OS0981

Intranasal immunization with a live vaccine protects against *Pseudomonas aeruginosa*-associated pneumonia in mice

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Transparency Declaration

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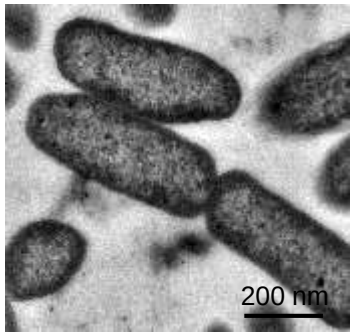
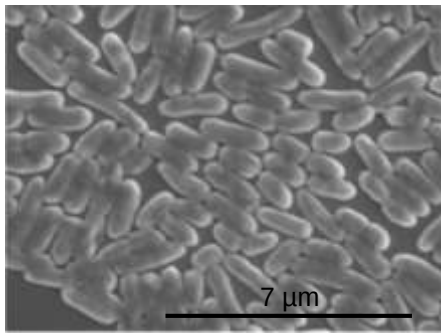
The background for a vaccine against *P. aeruginosa*

Pseudomonas aeruginosa

- Gram-negative rod, possesses a polar flagellum providing motility
- Ubiquitous, commonly found in soil and water
- Multitude of intrinsic and acquired resistance mechanisms
- Opportunistic pathogen in (those) humans:
  - Critically ill
  - Immunocompromised
  - With burn or combat-related wound
  - With cystic fibrosis (CF)
  - With non-CF bronchiectasis (nCFB)
  - With severe chronic obstructive pulmonary disease (COPD)
- Causative agent of different infections:
  - Contact-lens bacterial keratitis
  - **Acute pneumonia associated with MV (VAP) – high attributable mortality**
  - **Chronic pneumonia in CF patients – high associated morbidity and mortality**



P. aeruginosa cells observed using TEM
Cabral M P, García G, et al., 2017

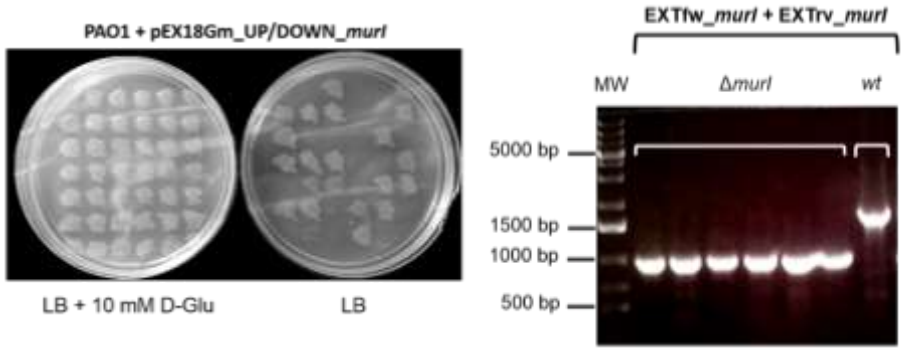


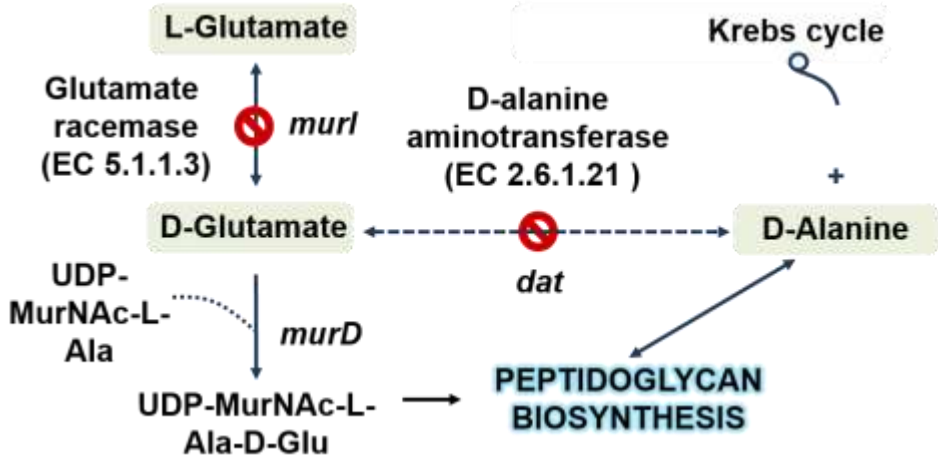
P. aeruginosa cells observed using SEM
Cabral M P, García G, et al. 2017

