Antiviral Research 108 (2014) 36–43

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Human monoclonal antibodies that neutralize vaccine and wild-type poliovirus strains

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ARTICLE INFO

Article history:
Received 6 January 2014
Revised 28 April 2014
Accepted 3 May 2014
Available online 10 May 2014

Keywords:
Poliovirus
Antiviral therapeutic
Neutralization
Epitope
Chronic viral infection
Eradication

ABSTRACT

An essential requirement for eradication of poliomyelitis is the elimination of circulating vaccine derived polioviruses (cVDPV) and polioviruses excreted by chronically infected individuals with immunodeficiencies (iVDPV). As part of a post-eradication risk management strategy, a human monoclonal antibody (mAb) therapeutic could play a role in halting excretion in asymptomatic carriers and could be used, in combination with vaccines and antiviral drugs, to protect polio-exposed individuals. Cross-neutralizing mAbs may be particularly useful, as they would reduce the number of mAbs needed to create a comprehensive PV therapeutic. We cloned a panel of IgG mAbs from OPV-vaccinated, IPV-boosted healthy subjects. Many of the mAbs had potent neutralizing activities against PV wild-type (WT) and Sabin strains, and two of the mAbs, 12F8 and 1E4, were significantly cross-reactive against types 1 and 2 and types 1 and 3, respectively. Mapping the binding epitopes using strains resistant to neutralization (escape mutants) suggested that cross-specific PV binding epitopes may primarily reside within the canyon region, which interacts with the cellular receptor molecule CD155 and the cross-neutralizing chimpanzee/human mAb, A12. Despite their close proximity, the epitopes for the 12F8 and 1E4 mAbs on Sabin 1 were not functionally identical to the A12 epitope. When tested together, 12F8 and 1E4 neutralized a diverse panel of clinically relevant PV strains and did not exhibit interference. Virus mutants resistant to the anti-poliovirus drug V-073 were also neutralized by the mAbs. The 12F8 and 1E4 mAbs may suitable for use as anti-PV therapeutics.

1. Introduction

The worldwide polio eradication campaign may soon end the circulation of wild type polioviruses (PV). The primary weapon in this fight has been vaccination with the attenuated live oral polio vaccine (OPV), performed in mass campaigns conducted on a national and sub-national level, coupled with surveillance for acute flaccid paralysis (AFP) (De Jesus, 2007). OPV is generally safe, but an estimated 200–250 cases of vaccine-associated paralytic poliomyelitis (VAPP) occur annually as a result of OPV vaccination (WHO, 2002). Furthermore, due to the highly mutable nature of the PV genome and the processes of natural selection, OPV virus strains can mutate to become virulent circulating vaccine-derived polio viruses (cVDPVs), which can spread in populations with insufficient levels of immunity (Kew et al., 2005). Multiple outbreaks of cVDPV-associated poliomyelitis caused by viruses of all three serotypes have been observed (Burns et al., 2013; Estivariz et al., 2008; Kew et al., 2002; Liang et al., 2006; Rakoto-Andrianarivelo et al., 2008; Yan et al., 2010). Halting the emergence and circulation of cVDPVs is therefore an absolute requirement for a successful polio eradication strategy. This will require universal replacement of OPV by IPV (Dowdle et al., 2003).

Abbreviations: AFP, acute flaccid paralysis; CPE, cytopathic effect; cVDPV, circulating vaccine derived polio virus; iVDPV, immunodeficiency-associated vaccine-derived poliovirus; aVDPV, ambiguous vaccine derived poliovirus; HRP, horseradish peroxidase; IPV, inactivated polio vaccine; IVIG, intravenous immunoglobulin; mAb, monoclonal antibody; OPV, oral polio vaccine; PV, polio virus; TCID, tissue culture infectious doses; VAPP, vaccine-associated paralytic poliovirus; VDPV, vaccine-derived polio virus; WT, wild type.

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http://dx.doi.org/10.1016/j.antiviral.2014.05.005
0166-3542/

An additional complication is that individuals with B-cell immune deficiencies may become chronic excretors of virus, termed immunodeficiency-associated vaccine-derived polioviruses (iVDPVs), and shed these viruses for years (Khetsuriani et al., 2003). Multiple cases of isolation from the environment of highly-evolved vaccine polioviruses of ambiguous origin (aVDPV) have also been documented (Al-Hello et al., 2013). These strains may be excreted by unidentified chronic shedders or represent a long-term cryptic circulation of ambiguous vaccine-derived polioviruses (aVDPV). While stopping emergence of cVDPVs can be achieved by cessation of OPV use (Dowdle et al., 2003), dealing with the threat posed by iVDPV and aVDPV is not straightforward. The prevalence of chronic excretors in the population is currently unknown, and their identification will require better understanding of the biology of chronic infection and massive surveillance efforts. But, more importantly, there is no known cure that would enable them to stop shedding the virus (MacLennan et al., 2004). It has been proposed that antiviral drugs effective against PV be developed and used to clear these patients from PV infection (Council, 2006). Development and clinical evaluation of small-molecule drugs effective against PVs are underway (Collett et al., 2008). Use of IgG and/or IgA mAbs could complement drug treatment.

