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A Single Chimpanzee-Human Neutralizing Monoclonal Antibody Provides Post-exposure Protection Against Type 1 and Type 2 Polioviruses

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Abstract

Background—Development of anti-poliovirus therapies to complement vaccination is an urgent priority. A number of antiviral drugs are in development. Recently we have developed human monoclonal antibodies that could be used for treatment of chronically infected individuals and emergency response to potential reappearance of polioviruses after eradication.

Objective—The aim of this study was to characterize neutralizing activity of anti-poliovirus monoclonal antibody A12 against wild type, vaccine-derived, and drug-resistant poliovirus strains, evaluate *in vivo* pre- and post-exposure protective properties of the antibody against polioviruses of serotypes 1 and 2, and to determine whether it interferes with response to immunization with poliovirus vaccine.

Study Design—Immunogenicity studies were performed in CD1 mice. Poliovirus neutralizing titers were determined in poliovirus microneutralization assay. Poliovirus immunization-challenge experiments were performed in poliovirus-susceptible TgPVR21 mice.

Results—We show that monoclonal antibody A12 effectively neutralizes *in vitro* a broad range of Type 1 and Type 2 wild and vaccine-derived polioviruses, provides effective pre- and post-exposure protection of TgPVR21 mice from challenge with a lethal dose of poliovirus. Treatment of animals with the antibody concurrent with IPV immunization does not prevent immune response to the vaccine.

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None

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Ethical approval was not required.

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Conclusions—Anti-poliovirus antibody A12 effectively neutralizes a range of wild and VDPV strains and protects transgenic mice susceptible to poliovirus against lethal challenge upon pre- and post-exposure administration. This suggests that the antibodies could be used in combination with drugs and/or vaccine to improve their efficacy and prevent emergence of resistant variants, and provides a justification for initiating their clinical evaluation.

Keywords

polio eradication; antiviral therapy; drug-resistance; chronic virus excretors; emergency prophylaxis

Introduction

1. Background

The worldwide polio eradication campaign depends on extensive use of oral poliovirus vaccine (OPV) ^{1,2} that is highly effective and safe. However in rare cases it may lead to emergence of vaccine-derived polioviruses (VDPV) that cause paralytic poliomyelitis and establish chronic infection in subjects with primary immunodeficiencies ^{3–5}. These patients can develop paralysis and persistently excrete virulent poliovirus capable of restarting circulation in poliovirus-free populations. Efforts are underway to develop new tools that could be used along with vaccines to stop circulation of all polioviruses ⁶ and for emergency response if poliovirus reappears. At least one antiviral drug is now in clinical development, and several other candidates are undergoing preclinical evaluation.

Recently we have isolated hybrid human/chimpanzee monoclonal antibodies (mAb) that are highly active against polioviruses of all three serotypes; some of these antibodies neutralize more than one serotype ⁷. We have also demonstrated that these antibodies protect TgPVR21 transgenic mice susceptible to poliovirus ⁸ from a lethal challenge, including post-exposure administration ⁷. This suggested that monoclonal antibodies could be used for emergency protection from poliovirus or to treat chronically infected immunodeficient patients.

Treatment with antiviral drugs or monoclonal antibodies can trigger emergence of resistant poliovirus variants ^{9–11}. Using a combination of drugs and antibodies could prevent the development of resistance. The synergistic effect depends on the mechanism of action of drugs and antibodies. Therefore several questions need to be answered before such combinations could be evaluated in clinical studies. How broad is the spectrum of sensitivity of different poliovirus strains to the antibodies? Could strains resistant to antiviral drugs be neutralized by antibodies? Can antibodies be used in combination with vaccines without interfering with immune response?

2. Objective

The aim of this study was to characterize neutralizing activity of anti-poliovirus monoclonal antibody A12 against spectrum of wild type, vaccine-derived, and drug-resistant poliovirus strains *in vitro*, evaluate the antibody's *in vivo* pre- and post-exposure protective properties

against polioviruses of serotypes 1 and 2, and to determine whether it interferes with immune response to poliovirus vaccine immunization.