MV – mechanical ventilation
VAP – ventilator-associated pneumonia

CONSTRUCTION AND CHARACTERIZATION OF *P. aeruginosa* Δ*murI*

P. aeruginosa* PAO1 Δ*murI





Universal target for bacterial attenuation

D-GLUTAMATE AUXOTROPHY = PLATFORM

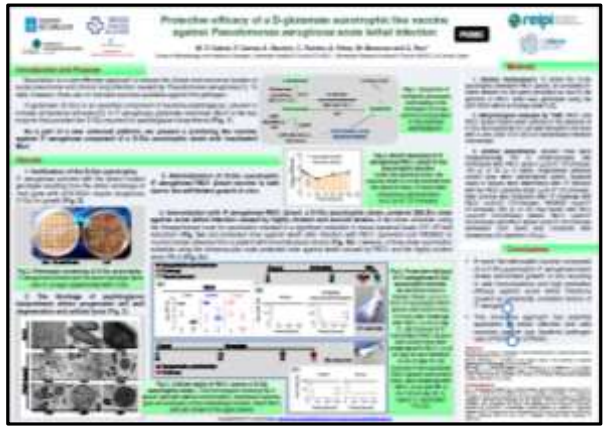
Live-attenuated bacterial vaccines

Inactivation of MurI (and Dat)

Generation of D-Glu auxotrophic strains

Impaired synthesis of the peptidoglycan

Lysis and bacterial death within mammals



26th ECCMID **P0885**
M. P. Cabral *et al*. Protective efficacy of a D-glutamate auxotrophic live vaccine against *Pseudomonas aeruginosa* acute lethal infection

α-PA VACCINE STIMULATES THE HUMORAL IMMUNE SYSTEM THROUGH THE INTRANASAL ROUTE

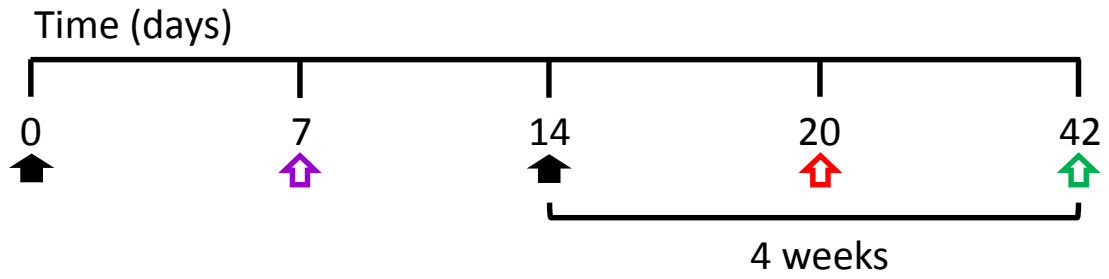
Vaccination

Pa Δ*murI* = α-Pa vaccine
2x10^8 CFU

Intranasal vaccination (IN)

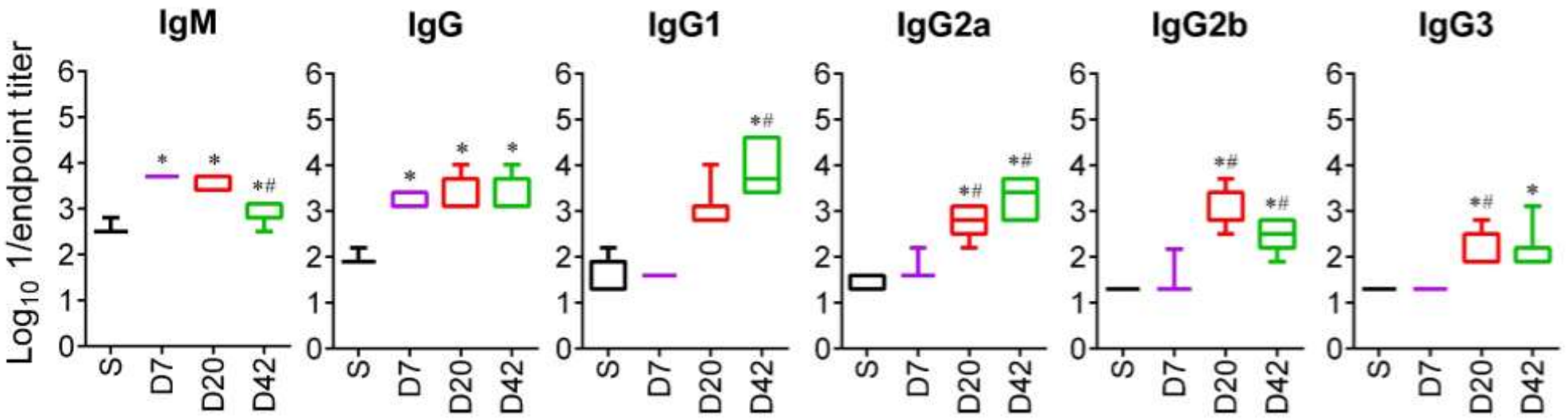
Blood extraction for serology

Two-dose vaccination schedule:



