in the OPV, but little by Sabin 1. The safety of the Sabin 1 was approved in clinical aspects during the last 4 decades. That's why we constructed Sabin 1-derived vaccine vector, and named RPS-Vax and integrated neutralizing epitopes of type 2 and 3 into this vector system to produce safe and effective rec-OPV. Another purpose of this experiment is to demonstrate the usability of the RPS-Vax vector system for the development of an effective live mucosal vaccines by showing preventative immunity in the challenge experiment to the recombinant chimeric virus-immunized Tg-PVR mice.

Poliovirus belonging to the Picornavirus family has a (+) sense ss-RNA viral genome, which encodes single long polyprotein processed by viral specific 3C-protease into structure and functional proteins. Structure proteins encapsidate viral genome, resulting in the production of icosahedral virons. We have constructed an RPS-Vax vector system by modification of Sabin 1 cDNA. RPS-Vax system contains MCS and artificial 3C-protease recognition site at the end of the N-terminal end of the Sabin 1 poliovirus cDNA. Foreign genes can be easily introduced into the MCS of the RPS-Vax vector system.

Type 2, 3 neutralizing epitope regions of poliovirus were incorporated into MCS of RPS-Vax, followed by production of replication-competent rec-OPVs by transfection of Hela cells with its RNA transcript. These chimeric viruses were tested for their immunogenicity against type 2, 3 polioviruses in Tg-PVR mice. Each chimeric virus produce 60 copies of introduced foreign proteins per virion during their replication in vivo. Neutrlizing epitope-containing VP1 region of type 2 or type2/type 3 heterodimer were introduced to generate a chimeric viruses. The region of VP1 around the rim of canyon area is the major neutralizing epitope of the poliovirus. Beside, many of these neutralizing epitopes were well defined in the poliovirus. Based on the

sequences of major neutralizing epitopes of type 2, 3 poliovirus, we have constructed 6 different neutralizing epitope concatamers and incorporated them into the RPS-Vax vector system, respectively, to enforce the preventative immunity against Type 2 and 3, followed by production of recombinant viruses. We used HIV-1 Tat as a folding unit to stabilize the expressed rec-protein against chaperon-mediated intracellular degradation. The recombinant proteins were nicely detected in the chimeric virus-infected HeLa cells by anti-Tat antibody or Type 2 or type 3-specific peptide antibody but not by each serotype-specific viral antiserum. Most of these concatamer-containing chimeric viruses were genetically unstable during the passages. The genetic instability of these chimeric viruses was remedied by modification and reconstruction of the nucleotide sequences following the novel insert-designing rules developed in our Lab and published in J. Virol (Lee et al, 76: 1649-1662, 2002).

These chimeric viruses were tested for their immunogenicity in Tg-PVR mice. Most of the control OPV-immunized mice were survived in the lethal dose challenge of wt poliovirus and the preventative immunity was very serotype-specific. However none of the mice immunized with chimeric polioviruses expressing type 2/3 epitope concatamers showed preventative immunity against the challenge of wt poliovirus. It means that neutralizing epitope concatamer was not effective to induce preventative immunity probably due to the disruptions of confrontation epitope shown in control virus. To introduce a stable conformational epitope of the B/C loop covering the major neutralizing epitope of VP1 of type 2, 3 poliovirus into the MCS of the RPS system, we have designed the inserts with the aid of the PC-based molecular modeling, and found that around 110 aa of internal VP1 is essential to retain the conformation of the epitope. Six different inserts covering the region of the major neutralizing epitope of serotype 2 poliovirus, were designed and integrated into the RPS system, followed by production of chimeric polioviruses. These were designed to have tagging sequence to detect easily when expressed. Also, 5 different inserts covering the same region of type 3 poliovirus were designed and integrated into the MCS of RPS system, followed by production of chimeric viruses, which were confirmed for their integrity of the foreign insert by RT-PCR of each clone, but 2 chimeric viruses for type 2 insert and 1 chimeric virus for type 3 insert were not detected for their expression by each serotype-specific peptide Ab. Those clones undetected in Western blot might be due to the conformational disruption or protein degradation of the introduced foreign inserts. The chimeric viruses were tested for their replication capacity by examining one-step growth curve. As expected, both chimeric viruses expressing type 2 epitope of 138 aa and type 3 epitope of 138 aa, showed the similar replication capacity to that of control Sabin 1 poliovirus.

Both chimeric viruses were also genetically stable at least 12 consecutive passages when checked by RT-PCR and Western blot experiments. All of the chimeric viruses were tested for their genetic stability and immunogenicity, and were summarized. Most of the chimeric viruses showed genetic stability during the 12 passages except this clone. Among the 6 chimeric viruses containing the type 2 inserts, 2 clones were effective enough to induce 100% protective immunity against wild type Lancing poliovirus when inoculated into the Tg-PVR mice. On the other hand, among the 5 chimeric viruses containing type 3 poliovirus insert, only one clone containing 138 aa insert induced 100% protective immunity against the challenge of wt Leon type 3 poliovirus. Whereas, those chimeric viruses containing short or genetically unstable inserts did not show any complete

protective immunity in the Tg-PVR mice. Neutralizing antibodies were measured in each chimeric virus-immunized mouse. In good agreement with the previous results, those mice demonstrating protective immunity also showed the high titer of the neutralizing antibody. Based on these experimental results, we have selected RPS-PV2-138 and RPS-PV3-138 as vaccine candidates for rec-OPV. Finally we have performed comparative studies for the immunogenicity of rec-OPV together with other vaccine program in Tg-PVR mice. OPV vaccine program was the best for the 100% protective immunity in the immunized mice against the challenge of wt poliovirus, Mahoney, Lancing and Leon.

IPV/OPV/OPV program used in USA and other developed countries showed preventative immunity, but was not so effective as shown in OPV/OPV/OPV program and the titer of neutrlizing Ab in the immunized mice was about 15% of that of OPV program. IPV/IPV/IPV program was not effective at all in our experiment. This result seemed to be, at least in part, due to the lower titer of IPV. Actually, we used 500 times lower titer of virus than in commercial IPV, moreover we used Sabin 1, 2, 3 as a source of IPV instead of wt poliovirus. Nevertheless, our rec-OPV immunized mice showed 100% protective immunity against wild type poliovirus when boosted twice with our inefficient IPV, and the titer of neutralizing Ab was over 85% of those shown in OPV/OPV/OPV program-immunized mice. It means that our rec-OPV/IPV/IPV program is very effective to induce the protective immunity against type 2 and 3 of poliovirus even though the rec-OPV expresses only an epitope of type 2, 3 polioviruses, respectively. It is a first report that shows the 100% preventative immunity against different serotypes of poliovirus with rec-polioviruses. In order to confirm the immunogenicity of rec-OPV, we have repeated the previous experiments with commercial IPV for boosting at a conc of 100 times lower in the titer of virus than those for the normal boosting. Our rec-OPV program was as efficient as that of traditional OPV program when boosted twice with lower titer of IPV. IPV program itself or IPV/OPV/OPV program induced nAb, but was not so efficient as our rec-OPV/IPV/IPV program for induction of protective immunity.

Taken together, our experimental results strongly suggest that the rec-OPV can be used as a safe and effective poliovaccine which has the similar immunogenicity to that of wild type OPV, but very unlikely to cause VAPP when vaccinated and boosted.