3. Study Design

Antibodies

Development and purification of the A12 monoclonal antibody was described in our previous manuscript ⁷. Briefly, Fab fragment libraries were produced from B-cells of chimpanzees immunized with poliovirus vaccines. Cross-reactive antibodies were isolated by sequentially panning Fab-displaying phage libraries against polioviruses of types 1, 2, and 3. After 2 cycles of panning, positive clones were screened for binding to poliovirus by ELISA with phages expressing poliovirus-binding Fab sequences. Resulting antibody A12 was shown to neutralize poliovirus serotype 1 and type 2.

Viruses/escape mutant generation for NT

Wild, iVDPV, and cVDPV strains of poliovirus were provided by Drs. Olen Kew and Steve Oberste, CDC, Atlanta. Sabin strains NA-4 (Type 1) and NB-2 (Type 2) were reference strains (CBER, FDA). A12-resistant mutant clone Es16a12-cl26 was generated as described previously⁷. Poliovirus titers were determined by poliovirus microtitration assay ¹².

Microneutralization test

Poliovirus-neutralizing antibody titers were determined in WHO micro-neutralization test ¹². The mAb were diluted to 5 µg/ml in DMEM supplemented with 2% FBS and 1% of antibiotic/antimycotic (Life Technologies, Grand Island, NY). Serial two-fold dilutions of the antibodies were incubated in triplicates with 100 TCID₅₀ of poliovirus for 3 h at 36°C, 5% CO₂. At the end of the incubation 2x10⁴ HEp-2C cells were added to the wells. The plates were incubated for 10 days at 36°C, 5% CO₂ and neutralizing titers were calculated using Kärber formula. Neutralizing titers were expressed as reciprocal of the highest antibody dilution at which 50% of cell monolayers are protected.

In vivo virus challenge experiments

TgPVR21 transgenic mice expressing human poliovirus receptor CD155 were obtained from the Central Institute for Experimental Animals (Tokyo, Japan). CD-1 mice were purchased from Charles River Laboratories (Wilmington, MA). Animal experiments were approved by institutional animal care committee and performed in accordance with the Guide for the Care and Use of Laboratory Animals ^{13, 14}.

TgPVR21 mice (5 males and 5 females in each group) were challenged intramuscularly (i.m.) with a lethal dose of five 50% paralytic doses (PD₅₀) of either wild-type poliovirus Type 1 (Mahoney) or Type 2 (MEF-1). Monoclonal antibody A12 (25 or 250 µg in 0.1 ml of PBS) was injected intravenously (i.v.) at 6 or 3 hours before the challenge, or at 3, 6, or 12 hours after the challenge. One control group of animals received PBS injected i.v. (0.1 ml) instead of the antibody. Mice were observed for signs of paresis or paralysis for 14 days and sacrificed after the symptoms developed. A separate group of animals received antibody

injections only; blood was collected from these animals to confirm serum neutralizing titers at the time of infection and throughout the experiment.

Immunization

Female CD-1 mice (in groups of 10) were immunized i.m. on days 0, 21, and 35 with IPV (IPOL, Sanofi Pasteur) in a volume of 0.05 ml in both legs (total of 8 D-antigen units (DU) of Type 1 poliovirus). MAb A12 (25 µg per mouse in 0.1 ml) was injected intravenously either 24 hours before, concurrently, or 24 hours after the first immunization. The anti-poliovirus antibody titers in the serum samples were measured by serum neutralization of 100 TCID₅₀ A12-resistant mutant clone of Mahoney.

Statistical analysis

Statistical analysis of the data was performed using Microsoft Excel. Seroconversion rates were compared using Fisher's exact test.

4. Results

Previously we have shown that mAb A12 neutralizes reference polioviruses of two serotypes (Types 1 and 2)⁷. It was interesting to study whether the antibody will also neutralize other strains. Candidate anti-poliovirus drug V-073 (Pocapovir) has been recently shown to be effective against poliovirus and is undergoing clinical evaluation⁶. To assess the spectrum of antiviral activity and potential synergy between Pocapovir and A12 antibodies we tested the ability of mAb A12 to neutralize a panel of wild and vaccine-derived strains as well as resistant forms of poliovirus selected by treatment with V-073. The results of these experiments showed that mAb A12 effectively neutralized a variety of poliovirus strains (Fig. 1), including those resistant to the compound V-073 (Fig. 2).

