Molecular Immunology 116 (2019) 98–105

Contents lists available at ScienceDirect

Molecular Immunology

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

Anti-PcrV IgY antibodies protect against *Pseudomonas aeruginosa* infection in both acute pneumonia and burn wound models

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ARTICLE INFO

Keywords:
Pseudomonas aeruginosa
Immunoglobulin Y (IgY)
PcrV
Burn
Acute pneumonia

ABSTRACT

Pseudomonas aeruginosa is a common nosocomial pathogen in burn patients, and rapidly acquires antibiotic resistance; thus, developing an effective therapeutic approach is the most promising strategy for combating infection. Type III secretion system (T3SS) translocates bacterial toxins into the cytosol of the targeted eukaryotic cells, which plays important roles in the virulence of *P. aeruginosa* infections in both acute pneumonia and burn wound models. The PcrV protein, a T3SS translocating protein, is required for T3SS function and is a well-validated target in animal models of immunoprophylactic strategies targeting *P. aeruginosa*. In the present study, we evaluated the protective efficacy of chicken egg yolk antibodies (IgY) raised against recombinant PcrV (r-PcrV) in both acute pneumonia and burn wound models. R-PcrV protein was generated by expressing the *pcrV* gene (cloned in pET-28a vector) in *E. coli* BL-21. Anti-PcrV IgY was obtained by immunization of hen. Anti-PcrV IgY induced greater protection in *P. aeruginosa*murine acute pneumonia and burn wound models than control IgY (C-IgY) and PBS groups. Anti-PcrV IgY improved opsonophagocytic killing and inhibition of bacterial invasion of host cells. Taken together, our data provide evidence that anti-PcrV IgY can be a promising therapeutic candidate for combating *P. aeruginosa* infections.

1. Introduction

Patients with burn wound infection caused by *P. aeruginosa* often suffer from high morbidity and mortality, which present a major challenge to healthcare systems throughout the world (Azzopardi et al., 2014). Multi-drug resistant (MDR) *P. aeruginosa* strains are predominant pathogens of burn wounds, and show remarkable ability to survive in various hospital environments, and cause widespread nosocomial outbreaks in hospital burn units worldwide, mainly by developing resistance to a wide spectrum of antimicrobial agents and producing various virulence factors (Falagas and Kopterides, 2006). Consequently, *P. aeruginosa* has recently been included in the World Health Organization (WHO) list of "Critical" priority pathogenic bacteria that pose the greatest threat to human health (Standley et al., 2017). Burn wounds infected with MDR-*P. aeruginosa* remain notoriously difficult to treat since *P. aeruginosa* can quickly disperse into deeper tissues causing sepsis and systemic infections, leading to high mortality (Ahmadi et al., 2017; Douglas et al., 2001; Mudau et al., 2013). The high mortality of burn wound *Pseudomonas* infections, combined with the paucity of effective antimicrobial agents (D'Avignon et al., 2010; Douglas et al., 2001; Mudau et al., 2013), highlights the urgent need for designing effective therapeutic approaches, such as vaccines, to aid the treatment and control of MDR-*P. aeruginosa* infections among patients and healthcare facilities worldwide.

Chicken egg yolk immunoglobulins (IgY) have been known as an economical and excellent source of polyclonal antibodies, which typically have no immunological cross-reactivity with mammalian IgG and the complement system; thus, IgY does not activate adverse inflammatory responses (Warr et al., 1995). In addition, the non-invasive, low-cost and simple collection method of IgY from egg yolks as well as high levels of antigen-specific production yield (up to 7 mg/ml of egg yolk) without side effects or disease resistance highlights a number of advantages over mammalian IgG antibodies, making it a favorable therapeutic agent to control infectious diseases (Tini et al., 2002). IgY has greater binding avidity to target antigens and can also be produced against conserved mammalian proteins more easily and successfully than IgG antibodies due to the evolutionary divergence between mammals and birds (Gassmann et al., 1990; Ikemori et al., 1993). IgY has been effectively eliminating infectious diseases in animals and also oral immunotherapy by gargling with anti-*P. aeruginosa* IgY antibodies decrease chronic colonization of *P. aeruginosa* in CF patients (Nilsson et al., 2008). In addition, the effective IgY immunotherapy has been

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https://doi.org/10.1016/j.molimm.2019.10.005
Received 22 February 2019; Received in revised form 4 October 2019; Accepted 7 October 2019
Available online 18 October 2019
0161-5890/

successful in preventing gastrointestinal infections (Rahman et al., 2012), as animal models demonstrated a favorable clinical impact of IgY therapy on influenza virus infection (Nguyen et al., 2010; Wallach et al., 2011) as well as the potential benefit of anti-*P. aeruginosa* IgY prophylaxis in non-chronically infected CF patients. The observed substantial clinical efficacy of IgY immunotherapy is associated with interference between pathogen and host epithelial cell interactions (Li et al., 2015; Rahman et al., 2013).

Most clinical isolates of *P. aeruginosa* possess type III secretion system (T3SS), which is a crucial virulence factor that contributes to the pathogenesis of *P. aeruginosa* in acute pneumonia and burn wound infection (Veesenmeyer et al., 2009). For instance, studies have shown that T3SS mutants of *P. aeruginosa* have lower virulence in terms of persistence in tissue as well as invasiveness, i.e. ability to directly inject effector molecules into host cells and disrupt cellular functions (Holder et al., 2001a). The *P. aeruginosa* V-antigen (PcrV) is an extracellular translocating conserved protein of the T3SS, which is essential for translocation of effector molecules that kill host epithelial cells (Song et al., 2012a). PcrV is a highly conserved protein of *P. aeruginosa* T3SS that stimulates innate and adaptive protective immune responses in both animal and human (Priebe and Goldberg, 2014). Anti-PcrV antibodies universally inhibit translocation of type III secretory toxins to host cells (Frank et al., 2002). Recently, it has been demonstrated that the phase II trial of KB001, a recombinant anti-PcrV Fab fragment, reduced airway inflammation and damage in CF patients with chronic P. aeruginosa infection (Sawa et al., 2014). In addition, antibodies to PcrV have been shown to exhibit potent, antibody-dependent complement-mediated, and opsonophagocytic killing activity against *P. aeruginosa* and protect infected animals from acute lung injury, bacteremia, and sepsis (Baer et al., 2009). Furthermore, studies have shown that active immunization with PcrV vaccine can provide enduring protection against *P. aeruginosa* infections in the lungs as well as burn wounds (Holder et al., 2001a; Nilsson et al., 2008; Sato and Frank, 2011; Sawa et al., 1999). In the present study, we have tested whether passive immunization with anti-PcrV IgY will inhibit the invasion of PA strains and induce opsonophagocytic killing to reduce mortality of infected mice with *P. aeruginosa* in both acute pneumonia and burn wound models.

2. Materials and methods

2.1. Bacterial strains and cultures

We used *P. aeruginosa* PAO1 strain to produce and purify PcrV proteins. PAO1 strain was kindly provided by the Central Medical Laboratory Services of the Burn Research Center (Tehran, Iran). Luria-Bertani (LB) medium, MacConkey agar (MAC), trypticase soy agar (TSA), and tryptic soy broth (TSB; all from Merck, Germany) were used for culture of *P. aeruginosa* strains.

2.2. Animal experiments, human cell culture assays and ethics committee approvals

Female 6–8-week-old BALB/C mice were purchased from the Royan Institute (Tehran, Iran). The 25 weeks old, brown Leghorn hens were purchased from Shahed University. All animal experiments were conducted in accordance with the protocols approved by the Animal Ethics Committee of Shahed University.

Human polymorphonuclear leukocytes (PMNs) were isolated from heparinized venous blood of healthy laboratory workers after obtaining consent. PMNs were purified by dextran sedimentation and Ficoll-Hypaque gradient centrifugation, as previously described (Boyum, 1968; Hatano et al., 2009). Cell viability and purity (> 95%) were determined using the trypan blue exclusion assay.

