CHARACTERISATION OF MONOCLONAL ANTIBODY RESISTANT POLIOVIRUS MUTANTS BY DEEP SEQUENCING ANALYSIS

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Poliovirus is a human enterovirus responsible for paralytic poliomyelitis and exists as three distinct serotypes. The antigenic regions recognised by neutralising antibodies on the surface of poliovirus have been extensively studied by the characterisation of plaque-purified monoclonal antibody (mAb) resistant viruses. However, the antigenic profile of poliovirus type-2 (PV2) is not fully established, as there are some conflicting results on the location of independent antigenic sites. It is important to fully characterise the antigenic structure of PV2 in order to better understand the impact of poliovirus evolution on antigenic changes in humans and the relevance of such mutations on vaccine immunogenicity/efficacy. This would help the design and characterisation of new vaccines that might still be required for the endgame of poliovirus eradication.

Objectives. This study aimed to use deep sequencing (DS) analysis of mAb-resistant poliovirus mutants as a means to fully refine the antigenic profile of PV2. We hypothesise that this technique will provide a more comprehensive structural profile of PV2, compared to conventional plaque purification techniques.

Methods. Two independent populations of Sabin strain PV2 were generated from purified vaccine seed virus preparations by passage in poliovirus sensitive HEp-2c cells at a multiplicity of infection (MOI) of 0.01 infectious virus per cell. A low MOI avoids “phenotypic hiding” of mutant genomes. These PV2 populations were challenged with a panel of type-2 specific mAbs. Serial viral dilutions were used to infect HEp-2c cells in the presence of antibody in successive passages. The virus RNA was then extracted and the whole genome amplified using a One-Step RT-PCR system. The viral genomes were purified and processed by DS on the Illumina MiSeq. Single nucleotide polymorphism (SNP) variations from the consensus Sabin type-2 sequence (GenBank AY184220) were analysed for mutations which likely confer antibody resistance.

Results. We found that DS techniques can much more sensitively detect mutations in the poliovirus genome: detecting variants present in less than 1% of the sample. This is in comparison to plaque purification techniques which would require the characterisation of numerous plaque-purified viruses before detection of mutations at this level.

Results revealed that mutant PV2 populations generated with a type-2 specific antibody contained 14 structural mutations which were likely selected for by antibody pressure, as visualised on a 3D protein model. The mutations mapped to amino acid residues previously located at antigenic sites 3a and 3b, confirming that they are part of a unique antigenic site. The number and proportion of mutants found in antibody-resistant populations varied depending on the MOI in the first passage, which was directly related to the proportion of mutants present in the original population. This was presumably generated by the high viral polymerase error rate.

Conclusion. This repeatable method provides evidence which is promising to more comprehensively define the antigenic sites of PV2 with our larger panel of mAbs. This would be useful in the post-eradication era of PV2, particularly in characterising circulating vaccine-derived PV2 strains which may reappear in the population and the environment.