IN immunization with PAO1 Δ*murI* (2x10^8 CFU) or saline

serology



Antibody titers against *P. aeruginosa* PAO1 in vaccinated and control mice (*n*=7). S, saline; D, day. **P*<0.05 (Student's t test) compared with saline group. #*P*<0.05, compared with the preceding condition (one-way ANOVA followed by Bonferroni's post hoc test).

α-PA VACCINE STIMULATES THE HUMORAL IMMUNE SYSTEM THROUGH THE INTRANASAL ROUTE

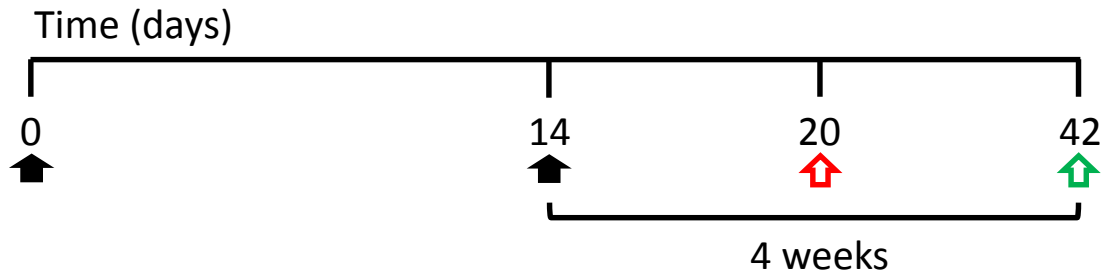
Vaccination

α-Pa vaccine 2x10^8 CFU

Intranasal vaccination (IN)