2.3. Preparation of recombinant protein

Specific primers were designed for the full-length PcrV-encoded gene (*pcrV)* of *P. aeruginosa* (GenBank Accession No: NC_002516.2), (forward 5'-CACGGAATTCATGGAAGTCAGAAACCTTA-3'; reverse 5'-ACCTAAGCTTCTAGATCGCGCTGAGAATG-3'). Amplifications were carried out using *Pfu* DNA polymerase (Fermentas, Lithonia) and the conditions were as: 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 61.6 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 5 min. The gene was inserted into an expression vector pET28a, in the frame with a T7 promoter, kanamycin-resistant gene and the N-terminal six-His-tagged sequence. *HindIII* and *EcoRI* (Fermentas, Lithonia) restriction sites were located at the 5′ end *pcrV* gene, respectively. The recombinant gene construct was overexpressed, and the protein was affinity purified by a Ni-NTA agarose-based procedure following an on-column re-solubilization protocol, as previously described (Behrouz et al., 2016; Goudarzi et al., 2009; Korpi et al., 2015). The purified recombinant protein was confirmed by Western blotting with mouse anti-His tag monoclonal antibody, as previously described (Behrouz et al., 2016). The presence of the *pcrV* gene recombinant construct (pET-28a/*pcrV*) was verified by *HindIII* and *EcoRI* digestion (Fig. S1). The protein expression of *E. coli* BL21 (DE3) carrying recombinant vector was induced with IPTG (1 mM). The PcrV was successfully purified by Ni–NTA affinity chromatography and 57 mg of highly purified r-PcrV was obtained from one liter of the induced culture. As illustrated in Fig. S1, based on western blot analysis, anti-His monoclonal antibody reacted specifically with a ~38 kDa protein in *E. coli* BL21 (DE3) lysates, corresponding to r-PcrV.

2.4. Preparation of anti-PcrV IgY antibodies

The hens were immunized intramuscularly with 150 µg of the r-PcrV in complete Freund's adjuvant (1:1; Sigma, USA) administered subcutaneously and boosted twice with 150 µg of the r-PcrV in incomplete Freund's adjuvant (1:1) at weeks 2 and 4. Two weeks after the last injection, the laid eggs were collected daily for 5–6 month and stored at 4 °C. The egg yolk was separated, pooled, and frozen prior to purification of IgY. Isolation of anti-PcrV IgY antibodies was performed as described previously (Akita and Nakai, 1993). The separated egg yolk was mixed with distilled water (pH 5) at −70 °C for 16 h and then filtered with Whatman cellulose filter paper (Sigma-Aldrich) to remove egg yolk fat. The suspension was mixed with 8.8% NaCl at pH 4 for 2 h and then centrifuged at 3380 *g* for 20 min. The pellet was dissolved in phosphate-buffered saline (PBS, pH 7.4), subsequently dialyzed against PBS with a molecular weight cutoff of 12 kDa overnight. Purity and yield of IgY were monitored at various stages and determined by 9% (w/v) SDS-PAGE (Bio-Rad, USA), followed by Coomassie Brilliant Blue G-250 staining. The total amount of IgY was quantitatively measured using a NanoDrop 2000 spectrophotometer system (Thermo Scientific, USA). The IgY raised against PcrV was precipitated by NaCl and 95 mg of anit-PcrV IgY was obtained per egg (Fig. S2).

2.5. Specificity of IgY antibodies

The specificity of IgY antibodies raised against the r-PcrV was analyzed by immunoblots using crude cell lysates prepared from PAO1 culture. Whole-cell lysate of PAO1 strain was electrophoresed by SDS-PAGE with 12.5% polyacrylamide mini-gels, and then transferred onto polyvinylidene fluoride (PVDF) membranes (Hi-bond Amersham Biosciences, USA) using a semi-dry blotting apparatus at 15 V/10 min (Labconco, Kansas City, MO, USA). The protein was electro-transferred to PVDF membranes (Invitrogen) in a semi-dry transfer cell at 15 V/10 min (Trans-Blot®SD, Bio-Rad, USA) and washed with tris-buffered saline with Tween 20 (TBS-T) containing 20 mM Tris–HCl (pH 7.4), 0.5 M NaCl, and 0.05% Tween 20 (Merck, Germany). Membrane blots were blocked for 1 h with PBS and 1% (w/v) skim milk, and then

incubated with anti-PcrV IgY, with 1:10,000 diluted HRP-conjugated rabbit anti-IgY antibody (Sigma-Aldrich). After several TBS-T washes, the membranes were developed using a 3,3'-Diaminobenzidine substrate (DAB, Sigma-Aldrich). Membrane blots were de-stained with 50% methanol plus 7% acetic acid (5–10 min RT). The images of DAB-stained blots were captured by a digital camera.

2.6. Assessment of IgY antibody titers

Antigen-specific IgY titers against whole-cell *P. aeruginosa*, as well as r-PcrV were assessed by ELISA. Antigen-specific ELISA assays were designed by coating 96-well microtiter plates (Nunc, USA) with either whole cell *P. aeruginosa* or r-PcrV, as described previously (DiGiandomenico et al., 2004). Briefly, 100 µL of whole-cell *P. aeruginosa* or r-PcrV (10 µg/mL in PBS) was incubated overnight at 4 °C, washed with 0.5% Tween-PBS (T-PBS), and blocked with PBS + 3% bovine serum albumin (Sigma-Aldrich). Different concentrations of IgY antibodies were incubated overnight at 4 °C, washed 3X with T-PBS, and 100 µL of 1:7000-diluted HRP-conjugated rabbit anti-IgY antibody was incubated (1 h, RT). Plates were then washed five times with T-PBS, and TMB substrate (Sigma-Aldrich) was added (100 µL/well; 30 min. at RT). Color development was stopped with 2 M H_2SO_4, and the absorbance at 405 nm (OD_{405}) was measured.

2.7. Invasion assay of P. aeruginosa strain

To determine the ability of IgY antibodies raised against r-PcrV to inhibit invasion of the A549 cell line by *P. aeruginosa*, a gentamicin protection assay was performed, as previously described (Neville et al., 2005). Briefly, IgY antibodies were mixed with PAO1 strain (10^7 CFUs) and then added to confluent A549 cells seeded in a 24-well plate (Nunc). Gentamicin was then added to kill extracellular bacteria, and the plates were incubated for 1 h, followed by quantification of PAO1 strain released from lysed cells. IgY from non-immunized hens (C-IgY) and PBS were used as negative controls.

2.8. Opsonophagocytic assay

The opsonophagocytic (OP) activity assay was performed, as described previously (Laghaei et al., 2016). Briefly, bacterial cultures were grown in TSB and incubated at 37 °C until the OD_{650} reached 0.20. PMNs concentration was adjusted to 2×10^9 cells/mL in RPMI-1640 medium (Gibco, Germany) containing 10% FBS and 25 mM HEPES. Different concentrations of anti-PcrV IgY antibodies were incubated (90 min) with fresh infant rabbit serum [complement source; affinity-purified at 4 °C (1 h) using target *P. aeruginosa* strain]. In the next step, samples (50 µL) were lysed with Triton X-100 (Merck), and serially diluted in 100-µL of T-PBS, and grown on TSA plates (triplicates), and bacterial colonies were enumerated. Negative controls consisted of 100 mL of RPMI-1640 medium/fetal calf serum [instead of antibodies], complement, and PMNs. The opsonic activities of anti-PcrV IgY antibodies were compared with that of control IgY (C-IgY), and OP activity was calculated as follows:

OP activity (%) = [1 − (bacterial CFUs of anti-PcrV IgY at 90 min/bacterial CFUs of C-IgY at 90 min)] × 100.

2.9. Murine burn infection model

The mice were burned and challenged as depicted by Stieritz and Holder (Neely et al., 1999). Briefly, mice were anesthetized and their backs were shaved. A burn wound covering 12%–15% of the body surface area was formed on their back using a 15 s ethanol flame. Immediately after the burn, 0.4 mL acetaminophen (0.25 mg/mL) was administered intraperitoneally as an analgesic, plus 0.5 mL of sterile saline to replace fluids. Infection challenge experiments were carried out using indicated *P. aeruginosa* strains as described previously (Neely et al., 1999); using 9 mice /group. Briefly, approximately 5 min after induction of burn wound, the site was subcutaneously injected with 3–5 × 10^7 CFUs of *P. aeruginosa*. Antibodies raised against PcrV were administered intraperitoneally as follows: prophylaxis (preinfection, 1 mg at 2 h before infection), therapeutic (postinfection, 1 mg 2 h after infection and 0.5 mg daily for 3 days), or combined (1 mg 2 h before infection and 0.5 mg daily for 3 days after infection). Ceftazidime (0.5 mg) was administered intraperitoneally 4 h after infection and twice daily for 3 days. Moreover, in another experiment, the mice were challenged subcutaneously at the burn site with *P. aeruginosa* that were preincubated with 0.1 and 10 mg of anti-PcrV IgY antibodies. During survival rate studies, all immunized and challenged mice were monitored twice a day for seven days post-challenge.

