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Characterization of human monoclonal antibodies that neutralize multiple poliovirus serotypes

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ABSTRACT

Following the eradication of wild poliovirus (PV), achieving and maintaining a polio-free status will require eliminating potentially pathogenic PV strains derived from the oral attenuated vaccine. For this purpose, a combination of non-cross-resistant drugs, such as small molecules and neutralizing monoclonal antibodies (mAbs), may be ideal. We previously isolated chimpanzee and human mAbs capable of neutralizing multiple PV types (cross-neutralization). Here, we describe three additional human mAbs that neutralize types 1 and 2 PV and one mAb that neutralizes all three types. Most bind conformational epitopes and have unusually long heavy chain complementarity determining 3 domains (HC CDR3). We assessed the ability of the mAbs to neutralize A12 escape mutant PV strains, and found that the neutralizing activities of the mAbs were disrupted by different amino acid substitutions. Competitive binding studies further suggested that the specific mAb:PV interactions that enable cross-neutralization differ among mAbs and serotypes. All of the cloned mAbs bind PV in the vicinity of the "canyon", a circular depression around the 5-fold axis of symmetry through which PV recognizes its cellular receptor. We were unable to generate escape mutants to two of the mAbs, suggesting that their epitopes are important for the PV life cycle. These data indicate that PV cross-neutralization involves binding to highly conserved structures within the canyon that binds to the cellular receptor. These may be facilitated by the long HC CDR3 domains, which may adopt alternative binding configurations. We propose that the human and chimpanzee mAbs described here could have potential as anti-PV therapeutics.

1. Introduction

The worldwide polio eradication campaign will soon end the circulation of wild type polioviruses (WPV) [1]. This will have been accomplished through the use of the Sabin oral attenuated polio vaccine (OPV). However, OPV can give rise to circulating vaccine-derived polioviruses (cVDPVs), which can acquire a neuroparalytic phenotype that is clinically similar to wild type PV [2]. cVDPVs have been responsible for multiple outbreaks of acute flaccid paralysis (AFP) among children in recent years [3]. An additional complication is that individuals with B-cell immune deficiencies who receive OPV may become chronic shedders of immunodeficiency-associated vaccine-derived polioviruses (iVDPVs) and potentially introduce these mutant viruses to the population [4–6]. The effort to eradicate clinical poliomyelitis will therefore require stopping the use of OPV use and eliminating any reservoirs of cVDPVs and iVDPVs.

The Global Polio Eradication Initiative (GPEI) is directing a transition from OPV to the inactivated polio vaccine (IPV) by 2020 [1]. Although IPV protects from AFP, it does not provide complete mucosal immunity. OPV and wild PV can readily propagate among populations that have been primarily vaccinated with IPV, giving

Abbreviations: AA, amino acid; AFP, acute flaccid paralysis; CDR3, complementary determining region 3; CPE, cytopathic effect; cVDPV, circulating vaccine derived poliovirus; GPEI, Global Polio Eradication Initiative; HC, immunoglobulin heavy chain; IPV, inactivated polio vaccine; LC, immunoglobulin light chain; iVAPP, immunodeficient vaccine associated paralytic poliomyelitis; iVDVP, immunodeficiency-associated vaccine derived poliovirus; mAb, monoclonal antibody; OPV, oral attenuated polio vaccine; PV, poliovirus; WPV, wild poliovirus.

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rise to AFP cases in an area of low vaccination rates, and iVDPVs pose a similar risk [7–10]. To contribute to a post-eradication risk management strategy, the National Research Council proposed that at least two mechanistically distinct PV drugs be developed [11]. These could be used to stop chronic iVDPV shedding in immunodeficient subjects, who cannot be cured by vaccination, and to stop circulation of cVDPVs, during and after the OPV to IPV transition [12,13]. Passive immunization with monoclonal antibodies (mAbs) may be effective against symptomatic PV infection, as well [14]. Therapeutic mAbs generally have excellent safety profiles and long half lives, and are not likely to be cross resistant with small molecules [15,16].

Although the existence of three serologically distinct PV types (1, 2, and 3) implies that few, if any, epitopes are shared among the types, our studies of the chimpanzee and human IgG response to PV antigens reveals that such epitopes exist and can be effective when targeted by passive immunization. The A12 mAb is a human/chimpanzee chimeric IgG that neutralizes both type 1 and type 2 Sabin PV, WPV and VDPV strains [17]. In the TgPVR21 transgenic mouse model of PV infection, A12 provided 100% protection from lethality when administered before WPV exposure and partial protection if given afterwards [18]. In studies of the human IgG response to PV, we identified mAb from volunteer P4, P4-12F8, which neutralizes types 1 and 2 Sabin and WPV strains, and 1E4, which neutralizes types 1 and 3 [16]. All three mAbs have strong activity against a wide variety of wild and vaccine-derived clinical PV isolates [16,18], indicating the potential clinical utility of mAbs that bind cross-neutralizing epitopes.

