Highly-neutralizing anti-PcrV antibodies as clinical candidates against Pseudomonas aeruginosa

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Background

The development of conventional antibiotics is extraordinarily challenging, and the success rate of the antibiotic drug discovery pipeline remains low. Antibodies that inhibit virulence factors or trigger the antibacterial immune response of the host represent a promising alternative approach. Targeting virulence factors such as the type III secretions (T3SS) system of P. aeruginosa emerges as an attractive alternative or supplement to traditional antibiotics.

Methods

Highly neutralizing monoclonal antibodies (mAbs) targeting PcrV, the needle-tip protein of the P. aeruginosa T3SS, were identified in a functional screening of plasma derived from cystic fibrosis (CF) patients chronically infected with P. aeruginosa. Single cell analytics and functional in vitro screening assays allowed for the production of highly potent mAbs which were evaluated for anti-pseudomonal activity in vitro and in vivo.

Results

Several CF patients with potent plasma neutralizing activity against T3SS-mediated cell lysis were identified. Extensive single cell analyses of their B cell receptor repertoires revealed a polyclonal antibody response towards PcrV and more than 43 patient-derived mAbs could be produced. Several of these fully human PcrV-binding mAbs demonstrated high in vitro neutralizing efficacy in lung-cell infection assays using both wild-type bacteria and MDR clinical isolates. Strikingly, the best-performing antibodies were as effective as conventional antibiotics in lung infection mouse models, significantly reducing lung CFUs and inflammatory cytokine levels. In addition, antibody treatment was highly protective for lung tissue of infected mice. Most importantly, patient-derived mAbs largely outperformed mouse-derived mAbs described by others both in vitro and in vivo (Figure 1).

Conclusion

Our study reveals that chronically infected patients represent an untapped source of neutralizing antibodies. Multiple studies show that human therapeutic IgG mAbs have favourable side effect profiles with minimal occurrences of severe adverse events and rare off-target effects. Consequently, our human mAbs targeting PA virulence factors could serve as a potent approach for treating acute infections, as well as for passive immunization strategies. Several preclinical studies are ongoing to advance these potent mAbs into clinical trials.

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Figure 1

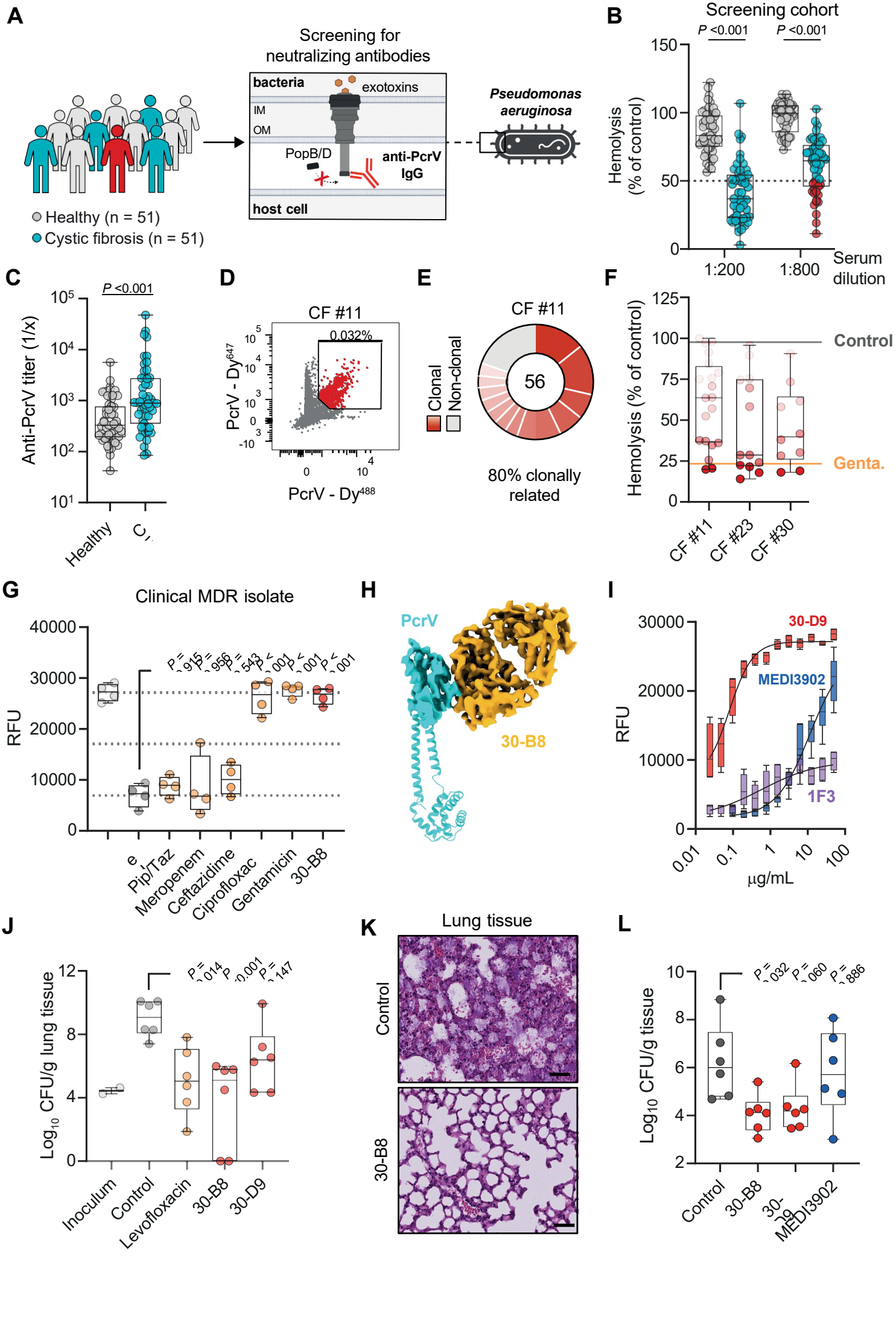
A
Screening for neutralizing antibodies
bacteria
exotoxins
IM
OM
PopB/D
anti-PcrV IgG
host cell
Pseudomonas aeruginosa
Healthy (n = 51)
Cystic fibrosis (n = 51)
B
Screening cohort
P <0.001
P <0.001
Hemolysis (% of control)
Serum dilution
1:200
1:800
C
P <0.001
Anti-PcrV titer (1/x)
Healthy
D
CF #11
0.032%
PcrV - Dy647
PcrV - Dy488
E
CF #11
Clonal
Non-clonal
56
80% clonally related
F
Hemolysis (% of control)
Control
Genta.
CF #11
CF #23
CF #30
G
Clinical MDR isolate
RFU
Pip/Taz
Meropenem
Ceftazidime
Ciprofloxac
Gentamicin
30-B8
H
PcrV
30-B8
I
30-D9
MEDI3902
1F3
RFU
μg/mL
J
Log10 CFU/g lung tissue
Inoculum
Control
Levofloxacin
30-B8
30-D9
K
Lung tissue
Control
30-B8
L
Log10 CFU/g tissue
Control
30-B8
MEDI3902