2.10. P. aeruginosa acute pneumonia mouse model

Mice were challenged i.n with 2 × 10^7 CFU of *P. aeruginosa* PAO1 strain that was preincubated with 0.1 and 1 mg of anti-PcrV IgY antibodies directly into each nostril (Priebe et al., 2008; Wu et al., 2012). All mice were closely monitored for one week.

2.11. Statistical analysis

Statistical analyses were performed by GraphPad Prism 6 (GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla California USA). Data were analyzed by one-way analysis of variance with Tukey's multiple comparison test. Survival data for different mouse groups were analyzed using the Mantel-Cox log-rank test. Results were expressed as the Mean ± Standard Deviation (SD), and *p*-value of < 0.05 was considered statistically significant.

3. Results

3.1. Specificity of IgY raised against r-PcrV

The specificities of IgY raised against r-PcrV were further verified using an indirect ELISA to analyze whole cell lysate. As shown in Fig. 1A–B, the IgY antibodies levels of hen immunized with r-PcrV against whole live cells of *P. aeruginosa* PAO1 strain or recombinant protein were significantly higher than those of C-IgY ($P < 0.01$). Also, the reactivity of IgY against live whole-cells of *P. aeruginosa* PAO1 strain or r-PcrV was significantly reduced by decreasing the concentration of IgY antibodies from 20 to 2.5 µg ($P < 0.01$, Fig. 1A–B). There was no significant difference between anti-PcrV IgY antibodies at concentration 1.25 µg and C-IgY ($P > 0.05$).

3.2. IgY raised against r-PcrV decrease the invasiveness of PAO1 strain

The inhibitory effects of IgY raised against r-PcrV on the invasion of A549 cells by PAO1 strain (Fig. 2) decreased in a dose-dependent manner. In contrast, IgY raised against r-PcrV, at 0.5 and 0.25 mg/mL decreased the invasion efficiencies of PAO1 (30.61% invasion, $P < 0.05$) more significantly than 1 and 2 mg/mL of anti-PcrV IgY and controls (73.80% invasion, Fig. 2). In the presence of 1 and 2 mg/mL of IgY raised against r-PcrV, invasion efficiencies of PAO1 were 25% and 38%, respectively, which was significantly higher than those of C-IgY and PBS ($P < 0.05$ vs. C-IgY and PBS, Fig. 2). In addition, C-IgY at 250 µg/mL reduced the invasion efficiencies of PAO1 (60% invasion, $P < 0.05$) more significantly than PBS (92% invasion, Fig. 2). In addition, in the presence of 0.05 and 0.1 mg/mL of anti-PcrV IgY, invasion efficiencies of PAO1 were 58% and 55%, respectively, which was significantly higher than those of PBS ($P < 0.05$). There was no significant difference between 0.05 and 0.1 mg/mL of anti-PcrV IgY and C-IgY ($P > 0.05$).

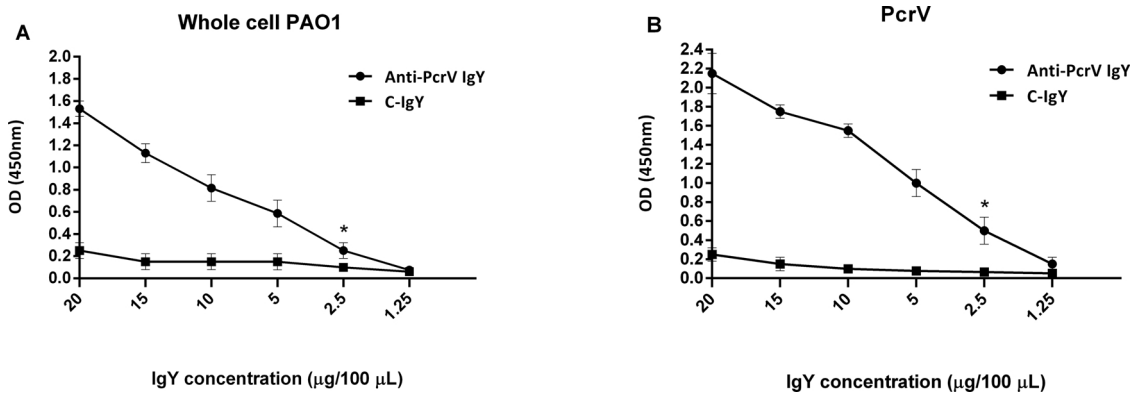

Fig. 1. An indirect ELISA was used to determine the reactivity of IgY antibodies against r-PcrV with the *P. aeruginosa* strain PAO1 (**A**) and r-PcrV (**B**). C-IgY served as negative controls. Values represent the mean of triplicate independent experiments ± standard deviation (SD). * $P < 0.05$.

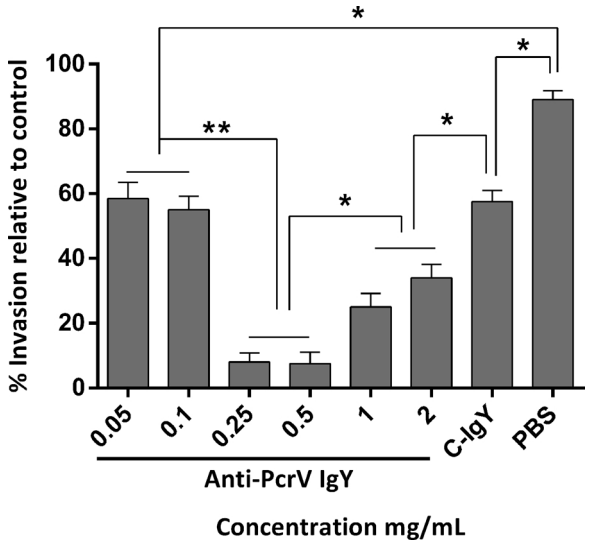

Fig. 2. The effects of IgY antibodies raised against PcrV on the invasion of A549 cells by *P. aeruginosa* PAO1 strain. PAO1 strain was incubated with different amounts of IgY antibodies (0.05, 0.1, 0.25, 0.5, 1 and 2 mg/mL). C-IgY and PBS served as negative controls. Values represent the mean of triplicate independent experiments ± SD. * $P < 0.05$.

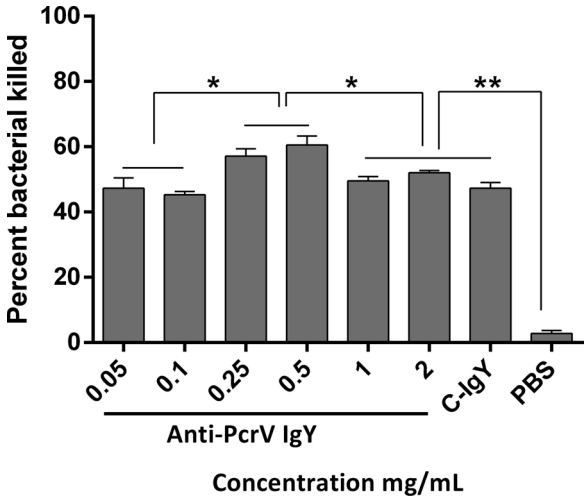

Fig. 3. Comparative analysis of opsonophagocytic killing activity against *P. aeruginosa* strain PAO1 by different amounts of IgY raised against r-PcrV. Opsono killing activity is measured by incubating anti-PcrV IgY antibodies with PAO1 strain with PMNs in the presence of rabbit complement. C-IgY and PBS served as controls. Data represent the Mean ± SD of triplicate independent experiments ± SD. * $P < 0.05$.

3.3. IgY raised against r-PcrV has high opsonophagocytic activity against P. aeruginosa

Since opsonophagocytic (OP) activity is essential to eradicate local and systemic *P. aeruginosa* infection, we assessed the *in vitro* OP activity of anit-PcrV IgY antibodies. As shown in Fig. 3, the presence of 250 and 500 μg/mL of anti-PcrV IgY antibodies significantly promoted the opsonophagocytosis of PAO1 strain (58%, $P < 0.05$) compared with other concentrations of anti-PcrV IgY and C-IgY. In the presence of 0.05 and 0.1 mg/mL of anti-PcrV IgY, opsonophagocytosis of PAO1 strain was significantly promoted (~48%, $P < 0.05$) compared with PBS. There was no significant difference between 0.05 and 0.1 mg/mL of anti-PcrV IgY and C-IgY ($P > 0.05$). Also, no significant difference was observed regarding *in vitro* OP activity between 0.25, 0.5, 1, 2 mg/mL of anti-PcrV IgY and C-IgY ($P < 0.05$, Fig. 3).