Passive immunization against PV will likely require two or more mAbs to prevent the outgrowth of resistant strains. It is therefore important to understand the nature and diversity of the cross-neutralizing antibody response to PV. A12 and P4-12F8 interact with amino acids (AAs) in the canyon domain, which surrounds the fivefold axis of symmetry and is where the virus interacts with its cellular receptor, CD155 [19,20]. The critical role of this domain in the viral life cycle may account for a relatively conserved antigen structure. Yet, A12 binds different AAs on type 1 and type 2 PV and at different angles [20]. In addition, P4-12F8 neutralized some A12 escape mutant PV strains, but not others [16]. These results suggest that multiple epitopes exist in type 1 and 2 PV that are capable of initiating a cross-neutralizing mAb response. To explore this hypothesis further, we cloned and studied additional human PV mAbs that neutralize types 1 and 2 PV.

2. Materials and methods

2.1. Volunteer blood sample donors

Two individuals, aged 30–35 years (Donors P3 and P4) and another individual, age > 60 years (Donor P6) were involved in the current study (Supplemental Table 1). Donor P3 and donor P4 formerly lived in a PV endemic country and were exposed to multiple doses of OPV vaccination. Donor P6 had a possible childhood exposure to WPV as well as multiple lifetime exposures to OPV and IPV. The donors each received a dose of trivalent IPV eight days prior to the blood sampling. Work with human peripheral blood cells was performed with full informed consent, under a protocol approved by the Main Line Hospitals Institutional Review Board and consistent with the principles set out in the WMA Declaration of Helsinki and the NIH Belmont Report.

2.2. Hybridoma generation and PV ELISAs

Hybridoma methods were performed and poliovirus specific clones were identified by immunocapture ELISA, as described previously [16]. Briefly, 1:10,000 dilution of pooled rabbit polyclonal PV antiserum was coated to ELISA plates in carbonate coating buffer and incubated at 4 °C overnight. The plates were washed 3 times with PBS-T (300 μl/well) and blocked (200 μl/well) with 5% calf serum, 3% normal goat serum and 2% normal rabbit serum. After blocking, pooled Sabin PV (~7 log 10 TCID50/ml, 50 μl/well) was added at 1:100 dilution in blocking buffer and incubated for 2 h at 37 °C. For experiments in which we assessed binding to heat-denatured Sabin (H antigen), we heated the Sabin PV at 56 °C for 1 h before testing. After washing the PV binding reactions with PBS-T, hybridoma supernatants or purified mAbs diluted in PBS-T (50 μg/ml) were added in triplicate (100 μl/well) and incubated at 37 °C for 1–2 h, followed by PBS-T wash; and mAb binding was detected with a 1:1500 dilution of HRP-conjugated mouse anti-human IgG Fc specific secondary mAb (9040-05; Southern Biotechnology, Birmingham, AL) [16]. As positive and negative controls, we used LX_10C6, a human mAb that binds D and H antigens from all three PV types, but has no neutralization activity (data not shown), and the human 3B3 mAb, which does not bind PV [21]. IgG heavy chain and light chain subtypes were determined by ELISA, and variable domain DNA sequences were obtained as described [16]. Sequences were analyzed with the IMGT website (http://www.imgt.org).

2.3. Virus strains

Sabin polioviruses were US reference stocks NA4 (Type 1), NB2 (Type 2), and NC2 (Type 3). Wild type poliovirus strains were Mahoney (Type 1), MEF1 (Type 2), and Saukett (Type 3) provided by Dr. Emmanuel Vidor, Sanofi-Pasteur. Wild and vaccine derived polioviruses (VDPV) of Type 1 were provided by Dr. Olen Kew, CDC. Dr. Jan Felix Drexler, University of Bonn, provided the RC2010 poliovirus strain [22].

Poliovirus escape mutant strains were generated as described previously [16,17]. Briefly, the antibodies were diluted to approximately 200 μg/ml in medium and sterilized through a Spin-X column. Four 5-fold dilutions of antibodies starting at 100 μg/ml were incubated for 3 h at room temperature with approximately 10^8 TCID50 PV, followed by incubation for 2 h at 36 °C. Monolayers of HEp-2c cells were inoculated with virus and incubated at 36 °C, 5% CO_2 until a cytopathic effect (CPE) developed. The virus-containing cell suspension was subjected to three freeze-thaw cycles to release intracellular virus and then clarified by centrifugation, and the viral titer was determined by microtitration [21]. MAb resistant viruses were plaque purified as described [16,17].

2.4. Poliovirus microneutralization test

PV-neutralizing mAb titers were determined in a microneutralization test [17,23]. The mAbs were diluted to the indicated concentrations (1 μg/ml) in maintenance medium (DMEM supplemented with 2% FBS and 1% antibiotic/antimycotic solution; Thermo Fisher Scientific) and sterilized by filtration through Spin-X columns (Corning, Corning, NY). The antibodies were tested at the concentration indicated in legends. Twofold serial dilutions of the mAbs (in duplicates or triplicates) were incubated for 3 h at 36 °C with 100 50% tissue culture infectious doses ($TCID_{50}$) of the respective poliovirus strain in an atmosphere of 5% CO_2. After the incubation, 1×10^4 HEp-2c cells were added to the wells. The plates were incubated for 10 days at 36 °C, 5% CO_2 and evaluated microscopically. Neutralizing antibody titers were calculated using the Kärber formula [23].

2.5. Antibody competitive binding assay

We used a competition assay to test whether biotinylated A12 could bind PV types 1 and 2 in the presence of the human mAbs.

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