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Mapping of the epitopes of poliovirus type 2 in complex with antibodies

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ABSTRACT

The inactivated polio vaccine (IPV) contains poliovirus (PV) samples that belong to serotypes 1, 2 and 3. All three serotypes contain the D-antigen, which induces protective antibodies. The antigenic structure of PVs consists of at least four different antigenic sites and the D-antigen content represents the combined activity of multiple epitopes (Ferguson et al., 1993; Minor, 1990; Minor et al., 1986). The potency of IPV vaccines is determined by measuring the D-antigen content. Several ELISA methods have been developed using polyclonal or monoclonal antibodies (Mabs) in order to quantify the D-antigen content. Characterization of the epitopes recognized by the different Mabs is crucial to map the entire virus surface and ensure the presence of epitopes able to induce neutralizing antibodies. Using a new approach that we developed to study the interaction between monoclonal antibodies and poliovirus type 2, which combines cryo-electron microscopy, image analysis and X-ray crystallography along with identification of exposed amino acids, we have mapped in 3D the epitope sites recognized by three specific Fabs at the surface of poliovirus type 2 (PV2) and characterized precisely the antigenic sites for these Fabs.

1. Introduction

Poliovirus (PV) is a member of the picornaviridae family and is the causative agent of poliomyelitis in human. PVs are human enteroviruses and like all enteroviruses, are pH stable and survive passage through the stomach and to the intestine were they replicate (Melnick, 1996). PVs cause the destruction of the motor neurons in the central nervous system. PV infection induces both innate and adaptive immune responses. Nevertheless, PV seems to weaken the innate response by interference with the Interferon system, allowing infection to be established. Adaptive immunity then takes over, producing neutralizing antibodies which induce in most cases viral clearance (Dotzauer and Kraemer, 2012). Due to an extensive vaccination campaign the incidence of poliomyelitis has been reduced drastically (Nathanson and Kew, 2010). For decades the successful fight against this disease has relied on the use of two different vaccines. The formalin-inactivated PV vaccine (IPV), developed by Salk et al. (1954), was the first vaccine to be licensed followed by the oral PV vaccine (OPV) developed by Sabin (1957). The last decade saw an increase in the use of IPV as compared to OPV which was due to the excellent safety and efficacy record of IPV as well as cost-effective production process for its production (Chumakov and Ehrenfeld, 2008). Even though IPV is less effective in stimulating enteric immunity than live vaccine. The IPV is formulated as a trivalent product containing a representative virus isolate of each serotype (Mahoney, MEF-1 and Saukett). All three serotypes contain the D-antigen, which induces protective antibodies. The antigenic structure of PVs consists of at least four different antigenic sites and the D-antigen content represents the combined activity of multiple epitopes (Ferguson et al., 1993; Minor, 1990; Minor et al., 1986). The potency of IPV vaccines is determined by measuring the D-antigen content. Several ELISA methods have been developed using polyclonal or monoclonal antibodies (Mabs) in order to quantify the D-antigen content. Characterization of the epitopes recognized by the different Mabs is crucial to map the entire virus surface and ensure the presence of epitopes able to induce neutralizing antibodies.

Picornaviruses are small, spherical (≈300 Å in diameter) and are composed of an RNA genome that is about 7500 nucleotides long and four protein capsid. It is a non-enveloped (+) RNA virus. The PV capsid is characterized by an icosahedral symmetry and composed

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of 60 copies of each of four capsid proteins designated VP1, VP2, VP3 and VP4.

The atomic three-dimension structure of types 1, 2 and 3 PVs have been determined (Filman et al., 1989; Hogle et al., 1985; Lentz et al., 1997) and also the chimeric type 2/type 1 PV (Yeates et al., 1991). It has been shown that capsid proteins VP1, VP2 and VP3, but not VP4 are exposed on the surface of the virus. VP1, VP2 and VP3 share a common core structure; all three polypeptides are composed of eight antiparallel β-strands which are connected by loops. These loops form protruding features on the surface of the virion. The comparison between the three serotypes revealed that the structural differences among the three PV serotypes occur primarily in the loop regions of the viral capsid proteins VP1, VP2 and VP3 (Lentz et al., 1997).

In previous work, using a new approach, combining cryo-electron microscopy and image analysis with X-ray crystallography along with identification of exposed amino acids, we have mapped in 3D the epitope sites recognized by five specific Fabs and one Mab and characterized precisely the antigenic sites for these Mabs on PV1. Mab is a monoclonal antibody Fab (fragment antigen-binding region). In contrast to Mab which has two heavy chains and two light chains, Fab is smaller and is composed of one constant and one variable domain from each heavy and light chain of the antibody. In particular, we mapped for the first time the Mab73-5-5 epitope, at the bottom of the canyon where the receptor CD155 binds the poliovirus (Bannwarth et al., 2015). CD155, a transmembrane glycoprotein, is of particular importance as it is a poliovirus receptor and is involved in the cellular poliovirus infection in primates.