3.4. Anti-PcrV IgY protects P. aeruginosa-infected mice and helps them survive

To evaluate the efficacy of anti-PcrV IgY immunotherapy to protect mice against *P. aeruginosa* infection, we compared the survival rates of C-PBS and C-IgY mice infected with PAO1 strain versus mice that were passively immunized with the anti-PcrV IgY (Fig. 4A–D). Moreover, prophylactic, therapeutic and combined administration of IgY antibodies against r-PcrV achieved 10% survival when mice were challenged with strain PAO1 (Fig. 4A–C). The survival rates of infected mice with neutralized *P. aeruginosa* with 0.1 and 10 mg of anti-PcrV IgY were determined 22% and 33%, respectively, while none of the C-PBS and C-IgY mice survived *P. aeruginosa* wound infections. All non-infected "C-Burn" mice survived for up to 9 weeks (data not shown).

3.5. Anti-PcrV IgY promote protection against P. aeruginosa lethal pneumonia

The survival rates of intranasally infected mice with neutralized *P. aeruginosa* with 0.1 and 1 mg of anti-PcrV IgY were determined 71.42%, while none of the C-PBS and C-IgY mice survived *P. aeruginosa* acute pneumonia (Fig. 5).

4. Discussion

The goal of this study was to evaluate the efficacy of a novel anti-PcrV IgY antibody against *P. aeruginosa* acute pneumonia and burn wound infection in murine models. *P. aeruginosa* commonly causes life-threatening burn wound infections that are very difficult to treat;

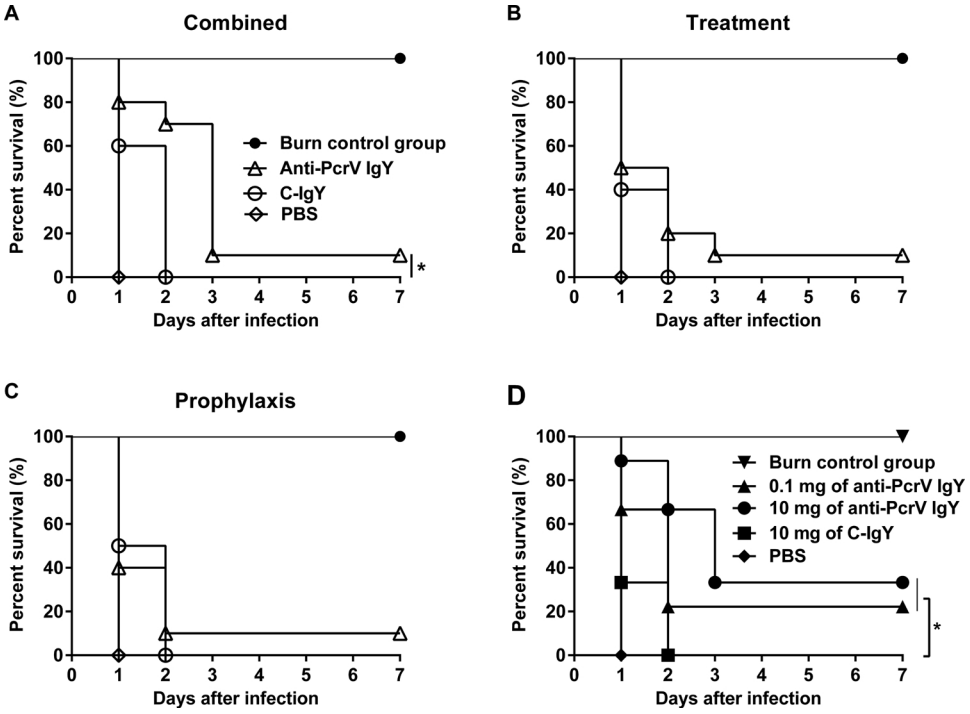

Fig. 4. The effect of treatment using different regimens of IgY antibodies against PcrV on the survival of mice infected (n = 9) with *P. aeruginosa* strains. Survival was assessed in infected mice with burn wounds 7 days after subcutaneous injection of bacterial. Mice received IgY antibodies to r-PcrV as follows: combined **(A)**, therapeutic **(B)**, or prophylaxis **(C)**. Also, Survival rate was evaluated in mice challenged subcutaneously at the burn site with *P. aeruginosa* that were preincubated with 0.1 and 10 mg of anti-PcrV IgY antibodies **(D)**. * $P < 0.05$ (Mantel-Cox log-rank test).

mainly due to the emergence of antimicrobial resistance and the production of virulence factors (Aloush et al., 2006; Banadkoki et al., 2016; Saffari et al., 2017). The remarkable ability of *P. aeruginosa* to survive and spread within patients combined with the scarcity of effective antimicrobial agents underscores the urgent need for alternative approaches to the treatment of burn wound infection, such as therapeutic vaccines (Holder, 2004). To date, numerous antigens have been evaluated as vaccine candidates against this formidable pathogen with varied outcomes (Behrouz et al., 2017; Golpasha et al., 2015; Manafi et al., 2009; Mansouri et al., 2003; Mousavi et al., 2016). Among *P. aeruginosa* antigens that show validated vaccine potential, PcrV proteins (individually, or in combination with antibiotic) hold the greatest promise; because they not only evoke effective innate immune responses; but also virtually all pathogenic strains of *P. aeruginosa* express this protein, which is essential in the pathogenesis of *P. aeruginosa* within the host (Holder et al., 2001a; Nilsson et al., 2008; Sato and Frank, 2011; Sawa et al., 1999).

In the present study, we have used acute pneumonia and burn wound mouse models to show that anti-PcrV IgY antibodies provide protection against potentially lethal *P. aeruginosa* infections. Our data revealed that challenged mice in both infection models were protected and their survival rates were higher than control groups. The results of the burned mouse model indicated that neutralization of *P. aeruginosa* by anti-PcrV IgY antibodies led to an increase of 33% in the survival rate of mice compared to the control group. Our findings are consistent with Holder et al (Neely et al., 2005a) who showed passive immunization with rabbit anti-PcrV IgG antibodies led to an increase of 33% in the survival rate of burned mice after challenge with *P. aeruginosa*. In addition, Holder et al. showed that active immunization with PcrV combined with anti-toxin A antibody therapy increased the survival rate of burned-infected mice to 40% (Holder et al., 2001a). Moreover, the low efficacy of anti-PcrV IgY antibodies in prophylaxis, treatment or combined intraperitoneal administration may be due to its incapability to trap or neutralize *P. aeruginosa* to prevent colonization in the target organ and cause a systemic lethal infection. On the other hand, not all bacteria are affected by anti-PcrV IgY antibodies and, after some time, they colonize in the target organ and cause lethal infection. Thus, we only observed an extended survival time in mice of treatment and prophylaxis group compared to the control group. Moreover, it seems that the highest protection in the neutralization of *P. aeruginosa* by anti-PcrV IgY antibodies due to it prevents bacterial colonization and invasion in the host organ. It was previously found that high hydrophobicity of anti-PcrV IgY antibodies aggregates bacteria, therefore, facilitating the clearance and phagocytosis by the host immune cells (Thomsen et al., 2015a). In addition, it was suggested that chicken IgY antibodies have inhibitory effects in bacterial pathogenesis and can be used as adjunct therapy to improve the antibiotic action. Furthermore, we tested whether anti-PcrV IgY antibodies therapy is able to increase the survival of *P. aeruginosa* infected mice in acute pneumonia model. Our findings reveal that anti-PcrV IgY antibodies provide robust

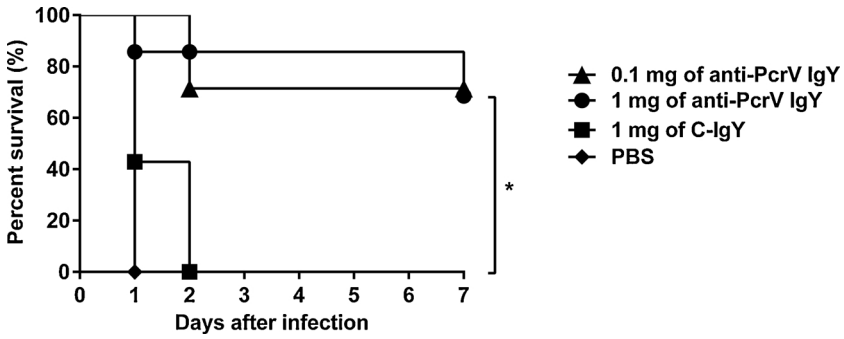

Fig. 5. Protective activity IgY antibodies against PcrV lethal pneumonia due to *P. aeruginosa* PAO1. Kaplan–Meier curves were plotted for mice (n = 7), which were challenged by 2×10^7CFUs of *P. aeruginosa* PAO1 strain that was preincubated with 0.1 and 1 mg of anti-PcrV IgY antibodies. Survival rates were monitored for seven days. * $P < 0.05$ (Mantel-Cox log-rank test).