The PV-specific antibody response is critical to PV immunity (Burnet and Macnamara, 1931; Flexner and Lewis, 1910). In PV-infected patients, even low titers of PV-specific IgG provided by IVIG may provide protection against neuroparalytic activity (Hammon et al., 1954). Studies have suggested that intestinal anti-poliovirus IgA titers correlate with decreased viral shedding (Valtanen et al., 2000). However, little is known about the potential activity of antibodies against chronic infection. Administration of breast milk (a source of secretory IgA) to a subject with common variable immunodeficiency and chronic VDPV infection significantly reduced excretion, although the effect was transient (MacLennan et al., 2004). Repeated dosing of intravenous immunoglobulin (IVIG) to patients with B-cell immunodeficiencies does not lead to viral clearance. However, even after IVIG administration, the level of polio-neutralizing antibodies in these patients remains relatively low. High doses of PV-specific mAbs (either IgG or IgA) may be more effective than IVIG in clearing virus from immune deficient subjects.

Another potential use of human mAbs is emergency prophylaxis for non-immune exposed subjects. While there is no doubt that such treatment will offer protection from developing paralytic disease (Hammon et al., 1954), it is less certain that it can prevent them from being infected and transmitting virus. Immunization with IPV prior to viral challenge leads to reductions in the prevalence of virus shedding, the duration of excretion, and its intensity, but these effects are smaller than those provided by immunization with OPV (Hird and Grassly, 2012). Availability of additional human mAbs would open a unique opportunity to study this question and to determine whether a high level of neutralizing antibodies could protect subjects, not only from the disease, but also from infection and viral replication. The human antibody response to PV has not been adequately characterized, but evidence suggests that it generates antibodies with important neutralizing functions. In one study, 4 human mAbs were cloned from tonsil B-cells, and each of these mAbs neutralized PV *in vitro* (Uhlig and Dernick, 1988). Two of the mAbs neutralized type 1 and 2 strains, and another neutralized type 1, 2, and 3 strains.

Recently, we generated chimeric chimpanzee/human anti-PV mAbs by combining Ig variable domains isolated from bone marrow of immunized chimpanzees with the constant domain of human IgG (Chen et al., 2011). These mAbs exhibited neutralization activity *in vitro* and protected transgenic mice against lethal challenge with wild type PV, even after post-exposure administration. Because of the close relatedness between human and chimpanzee IgG, these antibodies are not expected to induce an immune response in humans. Furthermore, two of the mAbs were cross-neutralizing, confirming the existence of conserved epitopes that may be targeted by mAbs to create PV therapeutics that have broad strain specificity (Chen et al., 2011, 2013). In this study, we used a highly efficient human hybridoma technology (Adekar et al., 2008) to clone a panel of mAbs from OPV-immunized human subjects boosted with IPV (Adekar et al., 2008). We identified additional cross-neutralizing mAbs and used PV escape mutant studies to begin to characterize their binding sites. Their properties suggest that they may be used to create cross-neutralizing mAb cocktails suitable for clinical application.

2. Materials and methods

2.1. Human monoclonal antibodies

Human monoclonal antibodies were cloned following methods previously described (Adekar et al., 2008). Peripheral blood mononuclear cells from healthy donors 8 days following vaccination with IPV were stored frozen in 90% Hyclone Defined FBS (Invitrogen, Carlsbad, CA) and 10% DMSO (Sigma–Aldrich) under liquid nitrogen. Prior to cell fusion, CD27-positive cells were isolated with anti-CD27 magnetic beads (Miltenyi Biotec, Auburn, CA) according to the manufacturer's instructions, and were cultured for 8 days on a monolayer of tCD40L cells (courtesy of Gordon Freeman, Dana Farber/Partners Cancer Care, Boston, MA) in IMDM supplemented with 10% FBS, IL4, IL10, transferrin, insulin, cyclosporine, and penicillin/streptomycin. The cultured cells were fused to the B5-6T heteromyeloma cell line and the nascent hybrid cells were selected with HAT (Sigma–Aldrich) in Advanced RPMI + 1% fetal calf serum. Hybridomas were stabilized by limiting dilution cloning, after which they were adapted to medium with 5% Ultra Low IgG fetal bovine serum medium (Life Technologies, Grand Island, NY), incubated for 5 days in a 500-ml roller bottle. Filtered supernatants were purified over protein G-Sepharose (Life Technologies). Antibody concentrations were determined using the NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE).

2.2. Blocking ELISA

Antibodies with PV immunoreactivity in hybridoma supernatants were identified using a blocking ELISA, as previously described (Chen et al., 2011). Amounts of 0.5–1.0 D antigen units/ml of PV were captured on 96-well ELISA plates coated with purified rabbit polyclonal serotype-specific IgG (2 μg/ml). Then, some wells containing captured antigen were incubated with mAb or polyclonal serum (blocking reaction), while control wells were treated with normal rabbit serum. Wells with no antigen served as a background control. Anti-PV antibodies reacted with their respective antigenic sites and blocked them from subsequent reaction with biotin-conjugated polyclonal antipolio IgG (0.5 μg/ml). Next, ExtrAvidin-peroxidase conjugate (1:1,000 dilution; Sigma) was added, and the reaction of bound peroxidase with tetramethylbenzidine substrate (TMB, Sigma) resulted in the development of a color reaction. After the addition of a stop reagent (Sigma), the plate was scanned at 450 nm. The OD450 in the background wells that contained hyper-immune rabbit serum corresponded to 100% blocking activity, and the OD450 for antigen-containing wells incubated with normal rabbit serum corresponded to 0% blocking activity. The ratio of blocking activity of a specific MAb or serum with a particular antigen was calculated from the difference between the average ODs of blocked and non-blocked wells containing the same antigen.