Prior to performing poliovirus challenge experiments in Tg mice, we determined whether the antibody must be dosed repeatedly during the experiment. mAb A12 was injected i.v. to CD1 mice at 25 µg or 100 µg per animal, and the neutralizing titers in mouse sera were monitored over the period of 21 days. Results for the 25 µg/mouse dose are presented on Fig. 3. The neutralizing titers decreased noticeably in the first 24 hours after injection (regardless of the concentration), which probably reflects initial redistribution of the antibodies among different tissues and is consistent with similar results by others^{15, 16}. The titers continued to decline gradually with a half-life of approximately 10 days (the neutralizing titers decreased by approximately 2 log₂ in 20 days), and were still detectable at the end of the monitoring period.

To study prophylactic and therapeutic efficacy of monoclonal human A12 antibody *in vivo*, we performed virus challenge experiments in TgPVR21 mice, which develop paralysis within the first 48–72 h following virus inoculation when challenged IM. Groups of ten mice were challenged with 5 PD₅₀ of either Type 1 (Mahoney) or Type 2 (MEF-1) strains of poliovirus.

In our first experiments we injected 25 µg of monoclonal antibody A12 per mouse i.v. (data not shown). This dose completely protected TgPVR21 mice from subsequent infection with

5 PD₅₀ of Type 1 poliovirus, and 9 out of 10 animals were protected from Type 2 infection. None of the animals in the placebo groups survived the challenge. Some mice were protected even when the antibody was administered after the virus challenge. However, the therapeutic effect of the antibody at that concentration was not complete: 80% or 40% of animals survived inoculation with Type 1 virus, when the mAb was injected 3 or 6 hours later, respectively; 30% survived inoculation with Type 2 virus (3 or 6 hours after the challenge).

We subsequently studied whether a higher dose (250 µg/mouse or ~10 mg/kg) of antibodies would increase the survival of animals in the post-exposure treatment groups. MAb A12 was administered 3 h before, or 3, 6, or 12 h following the virus inoculation (Table 1). All animals that received the mAb before the challenge were completely protected. The mAb protected some animals treated hours after the virus challenge: 60 to 80% of the animals (challenged with either of the two poliovirus serotypes) were protected from paralysis if the mAb was injected at 3 h or 6 h after the virus challenge, and 30% (Type 1) to 80% (Type 2) if treated with mAb 12 h following the virus challenge. This finding is consistent with our prior observations that infection of TgPVR21 mice challenged with Type 1 poliovirus progresses faster than Type 2 infection¹⁷.

Our results demonstrate that complete protection of mice can be achieved using 250 µg of the antibody, and that the mAb protects animals even if administered up to 12 hours after i.m. inoculation (and possibly later, especially for Type 2).

Use of mAbs in combination with vaccines in case of poliomyelitis outbreak is an attractive proposition. To find out whether the presence of mAb in would interfere with the immune response to IPV, groups of CD-1 mice were immunized and received intravenous injection of 25 µg of A12 mAb at various time points relative to the immunization (Table 2 and Fig. 4A), and serum samples were collected at different time points. To distinguish immune response to vaccine from neutralizing activity of A12 antibodies, neutralization test was performed with a Type 1 strain of poliovirus resistant to A12. Poliovirus-neutralizing antibodies were undetectable in all mouse sera collected at three weeks following first immunization (day 21; data not shown), with the exception of three mice in group that received vaccine only (titers 14, 11, and 11). Fig. 4 shows that mice immunized with IPV only or in the presence of A12 antibodies rapidly developed high titers of neutralizing antibodies after second immunization that further increased with booster immunization that followed. In unvaccinated mice, A12 antibody declined over time (Fig. 4B). Thus, the presence of protective amounts of A12 mAb in the serum of the animals did not affect development of poliovirus immunity in response to vaccination. This result suggests that subjects passively immunized with the mAb can develop protective immunity in response to vaccination with IPV or to contact with wild poliovirus while being protected from developing the symptoms of the disease immediately from the time of treatment.