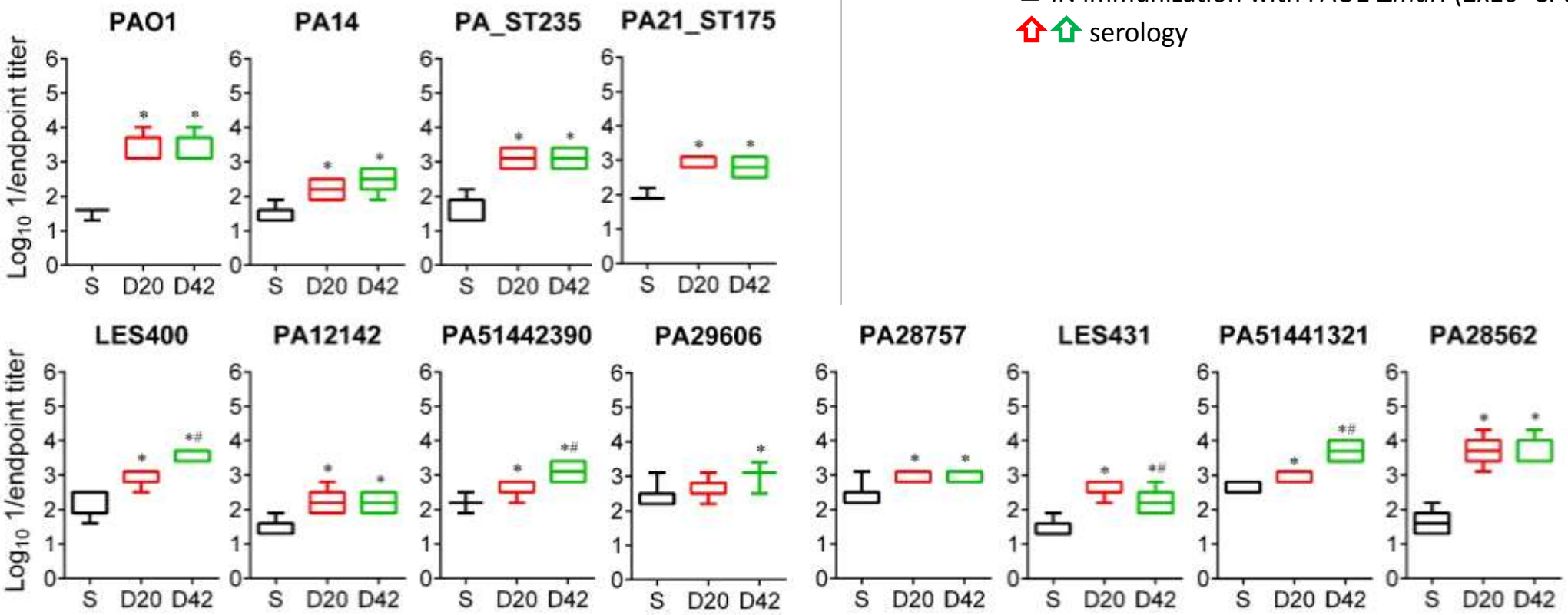
Blood extraction for serology

Two-dose vaccination schedule:



IN immunization with PAO1 Δ*murI* (2x10^8 CFU) or saline

serology



IgG titers against different *P. aeruginosa* strains in vaccinated and control mice (*n*=5-7). S, saline; D, day. **P*<0.05 (Student's t test) compared with saline group. #*P*<0.05, compared with the preceding condition (one-way ANOVA followed by Bonferroni's post hoc test).

α-PA VACCINE STIMULATES THE HUMORAL IMMUNE SYSTEM THROUGH THE INTRANASAL ROUTE

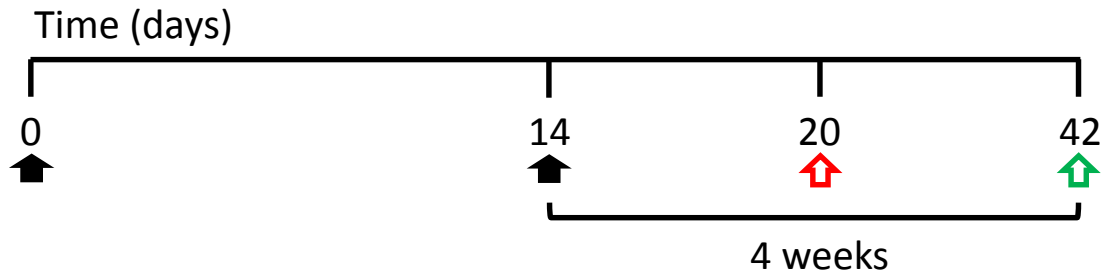
Vaccination

α-Pa vaccine 2x10^8 CFU

Intranasal vaccination (IN)

Blood extraction for serology

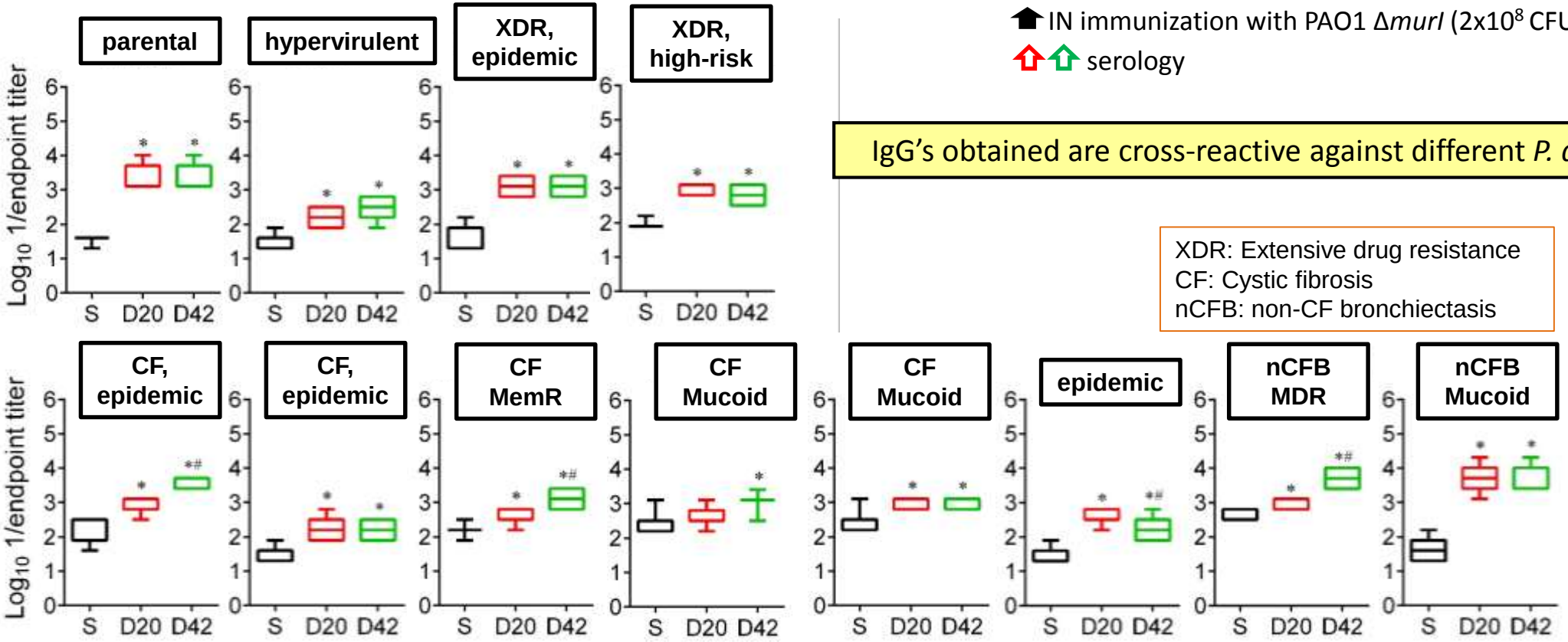
Two-dose vaccination schedule:



IN immunization with PAO1 Δ*murI* (2x10^8 CFU) or saline

serology

IgG's obtained are cross-reactive against different *P. aeruginosa* strains



XDR: Extensive drug resistance
CF: Cystic fibrosis
nCFB: non-CF bronchiectasis

IgG titers against different *P. aeruginosa* strains in vaccinated and control mice (*n*=5-7). S, saline; D, day. **P*<0.05 (Student's t test) compared with saline group. #*P*<0.05, compared with the preceding condition (one-way ANOVA followed by Bonferroni's post hoc test).

α-PA VACCINE PROTECTS AGAINST ACUTE PNEUMONIA

Vaccination

α-Pa vaccine
$2x10^8$ CFU

Intranasal vaccination (IN)

Challenge

PAO1 ExoU⁺
$7x10^5$ CFU

PA14 ExoU⁺
$1x10^6$ CFU

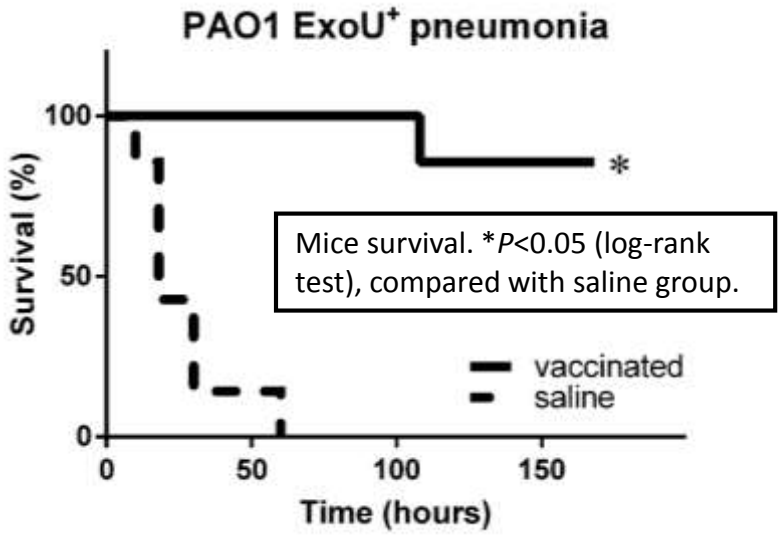
PA_ST235 ExoU⁺
$3x10^5$ CFU

(Diverse strains)

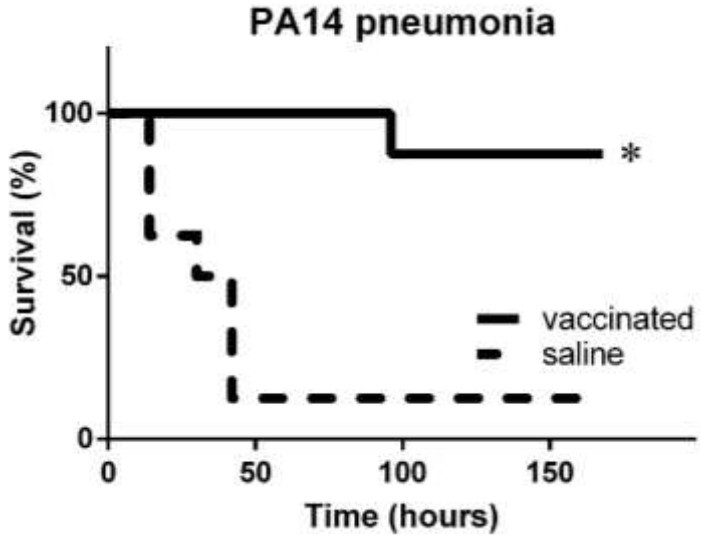
Intranasal challenge (IN)