Figure 1.
(**A**) Schematic representation of the workflow to identify and produce highly neutralizing PcrV mAbs. (**B**) Heat- inactivated sera of the CF group (n = 51) (cyan) and healthy control group (n = 51) (gray) were added at different concentrations (1:200 and 1:800) during infection of human red blood cells with PA. Percentage of hemolysis for each individual was calculated to infected and untreated red blood cells. Individuals with highly neutralizing serum activity are highlighted in red (hemolysis < 50% at 1:800 dilution). (**C**) PcrV-binding ELISA were performed with diluted serum samples from the cohort. Median effective concentrations (EC50) of the calculated binding curves were determined as PcrV titer. (**D**) PcrV-specific B cell population, isolated by Fluorescence Activated Cell Sorting (FACS), is shown for one donor. (**E**) PcrV-specific IgG+ B cell clones (identical VH/VJ gene and CDRH3 identity ≥75%) of one individual are shown in shades of red. B cells without corresponding clones are shown in gray. Clonal size is proportional to the number of clonal members (the total number of analyzed cells is shown in the center of the pie charts). (**F**) Neutralizing efficacy of patient-derived mAbs (n = 43) to the PcrV protein were determined by measuring bacteria-induced cell lysis of human erythrocytes in presence of the respective mAb (5 µg/mL). Degree of hemolysis was measured and calculated for each antibody compared to the control (infected, untreated cells). As control, cells were infected in presence of polyclonal IgG (IVIG) (grey line), 1F3 (5 µg/mL) (purple line) or gentamicin (20 µg/mL) (orange line). (**G**) A549 cells were infected using a drug resistant PA strain isolated from a patient with a blood-stream infections. Cells were treated with piperacillin/tazobactam (16 µg/mL), meropenem (8 µg/mL), ceftazidime (8 µg/mL), ciprofloxacin (1 µg/mL), gentamicin (4 µg/mL) (all orange) as well as the patient-derived monoclonal anti-PcrV antibody 30-B8 (50 µg/mL) (red). (**H**) Fab fragment of 30-B8 bind to one PcrV protomer. The PcrV protomer was fitted in the cryo-EM maps of 30-B8/PcrV as indicated. (**I**) A549 cells were infected as described before in presence of the human PcrV mAb 30-D9, a bispecifc PcrV – Psl antibody (MEDI3902), and 1F3 at a concentration ranging from 50 µg/mL to 24 ng/ml. RFUs were determined after adding resazurin. (**J**) CD-1 mice were treated with cyclophosphamide intraperitoneally at day -4 and day -1 to induce neutropenia. Subsequently, pulmonary infection was induced by nebulization of PA. To confirm successful application of bacteria, an inoculum group (gray) (n = 2) was used. A vehicle control (PBS) (dark gray) (n = 6), levofloxacin (100 mg/kg) (orange) (n = 6), or mAbs (5 mg/kg) (red) (each n = 6) were administered 2 h later intraperitoneally. After 24 h, experiments were terminated and lungs were homogenized, followed by quantifications of CFUs. (**K**) Lungs of animals treated with a control (n = 3) or 30-B8 (n = 3) were fixed, embedded in paraffin, and stained with H&E. Two representative images of an animal treated with the control or the human anti-PcrV mAb 30-B8 are shown. (**L**) CD-1 mice were treated with cyclophosphamide prior pulmonary infection with PA (Boston 41501 strain). A vehicle control (PBS) (grey) (n = 6), levofloxacin (100 mg/kg) (orange) (n = 6), human anti-PcrV mAbs (1.5 mg/kg) (red) or MEDI3902 (1.5 mg/kg) (each n = 6) were administered 2 h later intraperitoneally. After 24 h experiments were terminated and lungs were homogenized followed by quantifications of CFUs.