5. Discussion

The worldwide campaign to stop transmission of wild polioviruses may soon eliminate all sources of pathogenic virus except for an insufficiently characterized group of

immunodeficient patients incapable of resolving poliovirus infection. They are not only at significant risk of developing paralysis, but are also a source of virulent polioviruses that could restart virus circulation. No effective treatment is known that would help the patients to clear the infection, and therefore search for new antiviral strategies is underway^{6, 18–20}. In addition to antiviral drugs, passive immunization with intravenous immunoglobulin (IVIG) was shown more than 60 years ago to be highly effective in protecting from paralytic disease²¹. Subsequent introduction of two highly effective vaccines prevented passive immunotherapy from being widely used. Since most known chronic poliovirus carriers have defects in production of antibodies^{22, 23}, they are sustained by regular injections of intravenous immunoglobulin (IVIG), which may be insufficient to clear poliovirus infection. Monoclonal antibodies can be produced and administered in significantly higher quantities and increase the neutralizing antibody titer to levels unattainable through vaccination, and which could potentially clear chronic poliovirus infection. Another potential use for passive immunization is emergency response to virus exposure of insufficiently immunized individuals or contacts of a polio case. For both indications, mAbs could be used in combination with antiviral drugs, potentiating each other and preventing development of resistant virus variants. This communication focused on some of the questions that must be addressed before clinical evaluation of anti-poliovirus mAbs.

Here we demonstrate that A12 mAb injected intravenously at different time points relative to the viral challenge protects transgenic mice from challenge with 5 PD₅₀ of poliovirus (Type 1 Mahoney, Type 2 MEF-1) in a dose-dependent manner. A higher dose of A12 (about 10 mg/kg) also had a remarkable therapeutic effect upon post-exposure treatment. The effect was more pronounced for type 2 virus, consistent with the slower rate of progression of clinical signs caused by the serotype¹³. Since TgPVR21 mice are not sensitive to poliovirus if infected through the oral route, immunization-challenge experiments were performed by intramuscular injection, which leads to a rapid development of the clinical signs of poliovirus infection. Progress of the infection may be slower after oral infection, which may allow passive immunization to be more effective, even at later time points. The challenge experiments will need to be performed in a species sensitive to the natural route of infection (e.g., monkeys) to completely characterize effectiveness of the mAb in order to determine its potential for clinical use. Dose-dependence of the protective effect of the antibodies also suggests that the immunity level induced by vaccination is important for the robustness of protection. Therefore for evaluation of vaccines efficacy geometric mean titers of neutralizing antibodies must be considered along with the seroconversion rate.

In conclusion, our results indicate that the treatment with A12 monoclonal antibody successfully protects animals from challenge with a lethal dose of wild type poliovirus of serotypes 1 and 2. The antibody is effective *in vitro* against most tested wild type and vaccine-derived poliovirus strains with only a few strains being relatively resistant. Importantly, the pattern of partial resistance is different for different antibodies²⁴, suggesting that a combination or a cocktail of antibodies might provide impenetrable protection against the virus. Poliovirus strains that developed resistance to antiviral compound V-073 were fully susceptible to neutralization with A12 antibody, suggesting that

their joint administration can prevent or significantly slow down the emergence of the resistance, improving therapeutic characteristics. Finally, the mAb did not prevent development of neutralizing antibodies in response to concurrent IPV immunization. The findings presented in this and our previous communications make a strong case for initiating clinical evaluation of the A12 and other human antibodies.