protection against *P. aeruginosa* in acute pneumonia model. In fact, noting the survival rate (70%) among treated mice that were challenged with *P. aeruginosa*, the anti-PcrV IgY antibodies efficacy surpasses the effects of anti-PcrV IgY antibodies against *P. aeruginosa* burn wound infection. Results from the survival rates indicate that neutralization of *P. aeruginosa* by anti-PcrV IgY antibodies mounts a high protection level against *P. aeruginosa,* leading to the eventual clearance of *P. aeruginosa*. We speculate that the IgY antibodies act against PcrV virulence factor of *P. aeruginosa*, and interfere with the early stages of its pathogenesis like lung invasion, as well as the later stages such as systemic dissemination to organs (Dacheux et al., 2001; Sawa et al., 1999). Interestingly, the data showing the significant increase in the survival of anti-PcrV IgY antibodies-treated mice corresponds well with the enhanced specific opsonophagocytic activity, accompanied by the substantial reduction in bacterial invasion.

In the murine model, we show that passive therapy with anti-PcrV IgY antibodies led to robust protection, as elucidated by elevated opsonic killing and anti-invasion activities of IgY antibodies against *P. aeruginosa*, which confer effective protection against lethal infection by *P. aeruginosa* strains. This outcome is consistent with reports that monoclonal or polyclonal anti-PcrV antibody has an inhibitory effect on T3SS and increases bacterial clearance, which ultimately leads to protecting against *P. aeruginosa* infections and preventing sepsis and rat death in acute pneumonia models (Frank et al., 2002; Sato and Frank, 2011). In evaluating the *in vitro* opsonic killing activity of IgY antibodies against *P. aeruginosa*, we found that anti-PcrV IgY antibodies have specific opsonic activity against *P. aeruginosa*. It has been recently demonstrated that phagocytosis of IgY-opsonized *P. aeruginosa* is not promoted via the surface receptors of polymorphonuclear cells, since this antibody is not capable of binding to the FCR on the mammalian cell surface (Schade et al., 2005). In addition, the hydrophobic nature of IgY antibodies leads to physical alteration, immobility and formation of *P.aeruginosa* aggregates, which ultimately facilitates the phagocytic activity of polymorphonuclear cells (Thomsen et al., 2015b). Similarly, it was revealed that some antibiotics such as Aztreonam increase hydrophobicity and facilitate phagocytosis via alteration of the bacterial cell wall (Pruul et al., 1988a). This suggests that anti-PcrV IgY antibodies inhibit essential *P. aeruginosa* virulence factor; PcrV, which facilitates bacterial colonization, invasiveness, and the eventual systemic spread of *P. aeruginosa* in acute pneumonia and burn wound mouse models (Holder et al., 2001a; Neely et al., 2005a; Sawa et al., 2014; Shime et al., 2001a). It is worth mentioning that increasing the concentrations of anti-PcrV IgY antibodies lead to a decrease in opsonophagocytosis of *P. aeruginosa*. The effective concentration of IgY antibodies has been varied to improve opsonophagocytic killing activity against pathogenic bacteria (Thomsen et al., 2015b; Zhen et al., 2008). Zhen et al demonstrated that 10 mg/mL of anti-*Staphylococcus aureus* IgY antibodies improve the opsonophagocytic killing activity against bacteria however concentration lower than 1 mg/mL of the IgY *S. aureus* shows no significant opsonophagocytic killing activity against bacteria compared to control (Zhen et al., 2008). In addition, the proportion of viable *P. aeruginosa* PAO1 at a concentration of 1:10 (antibodies: media) of anti-*P. aeruginosa* IgY antibodies was reduced by 87% and by 79% in comparison to C-IgY opsonized bacteria (Thomsen et al., 2015b). It is worth mentioning that due to the hydrophobic nature of IgY antibodies, the concentration of IgY antibodies is the critical factor in the effective opsonic killing activity of IgY antibodies against *P. aeruginosa* (Pruul et al., 1988b). Moreover, lower concentrations of anti-PcrV IgY antibodies resulted in maximal inhibition of *P. aeruginosa* invasion of A549 cells, which indicated a key role in reducing the local and systemic distribution of *P. aeruginosa.* It seems likely that due to the hydrophobic nature of IgY, antibodies form aggregates in higher concentrations; thus, fewer antibodies bind to bacteria to inhibit invasion (Tini et al., 2002). The effective concentration of IgY antibodies has been varied to provide optimal inhibitory effects against the invasion of pathogenic bacteria. (Borhani et al., 2015; Pizarro-Guajardo et al., 2017). Borhani et al. showed that 0.5 and 1 mg/mL of anti-OipA IgY had the optimal inhibitory effects towards the attachment and invasion of *H. pylori* to AGS cells and 0.1 mg/mL of anti-OipA IgY had no significant effects on attachment of bacteria to AGS cells compared to control (Borhani et al., 2015). IgY Specific to *Clostridium difficile* R20291 spores at a concentration of 0.6 mg/mL effectively reduced adherence and invasion of *C. jejuni* to intestinal epithelial cells and by increasing concentration of IgY to 1.2 mg/mL, the adherence and invasion of *C. jejuni* to intestinal epithelial cells was augmented (Pizarro-Guajardo et al., 2017). The opsonophagocytosis activity of C-IgY could be due to the exposition of chickens with *P. aeruginosa*, which is a ubiquitous environmental bacterium. The observation that IgY antibodies have nonspecific impacts, as demonstrated by the effects of C-IgY on the cell invasion and bacterial killing, may be rationalized by the polyclonal nature of IgY encompassing cross-reactivity, which may assist in controlling colonization with non-P. aeruginosa pathogens (Thomsen et al., 2015b). Our result has underlined the importance of dose and time of chicken antibody administration. Given the mechanism of protective effects of chicken antibodies, they could be more effective in mucus and gastrointestinal tract; this could be the reason that systematic administration of antibodies in treating bacterial infection was not the focus of previous studies (Ebina et al., 1990; Ikemori et al., 1997; Jahangiri et al., 2018; Nilsson et al., 2008; Owusu-Asiedu et al., 2002; Schade et al., 2005; Shin et al., 2002; Vega et al., 2011; Wiedemann et al., 1990). Thus, anti-PcrV IgY antibodies showed a great activity against *P. aeruginosa* strains and interfere with the *P. aeruginosa* virulence factor for improving the opsonophagocytic killing and inhibiting cell invasion of *P. aeruginosa*.

Previous studies have shown that passive immunization with rabbit polyclonal anti-PcrV IgG, murine monoclonal anti-PcrV IgG mAb166, and mAb166 Fab fragments raised against either full-length or partial-length of PcrV protein provides robust protection against *P. aeruginosa* in acute pneumonia model (DiGiandomenico et al., 2014; Holder et al., 2001b; Imamura et al., 2007; Kinoshita et al., 2016; Neely et al., 2005b; Shime et al., 2001b; Warrener et al., 2014). In another strategy, the administration of monoclonal anti-PcrV antibody in combination with an antibiotic confers synergistic protection against large numbers of highly virulent MDR *P. aeruginosa* strains, which cause airway and systemic infections (Song et al., 2012b). More recent evidence highlights that targeting the *P. aeruginosa* PcrV with KB001, a recombinant, PEGylated, engineered, human Fab' anti-PcrV fragment, reduces airway inflammation and damage associated with *P. aeruginosa* chronic pneumonia in cystic fibrosis patients (Jain et al., 2018). These observations indicate that using polyclonal or monoclonal anti-PcrV antibodies can be regarded as potential non-antibiotic immunostrategies to reduce airway inflammation and damage in P. aeruginosa pneumonia. However, IgY antibody, as a candidate for the immunoprophylaxis and therapy of *P. aeruginosa* infection, also has general advantages compared to polyclonal and monoclonal IgG. Studies have demonstrated that the levels of pro-inflammatory cytokines of human lung epithelial cells treated with IgY were significantly lower than that of human and goat IgG, indicating that IgYs do not cause inflammatory responses in lung cells and can thus be safely used for prevention of airway infections (Kubickova et al., 2014; Thomsen et al., 2016a). In the same manner, IL-1β and TNF-α, as potent mediators of inflammation, were significantly reduced in anti-*P. aeruginosa* IgY-treated mice compared to controls after 24 h of infection, suggesting a moderation of neutropoiesis consistent with reduced numbers of bacteria via opsonophagocytic process, which ultimately reduced its interaction with the IgY opsonized pathogens. Binding of IgY to *P. aeruginosa* early in the course of infection prevents the adhesion of bacteria to the oropharynx, which can prevent bacterial colonization at the mucosal surface in lung diseases (Thomsen et al., 2016a, b). Consequently, CF patients with IgA deficiency could have a greater susceptibility to bacterial colonization; thus, passive immunization with IgY could prophylactically augment mucosal IgA immunity to increase the resistance rate against *P.*

aeruginosa colonization (Brett et al., 1990; Thomsen et al., 2016b). As indicated by the above-mentioned findings, IgY antibodies have great potential therapeutic applications as compared to IgG for the prevention and treatment of respiratory infections.