In this study, we were interested in the binding of four Fabs (1037, 1050, 1121 and 159-7) to PV2. Using the same method described in (Bannwarth et al., 2015), we describe the precise amino acid residues and the epitopes required for the binding of three out of four Fabs (1037, 1050 and 1121) while binding of Fab159-7 to IPV2 was too weak to allow precise identification of the amino acids involved. This method combines medium-resolution 3D maps of complexes between PV type 2 (PV2) and monoclonal antibody fragments obtained from cryo-electron microscopy (cryoEM) images and known structure of the PV2 at high resolution determined using X-ray crystallography.

This structural information is complementary to the data obtained by analysis of antigenic mutants (Blondel et al., 1986; Diamond et al., 1985; Minor et al., 1983, 1985; Page et al., 1988), chimera (Minor et al., 1991) and escape mutants (Ketterlinus et al., 1993; Minor et al., 1986; Wiegers et al., 1988), which have been used to map the viral antigenic sites. These combined studies could be used as a guide in order to select antibodies able to map the entire "epitopic" surface of the virus.

2. Materials and methods

2.1. Poliovirus, monoclonal antibody and Fab Production

2.1.1. PV samples

Samples were provided by the Production Department of Sanofi Pasteur. They included inactivated monovalent poliovirus type 2 (MEF-I strain) (IPV2).

2.1.2. Monoclonal antibodies (Mabs)

The following purified Mabs where obtained from the National Institute for Biological Standards and Control (NIBSC): anti-type 2 IPV Mabs 1037, 1050, 1121. The generation and characterization of these Mabs have been reported by Minor et al. (1986). Mab 159-7 was obtained from the Health Canada's Biologics and Genetic Therapies Directorate (BGTD). All these Mabs neutralize the MEF-I type 2 strain (data not shown).

2.1.3. Fab production

Fabs were generated from the corresponding Mabs by papain digestion and then protein A chromatography purification. SDS-PAGE analysis for the Fabs are shown in Fig. S1 in non-reducing and reducing conditions. Light and heavy chains are intact.

2.2. Virus–Fab complex formation, CryoEM, and 3D image reconstruction

2.2.1. Preparation of Polio/Fab complexes

Before incubation with IPV2, Fabs were concentrated using Amicon Ultra Cell (0.5 mL) with a cut-off of 10 kDa. Final concentrations were 2.3 mg/mL for Fab1050, 3.28 mg/mL for Fab1037, 0.4 mg/mL for Fab1121 and 0.1 mg/mL for Fab 159-7.

IPV2 and Fabs were incubated for 2 h (Fab1037 and Fab1050) or 3 h (Fab1121 and Fab159-7) at room temperature at a molar ratio of 1:600. Formation of the virus–antibody complex was monitored by taking electron micrographs of negatively stained specimen every 30 min. Excess (unbound) Fabs were removed by passing through an Illustra MicroSpin^{TM} S-300 HR column (GE Healthcare).

2.2.2. Preparation of hydrated frozen specimens

Small aliquots (3.5 μL) of the complexes were deposited on carbon-coated electron microscope grids for routine observation using negative staining. For observation in cryo conditions, 3.5 μL of complex was applied to holey carbon-coated grids blotted with filter paper for 3 s and rapidly plunged into liquid ethane, cooled to −175 °C by liquid nitrogen. Specimens were kept at −180 °C using a Gatan cryo-holder in a JEOL 2100F microscope operating at 200 kV. Images were recorded under low-dose conditions (<20 e^-/A^2) using an ultrascan 4k Gatan camera at a nominal magnification of ×39160 with a pixel size of 3.5 Å at the given magnification.

2.2.3. Image analysis

Particles were selected and normalized using EMAN software (Ludtke, 2010). The number of IPV2–Fab complexes selected for the image analysis varied according to the experiment, i.e. 2238 IPV2–Fab1037 complexes, 3892 IPV2–Fab1050 complexes, 356 IPV2–Fab1121 complexes and 3023 IPV2–Fab159-7 complexes. In addition, 750 particles were selected for the reconstruction of the virus IPV2 alone.

The 3D reference model used to start the image analysis was calculated from the available PV2 structure (PDB code: 1eah for type 2) (Lentz et al., 1997). The PDB structure was converted into a volume and filtered with a low-pass filter to a resolution of 30 Å.

3D reconstruction from the picked single-particle images was performed with XMIPP software (Scheres et al., 2008), using the projection-matching angular refinement method that aligns experimental images with projections of the 3D model obtained in the previous iteration (3D reference). Orientation refinement was monitored by correlation coefficients.

The resolution of the resulting reconstruction was estimated by splitting the image data into two sets and comparing Fourier Shell Correlation (FSC) obtained in the separate reconstructions using the criterion of 0.5. Fourier Shell curves are shown in Fig. S2. The class averages of the various 3D reconstructions are also shown in Fig. S3. The defocus level was calculated for each image and used to calculate the phase-contrast transfer function. The defocus in μm was between 1 and 4 μm. Isosurface representations of the reconstructed density were visualized using UCSF Chimera (Pettersen et al., 2004)

2.2.4. Fitting X-ray structures into the CryoEM Volumes

The X-ray atomic structure of the PV2 (PDB code 1eah) was fitted back into the cryoEM volumes using the UCSF Chimera software. The cryo-electron microscope reconstructed density map of the