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Highlights

- Monoclonal antibody A12 neutralizes a range of type 1 and type 2 wild and vaccine polioviruses
- The A12 antibody is effective against virus strains resistant to anti-poliovirus drug V-073
- Antibody A12 does not interfere with immune response to poliovirus vaccine
- A12 protects mice susceptible to poliovirus against challenge with type 1 and type 2 strains
- Antibody protects mice against poliovirus challenge upon pre- or post-exposure administration

Figure 1. Neutralization of wild type (WT) and vaccine-derived poliovirus (VDPV) strains by A12 monoclonal antibody

A12 (5 µg/ml) was tested in microneutralization assay with either Type 1 (A) or Type 2 (B) WT and VDPV strains as challenge viruses. Results are expressed as reciprocal neutralization titers against the indicated strains.

Figure 2. Neutralization of V073-resistant strains by A 12 monoclonal antibody

A12 monoclonal antibody was tested in microneutralization assay with 100 TCID₅₀ of V073-resistant VDPV strains (patterned bars: Type 1 - a=10235.1, b=10235.3; Type 2 - c=10230.4, d=10230.8) and Sabin strains (solid bars) as challenge virus. Neutralizing titer is a reciprocal of the dilution of the antibody that protects 50% of cell cultures.

Figure 3. A12 monoclonal antibody persistence in mice

CD-1 mice received a single i.v. injection of A12 monoclonal antibody (25 µg/mouse). Sera from the mice were collected at different time points after injection for 21 day and tested in microneutralization assay with Sabin Type 1 (open circles) or Type 2 (closed circles) poliovirus strains as challenge viruses.

Figure 4. Poliovirus Type 1 neutralizing antibodies developed in response to IPV immunization in the presence of A12 monoclonal antibody

CD1 mice received mAb A12 injections (i.v., 25 μ g/mouse; patterned bars) at different time points relative to immunizations with IPV (8 DU/mouse Type 1, solid bars). (A) The sera were tested in microneutralization assay with A12 escape mutant strain as challenge virus. The results are presented as averages of Log2 transformed titers $\pm$ SD. The differences between the groups were not statistically significant (t-test). 1, no mAb, IPV only; 2, mAb and IPV; 3 mAb 24 hours before IPV; 4 mAb 24 hours following IPV. (B) A separate group of mice received A12 antibody only. The serum neutralizing titers were determined with Mahoney poliovirus strain as challenge virus at the same time points as in Figure 4(A). Individual results are shown. Bars are GMTs calculated for each group of ten animals. Undetectable titers (titers <8) were assigned value of 4 for calculation of GMT.

Table 1

Protection of PVRTg21 mice from lethal wild-type poliovirus challenge by mAb A12.

Antibody administration (250 µg/mouse)	Time ^a	Poliovirus type1 (Mahoney), 5 PD50	Poliovirus type 2 (MEF-1), 5 PD50
mAb A12		Unaffected/challenged mice	Unaffected/challenged mice
		p-value ^b	p-value ^b
+	- 3 h	10/10	10/10
		0.00001	0.00001
+	+ 3 h	8/10	6/10
		0.0007	0.01
+	+ 6 h	6/10	8/10
		0.01	0.0007
+	+ 12 h	3/10	8/10
		0.21	0.0007
PBS	- 3 h	0/10	0/10

^a mAb A12 was injected prior to (-) or post (+) viral challenge.

^b Fisher's exact test, 2-tailed.

Table 2

Seroconversion rates in animals immunized with IPV in presence of mAb A12.

Treatment ^a		Animals with Ab production/animals treated ^b			
IPV Immunization	mAb A12 injection, 25 µg/mouse	day 21	day 35	day 42	day 49
+	+	0/10	7/10	10/10	10/10
+	+	0/10	4/10	9/10	10/10
+	+	0/10	7/10	9/10	9/10
+	-	3/10	6/10	9/10	10/10

^a CD-1 mice were immunized with IPV and injected with mAb A12 at the same time (0 h), 24 h prior to (+) or 24 h post (-) immunization. The mice that were immunized with IPV but did not receive A12 were used as control group.

^b Titers of neutralizing antibodies against Type 1 were determined in poliovirus neutralization assay with A12 escape mutant as challenge virus. The animals that developed endogenous poliovirus-neutralizing antibodies with titers higher than 1:8 were designated as animals with Ab production.