Monitoring of survival

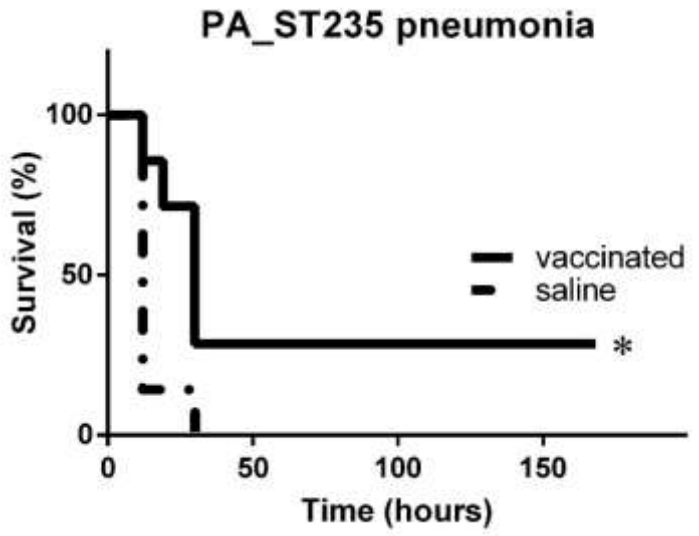
(Two-dose vaccination schedule)



Outcome
Saline group: 7 exitus/7 (0% survival)
Vaccinated: 1 exitus/7 (86% survival)



Outcome
Saline group: 7 exitus/8 (13% survival)
Vaccinated: 1 exitus/8 (88% survival)



Outcome
Saline group: 7 exitus/7 (0% survival)
Vaccinated: 5 exitus/7 (29% survival)

α-PA VACCINE CONFERS SUPERIOR VACCINE PROTECTION THAN PAO1-INACTIVATED VACCINE

Vaccination

α-Pa vaccine
$2x10^8$ CFU

Formalin-inactivated PAO1
$2x10^8$ CFU

Intranasal vaccination (IN)

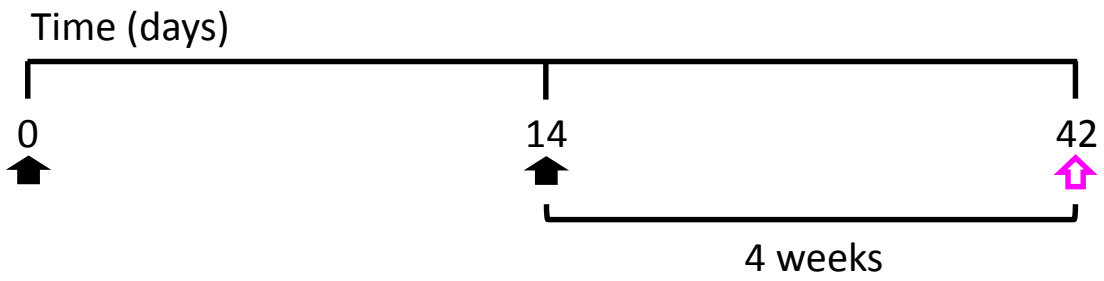
Challenge

PA14 ExoU⁺
$1x10^6$ CFU

Intranasal challenge (IN)