In conclusion, here we report that a novel anti-PcrV IgY antibody qualifies as a therapeutic option against *P. aeruginosa* infections, since it elicits robust protective effects that inhibits the invasion of *P. aeruginosa*. In light of these findings, a thorough evaluation of anti-PcrV IgY antibodies under controlled clinical settings against a broad spectrum of *P. aeruginosa* clinical isolates seems warranted. Purportedly, anti-PcrV IgY antibodies may work in combination with the current antimicrobial therapies, as an alternative approach towards effective control of life-threatening *P. aeruginosa* infections. Our results reinforce the promise that, perhaps soon, clinical applications of passive immunotherapy using a specific IgY against *P. aeruginosa* PcrV can be scaled-up to inexpensively produce large amounts of antibodies under hygienic, convenient and humane conditions using egg yolks from immunized laying hens. This practical approach has the potential to efficiently provide patients with extended range of protection, and help reduce the high morbidity and mortality associated with severe infections around the world.

Declaration of Competing Interest

There are no conflicts of interest.

Acknowledgment

This study was supported, in part, by a grant from Shahed University of Medical Sciences; grant No. A/P/106/94.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.molimm.2019.10.005.

References

Ahmadi, H., Behrouz, B., Irajian, G., Amirmozafari, N., Naghavi, S., 2017. Bivalent flagellin immunotherapy protects mice against Pseudomonas aeruginosa infections in both acute pneumonia and burn wound models. Biologicals 46, 29–37.

Akita, E.M., Nakai, S., 1993. Comparison of four purification methods for the production of immunoglobulins from eggs laid by hens immunized with an enterotoxigenic E. coli strain. J. Immunol. Methods 160, 207–214.

Aloush, V., Navon-Venezia, S., Seigman-Igra, Y., Cabili, S., Carmeli, Y., 2006. Multidrug-resistant Pseudomonas aeruginosa: risk factors and clinical impact. Antimicrob. Agents Chemother. 50, 43–48.

Azzopardi, E.A., Azzopardi, E., Camilleri, L., Villapalos, J., Boyce, D.E., Dziewulski, P., Dickson, W.A., Whitaker, I.S., 2014. Gram negative wound infection in hospitalised adult burn patients–systematic review and metanalysis. PLoS One 9, e95042.

Baer, M., Sawa, T., Flynn, P., Luehrsen, K., Martinez, D., Wiener-Kronish, J.P., Yarranton, G., Bebbington, C., 2009. An engineered human antibody fab fragment specific for Pseudomonas aeruginosa PcrV antigen has potent antibacterial activity. Infect. Immun. 77, 1083–1090.

Banadkoki, A.Z., Keshavarzmehr, M., Afshar, Z., Aleyasin, N., Fatemi, M.J., Behrouz, B., Hashemi, F.B., 2016. Protective effect of pilin protein with alum + naloxone adjuvant against acute pulmonary Pseudomonas aeruginosa infection. Biologicals 44, 367–373.

Behrouz, B., Amirmozafari, N., Khoramabadi, N., Bahroudi, M., Legaee, P., Mahdavi, M., 2016. Cloning, expression, and purification of Pseudomonas aeruginosa flagellin, and characterization of the elicited anti-flagellin antibody. Iran. Red Crescent Med. J. 18, e28271.

Behrouz, B., Hashemi, F.B., Fatemi, M.J., Naghavi, S., Irajian, G., Halabian, R., Imani Fooladi, A.A., 2017. Immunization with bivalent flagellin protects mice against fatal Pseudomonas aeruginosa pneumonia. J. Immunol. Res., 5689709 2017.

Borhani, K., Mobarez, A.M., Khabiri, A.R., Behmanesh, M., Khoramabadi, N., 2015. Production of specific IgY Helicobacter pylori recombinant OipA protein and assessment of its inhibitory effects towards attachment of H. Pylori to AGS cell line. Clin. Exp. Vaccine Res. 4, 177–183.

Boyum, A., 1968. Isolation of mononuclear cells and granulocytes from human blood. Isolation of monuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. Scand. J. Clin. Lab. Invest. (Suppl. 97), 77–89.

Brett, M.M., Ghoneim, A.T., Littlewood, J.M., 1990. Serum IgA antibodies against Pseudomonas aeruginosa in cystic fibrosis. Arch. Dis. Child. 65, 259–263.

D'Avignon, L.C., Hogan, B.K., Murray, C.K., Loo, F.L., Hospenthal, D.R., Cancio, L.C., Kim, S.H., Renz, E.M., Barillo, D., Holcomb, J.B., Wade, C.E., Wolf, S.E., 2010. Contribution of bacterial and viral infections to attributable mortality in patients with severe burns: an autopsy series. Burns 36, 773–779.

Dacheux, D., Goure, J., Chabert, J., Usson, Y., Attree, I., 2001. Pore-forming activity of type III system-secreted proteins leads to oncosis of Pseudomonas aeruginosa-infected macrophages. Mol. Microbiol. 40, 76–85.

DiGiandomenico, A., Keller, A.E., Gao, C., Rainey, G.J., Warrener, P., Camara, M.M., Bonnell, J., Fleming, R., Bezabeh, B., Dimasi, N., Sellman, B.R., Hilliard, J., Guenther, C.M., Datta, V., Zhao, W., Yu, X.Q., Suzich, J.A., Stover, C.K., 2014. A multifunctional bispecific antibody protects against Pseudomonas aeruginosa. Sci. Transl. Med. 6, 262ra155.

DiGiandomenico, A., Rao, J., Goldberg, J.B., 2004. Oral vaccination of BALB/c mice with Salmonella enterica serovar Typhimurium expressing Pseudomonas aeruginosa O antigen promotes increased survival in an acute fatal pneumonia model. Infect. Immun. 72, 7012–7021.

Douglas, M.W., Mulholland, K., Denyer, V., Gottlieb, T., 2001. Multi-drug resistant Pseudomonas aeruginosa outbreak in a burns unit—An infection control study. Burns 27, 131–135.

Ebina, T., Tsukada, K., Umezu, K., Nose, M., Tsuda, K., Hatta, H., Kim, M., Yamamoto, T., 1990. Gastroenteritis in suckling mice caused by human rotavirus can be prevented with egg yolk immunoglobulin (IgY) and treated with a protein-bound polysaccharide preparation (PSK). Microbiol. Immunol. 34, 617–629.

Falagas, M.E., Kopterides, P., 2006. Risk factors for the isolation of multi-drug-resistant Acinetobacter baumannii and Pseudomonas aeruginosa: a systematic review of the literature. J. Hosp. Infect. 64, 7–15.

Frank, D.W., Vallis, A., Wiener-Kronish, J.P., Roy-Burman, A., Spack, E.G., Mullaney, B.P., Megdoud, M., Marks, J.D., Fritz, R., Sawa, T., 2002. Generation and characterization of a protective monoclonal antibody to Pseudomonas aeruginosa PcrV. J. Infect. Dis. 186, 64–73.

Gassmann, M., Thommes, P., Weiser, T., Hubscher, U., 1990. Efficient production of chicken egg yolk antibodies against a conserved mammalian protein. FASEB J. 4, 2528–2532.

Golpasha, I.D., Mousavi, S.F., Owlia, P., Siadat, S.D., Irani, S., 2015. Immunization with 3-oxododecanoyl-L-homoserine lactone-r-PcrV conjugate enhances survival of mice against lethal burn infections caused by Pseudomonas aeruginosa. Bosn. J. Basic Med. Sci. 15, 15–24.