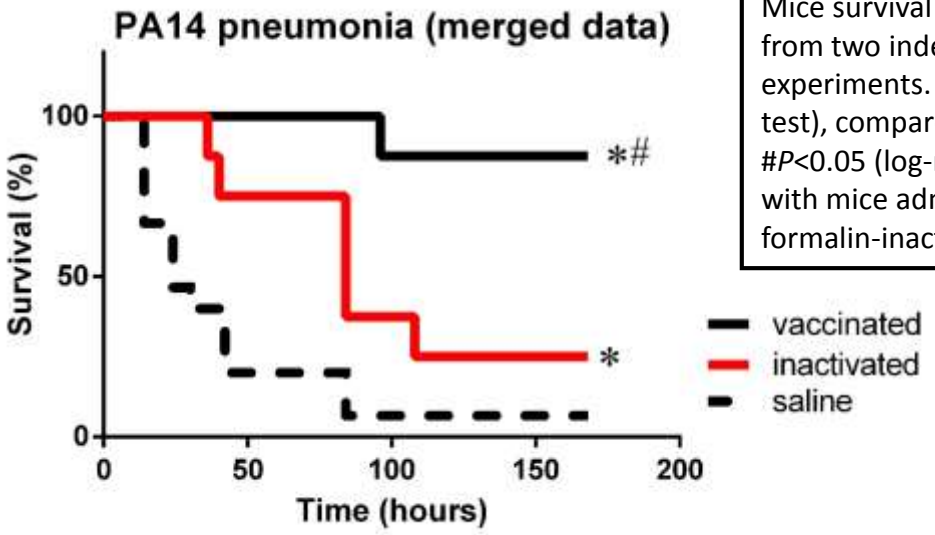
Monitoring of survival

Two-dose vaccination schedule:



IN immunization with PAO1 Δ*murI* ($2x10^8$ CFU), formalin-inactivated PAO1 ($2x10^8$ CFU) or saline

challenge



Mice survival with merged data from two independent experiments. **P*<0.05 (log-rank test), compared with saline group. #*P*<0.05 (log-rank test), compared with mice administered the formalin-inactivated vaccine.

Outcome
Saline group: 14 exitus/15 (7% survival)
PAO1-inactivated: 6 exitus/8 (25% survival)
Vaccinated: 1 exitus/8 (88% survival)

α-PA VACCINE ACTIVATES CELL-MEDIATED IMMUNITY THROUGH THE INTRANASAL ROUTE

Cytokine production BEFORE CHALLENGE

2 WEEKS | **4 WEEKS**

D0
IN immunization (2x10^8 CFU) *n*=6
Saline *n*=6

D14
IN immunization (2x10^8 CFU) *n*=6
Saline *n*=6

D42
Spleens collection

Cytokine production AFTER CHALLENGE

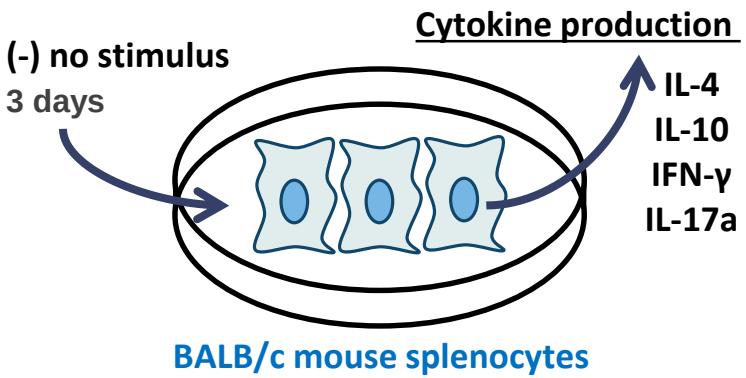
2 WEEKS | **4 WEEKS**

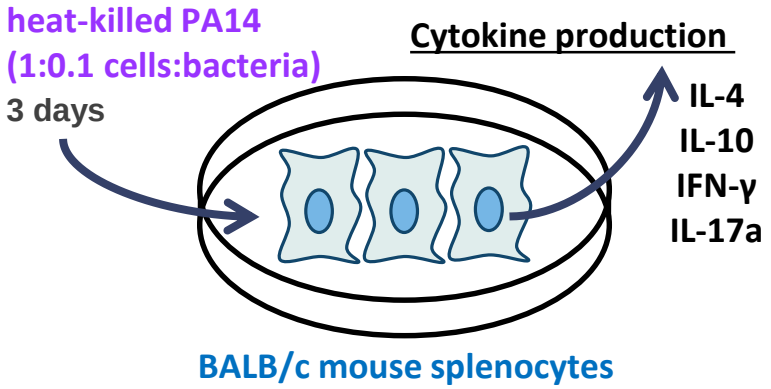
D0
IN immunization (2x10^8 CFU) *n*=6
Saline *n*=6

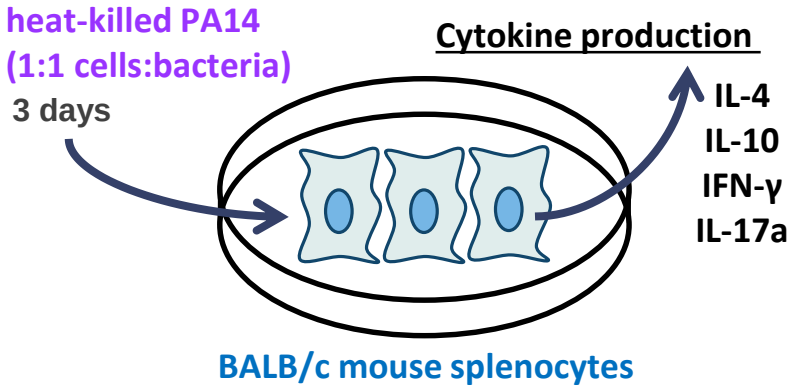
D14
IN immunization (2x10^8 CFU) *n*=6
Saline *n*=6