Goudarzi, G., Sattari, M., Roudkenar, M.H., Montajabi-Niyat, M., Zavaran-Hosseini, A., Mosavi-Hosseini, K., 2009. Cloning, expression, purification, and characterization of recombinant flagellin isolated from Pseudomonas aeruginosa. Biotechnol. Lett. 31, 1353–1360.

Hatano, Y., Taniuchi, S., Masuda, M., Tsuji, S., Ito, T., Hasui, M., Kobayashi, Y., Kaneko, K., 2009. Phagocytosis of heat-killed Staphylococcus aureus by eosinophils: comparison with neutrophils. APMIS 117, 115–123.

Holder, I.A., 2004. Pseudomonas immunotherapy: a historical overview. Vaccine 22, 831–839.

Holder, I.A., Neely, A.N., Frank, D.W., 2001a. PcrV immunization enhances survival of burned Pseudomonas aeruginosa-infected mice. Infect. Immun. 69, 5908–5910.

Holder, I.A., Neely, A.N., Frank, D.W., 2001b. PcrV immunization enhances survival of burned Pseudomonas aeruginosa-infected mice. Infect. Immun. 69, 5908–5910.

Ikemori, Y., Ohta, M., Umeda, K., Icatlo Jr., F.C., Kuroki, M., Yokoyama, H., Kodama, Y., 1997. Passive protection of neonatal calves against bovine coronavirus-induced diarrhea by administration of egg yolk or colostrum antibody powder. Vet. Microbiol. 58, 105–111.

Ikemori, Y., Peralta, R.C., Kuroki, M., Yokoyama, H., Kodama, Y., 1993. Research note: avidity of chicken yolk antibodies to enterotoxigenic Escherichia coli fimbriae. Poult. Sci. 72, 2361–2365.

Imamura, Y., Yanagihara, K., Fukuda, Y., Kaneko, Y., Seki, M., Izumikawa, K., Miyazaki, Y., Hirakata, Y., Sawa, T., Wiener-Kronish, J.P., Kohno, S., 2007. Effect of anti-PcrV antibody in a murine chronic airway Pseudomonas aeruginosa infection model. Eur. Respir. J. 29, 965–968.

Jahangiri, A., Owlia, P., Rasooli, I., Salimian, J., Derakhshanifar, E., Naghipour Erami, A., Darzi Eslam, E., Darvish Alipour Astaneh, S., 2018. Specific egg yolk antibodies (IgY) confer protection against Acinetobacter baumannii in a murine pneumonia model. J. Appl. Microbiol (in press).

Jain, R., Beckett, V.V., Konstan, M.W., Accurso, F.J., Burns, J.L., Mayer-Hamblett, N., Milla, C., VanDevanter, D.R., Chmiel, J.F., 2018. KB001-A, a novel anti-inflammatory, found to be safe and well-tolerated in cystic fibrosis patients infected with Pseudomonas aeruginosa. J. Cyst. Fibros. 17, 484–491.

Kinoshita, M., Kato, H., Yasumoto, H., Shimizu, M., Hamaoka, S., Naito, Y., Akiyama, K., Moriyama, K., Sawa, T., 2016. The prophylactic effects of human IgG derived from sera containing high anti-PcrV titers against pneumonia-causing Pseudomonas aeruginosa. Hum. Vaccine Immunother. 12, 2833–2846.

Korpi, F., Irajian, G., Mahadavi, M., Motamedifar, M., Mousavi, M., Laghaei, P., Raei, N., Behrouz, B., 2015. Active immunization with recombinant PilA protein protects against Pseudomonas aeruginosa infection in a mouse burn wound model. J. Microbiol. Biotechnol (in press).

Kubickova, B., Majerova, B., Hadrabova, J., Noskova, L., Stiborova, M., Hodek, P., 2014. Effect of chicken antibodies on inflammation in human lung epithelial cell lines. Neuro Endocrinol. Lett. 35 (Suppl. 2), 99–104.

Laghaei, P., Hashemi, F.B., Irajian, G., Korpi, F., Amirmozafari, N., Behrouz, B., 2016. Immunogenicity and protective efficacy of Pseudomonas aeruginosa type a and b flagellin vaccines in a burned mouse model. Mol. Immunol. 74, 71–81.

Li, X., Wang, L., Zhen, Y., Li, S., Xu, Y., 2015. Chicken egg yolk antibodies (IgY) as non-antibiotic production enhancers for use in swine production: a review. J. Anim. Sci.

Biotechnol. 6, 40.
Manafi, A., Kohanteb, J., Mehrabani, D., Japoni, A., Amini, M., Naghmachi, M., Zaghi, A.H., Khalili, N., 2009. Active immunization using exotoxin A confers protection against Pseudomonas aeruginosa infection in a mouse burn model. BMC Microbiol. 9, 23.
Mansouri, E., Blome-Eberwein, S., Gabelsberger, J., Germann, G., von Specht, B.U., 2003. Clinical study to assess the immunogenicity and safety of a recombinant Pseudomonas aeruginosa OprF-OprI vaccine in burn patients. FEMS Immunol. Med. Microbiol. 37, 161–166.
Mousavi, M., Behrouz, B., Irajian, G., Mahdavi, M., Korpi, F., Motamedifar, M., 2016. Passive immunization against Pseudomonas aeruginosa recombinant PilA in a murine burn wound model. Microb. Pathog. 101, 83–88.
Mudau, M., Jacobson, R., Minenza, N., Kuonza, L., Morris, V., Engelbrecht, H., Nicol, M.P., Bamford, C., 2013. Outbreak of multi-drug resistant Pseudomonas aeruginosa bloodstream infection in the haematology unit of a South African Academic Hospital. PLoS One 8, e55985.
Neely, A.N., Holder, I.A., Warden, G.D., 1999. Then and now: studies using a burned mouse model reflect trends in burn research over the past 25 years. Burns 25, 603–609.
Neely, A.N., Holder, I.A., Wiener-Kronish, J.P., Sawa, T., 2005a. Passive anti-PcrV treatment protects burned mice against Pseudomonas aeruginosa challenge. Burns 31, 153–158.
Neely, A.N., Holder, I.A., Wiener-Kronish, J.P., Sawa, T., 2005b. Passive anti-PcrV treatment protects burned mice against Pseudomonas aeruginosa challenge. Burns 31, 153–158.
Neville, L.F., Barnea, Y., Hammer-Munz, O., Gur, E., Kuzmenko, B., Kahel-Raifer, H., Eren, R., Elkeles, A., Murthy, K.G., Szabo, C., Salzman, A.L., Dagan, S., Carmeli, Y., Navon-Venezia, S., 2005. Antibodies raised against N'-terminal Pseudomonas aeruginosa flagellin prevent mortality in lethal murine models of infection. Int. J. Mol. Med. 16, 165–171.
Nguyen, H.H., Tumpey, T.M., Park, H.J., Byun, Y.H., Tran, L.D., Nguyen, V.D., Kilgore, P.E., Czerkinsky, C., Katz, J.M., Seong, B.L., Song, J.M., Kim, Y.B., Do, H.T., Nguyen, T., Nguyen, C.V., 2010. Prophylactic and therapeutic efficacy of avian antibodies against influenza virus H5N1 and H1N1 in mice. PLoS One 5, e10152.
Nilsson, E., Larsson, A., Olesen, H.V., Wejaker, P.E., Kollberg, H., 2008. Good effect of IgY against Pseudomonas aeruginosa infections in cystic fibrosis patients. Pediatr. Pulmonol. 43, 892–899.
Owusu-Asiedu, A., Baidoot, S.K., Nyachoti, C.M., Marquardt, R.R., 2002. Response of early-weaned pigs to spray-dried porcine or animal plasma-based diets supplemented with egg-yolk antibodies against enterotoxigenic Escherichia coli. J. Anim. Sci. 80, 2895–2903.
Pizarro-Guajardo, M., Diaz-Gonzalez, F., Alvarez-Lobos, M., Paredes-Sabja, D., 2017. Characterization of chicken IgY specific to Clostridium difficile R20291 spores and the effect of oral administration in mouse models of initiation and recurrent disease. Front. Cell. Infect. Microbiol. 7, 365.
Priebe, G.P., Goldberg, J.B., 2014. Vaccines for Pseudomonas aeruginosa: a long and winding road. Expert Rev. Vaccines 13, 507–519.
Priebe, G.P., Walsh, R.L., Cederroth, T.A., Kamei, A., Coutinho-Sledge, Y.S., Goldberg, J.B., Pier, G.B., 2008. IL-17 is a critical component of vaccine-induced protection against lung infection by lipopolysaccharide-heterologous strains of Pseudomonas aeruginosa. J. Immunol. 181, 4965–4975.
Pruul, H., Lewis, G., McDonald, P.J., 1988a. Enhanced susceptibility of gram-negative bacteria to phagocytic killing by human polymorphonuclear leucocytes after brief exposure to aztreonam. J. Antimicrob. Chemother. 22, 675–686.
Pruul, H., Lewis, G., McDonald, P.J., 1988b. Enhanced susceptibility of gram-negative bacteria to phagocytic killing by human polymorphonuclear leucocytes after brief exposure to aztreonam. J. Antimicrob. Chemother. 22, 675–686.
Rahman, S., Higo-Moriguchi, K., Htun, K.W., Taniguchi, K., Icatlo Jr., F.C., Tsuji, T., Kodama, Y., Van Nguyen, S., Umeda, K., Oo, H.N., Myint, Y.Y., Htut, T., Myint, S.S., Thura, K., Thu, H.M., Fatmawati, N.N., Oguma, K., 2012. Randomized placebo-controlled clinical trial of immunoglobulin Y as adjunct to standard supportive therapy for rotavirus-associated diarrhea among pediatric patients. Vaccine 30, 4661–4669.
Rahman, S., Van Nguyen, S., Icatlo Jr., F.C., Umeda, K., Kodama, Y., 2013. Oral passive IgY-based immunotherapeutics: a novel solution for prevention and treatment of alimentary tract diseases. Hum. Vaccines Immunother. 9, 1039–1048.
Saffari, M., Behbood, S., Irajian, G., Khorshidi, A., Moniri, R., Behrouz, B., 2017. Antibodies raised against divalent type b flagellin and pilin provide effective immunotherapy against Pseudomonas aeruginosa infection of mice with burn wounds. Biologicals 45, 20–26.
Sato, H., Frank, D.W., 2011. Multi-functional characteristics of the Pseudomonas aeruginosa type III needle-tip protein, PcrV; comparison to orthologs in other gram-negative Bacteria. Front. Microbiol. 2, 142.
Sawa, T., Ito, E., Nguyen, V.H., Haight, M., 2014. Anti-PcrV antibody strategies against virulent Pseudomonas aeruginosa. Hum. Vaccines Immunother. 10, 2843–2852.
Sawa, T., Yahr, T.L., Ohara, M., Kurahashi, K., Gropper, M.A., Wiener-Kronish, J.P., Frank, D.W., 1999. Active and passive immunization with the Pseudomonas V antigen protects against type III intoxication and lung injury. Nat. Med. 5, 392–398.
Schade, R., Calzado, E.G., Sarmiento, R., Chacana, P.A., Porankiewicz-Asplund, J., Terzolo, H.R., 2005. Chicken egg yolk antibodies (IgY-technology): a review of progress in production and use in research and human and veterinary medicine. Altern. Lab. Anim. 33, 129–154.
Shime, N., Sawa, T., Fujimoto, J., Faure, K., Allmond, L.R., Karaca, T., Swanson, B.L., Spack, E.G., Wiener-Kronish, J.P., 2001a. Therapeutic administration of anti-PcrV F (ab')(2) in sepsis associated with Pseudomonas aeruginosa. J. Immunol. 167, 5880–5886.
Shime, N., Sawa, T., Fujimoto, J., Faure, K., Allmond, L.R., Karaca, T., Swanson, B.L., Spack, E.G., Wiener-Kronish, J.P., 2001b. Therapeutic administration of anti-PcrV F (ab')(2) in sepsis associated with Pseudomonas aeruginosa. J. Immunol. 167, 5880–5886.
Shin, J.H., Yang, M., Nam, S.W., Kim, J.T., Myung, N.H., Bang, W.G., Roe, I.H., 2002. Use of egg yolk-derived immunoglobulin as an alternative to antibiotic treatment for control of Helicobacter pylori infection. Clin. Diagn. Lab. Immunol. 9, 1061–1066.
Song, Y., Baer, M., Srinivasan, R., Lima, J., Yarranton, G., Bebbington, C., Lynch, S.V., 2012a. PcrV antibody-antibiotic combination improves survival in Pseudomonas aeruginosa-infected mice. Eur. J. Clin. Microbiol. Infect. Dis. 31, 1837–1845.
Song, Y., Baer, M., Srinivasan, R., Lima, J., Yarranton, G., Bebbington, C., Lynch, S.V., 2012b. PcrV antibody-antibiotic combination improves survival in Pseudomonas aeruginosa-infected mice. Eur. J. Clin. Microbiol. Infect. Dis. 31, 1837–1845.
Standley, M., Allen, J., Cervantes, L., Lilly, J., Camps, M., 2017. Fluorescence-based reporters for detection of mutagenesis in E. coli. Methods Enzymol. 591, 159–186.
Thomsen, K., Christophersen, L., Bjarnsholt, T., Jensen, P.O., Moser, C., Hoiby, N., 2015a. Anti-pseudomonas aeruginosa IgY antibodies induce specific bacterial aggregation and internalization in human polymorphonuclear neutrophils. Infect. Immun. 83, 2686–2693.
Thomsen, K., Christophersen, L., Bjarnsholt, T., Jensen, P.O., Moser, C., Hoiby, N., 2015b. Anti-pseudomonas aeruginosa IgY antibodies induce specific bacterial aggregation and internalization in human polymorphonuclear neutrophils. Infect. Immun. 83, 2686–2693.
Thomsen, K., Christophersen, L., Bjarnsholt, T., Jensen, P.O., Moser, C., Hoiby, N., 2016a. Anti-Pseudomonas aeruginosa IgY antibodies augment bacterial clearance in a murine pneumonia model. J. Cyst. Fibros. 15, 171–178.
Thomsen, K., Christophersen, L., Jensen, P.O., Bjarnsholt, T., Moser, C., Hoiby, N., 2016b. Anti-Pseudomonas aeruginosa IgY antibodies promote bacterial opsonization and augment the phagocytic activity of polymorphonuclear neutrophils. Hum. Vaccine Immunother. 12, 1690–1699.
Tini, M., Jewell, U.R., Camenisch, G., Chilov, D., Gassmann, M., 2002. Generation and application of chicken egg-yolk antibodies. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 131, 569–574.
Veesenmeyer, J.L., Hauser, A.R., Lisboa, T., Rello, J., 2009. Pseudomonas aeruginosa virulence and therapy: evolving translational strategies. Crit. Care Med. 37, 1777–1786.
Vega, C., Bok, M., Chacana, P., Saif, L., Fernandez, F., Parreño, V., 2011. Egg yolk IgY: protection against rotavirus induced diarrhea and modulatory effect on the systemic and mucosal antibody responses in newborn calves. Vet. Immunol. Immunopathol. 142, 156–169.
Wallach, M.G., Webby, R.J., Islam, F., Walkden-Brown, S., Emmoth, E., Feinstein, R., Gronvik, K.O., 2011. Cross-protection of chicken immunoglobulin Y antibodies against H5N1 and H1N1 viruses passively administered in mice. Clin. Vaccine Immunol. 18, 1083–1090.
Warr, G.W., Magor, K.E., Higgins, D.A., 1995. IgY: clues to the origins of modern antibodies. Immunol. Today 16, 392–398.
Warrener, P., Varkey, R., Bonnell, J.C., DiGiandomenico, A., Camara, M., Cook, K., Peng, L., Zha, J., Chowdury, P., Sellman, B., Stover, C.K., 2014. A novel anti-PcrV antibody providing enhanced protection against Pseudomonas aeruginosa in multiple animal infection models. Antimicrob. Agents Chemother. 58, 4384–4391.
Wiedemann, V., Kühlmann, R., Schmidt, P., Erhardt, W., Lösch, U., 1990. Chicken Egg Antibodies for Prophylaxis and Therapy of Infectious Intestinal Diseases 37, 163–172.
Wu, W., Huang, J., Duan, B., Traficante, D.C., Hong, H., Risech, M., Lory, S., Priebe, G.P., 2012. Th17-stimulating protein vaccines confer protection against Pseudomonas aeruginosa pneumonia. Am. J. Respir. Crit. Care Med. 186, 420–427.
Zhen, Y.H., Jin, L.J., Guo, J., Li, X.Y., Li, Z., Fang, R., Xu, Y.P., 2008. Characterization of specific egg yolk immunoglobulin (IgY) against mastitis-causing Staphylococcus aureus. J. Appl. Microbiol. 105, 1529–1535.