D42-9
Challenge with PA14 ExoU$^+$ (1x10^6 CFU)

+9h
Spleens collection

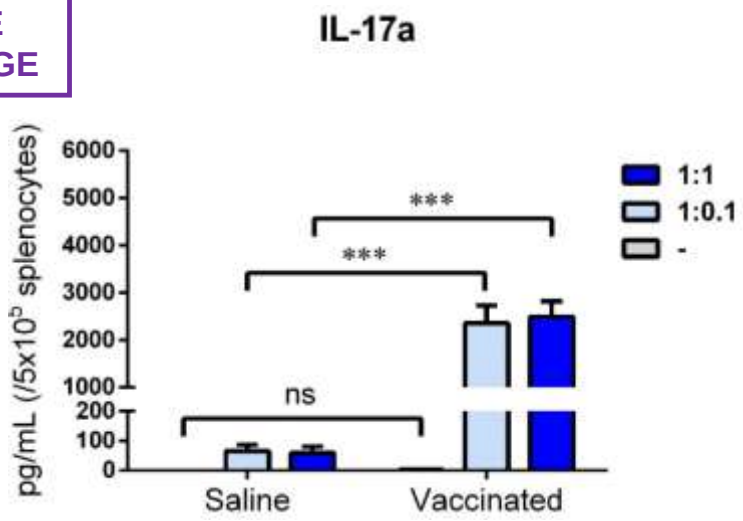


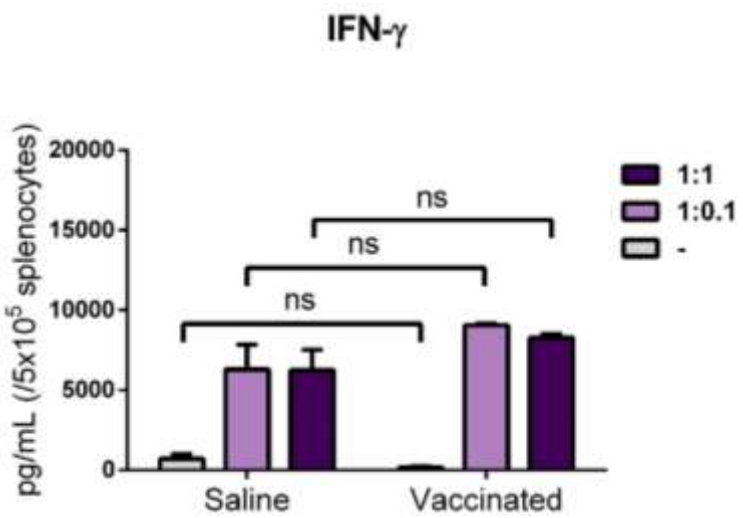


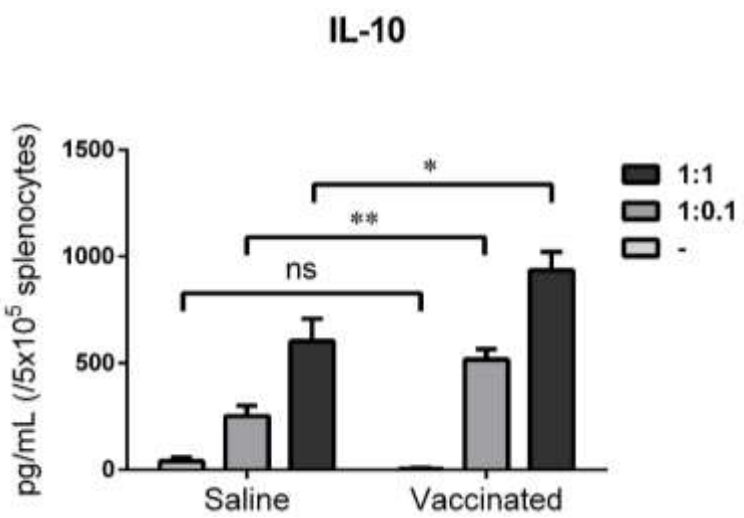


α-PA VACCINE ACTIVATES CELL-MEDIATED IMMUNITY THROUGH THE INTRANASAL ROUTE

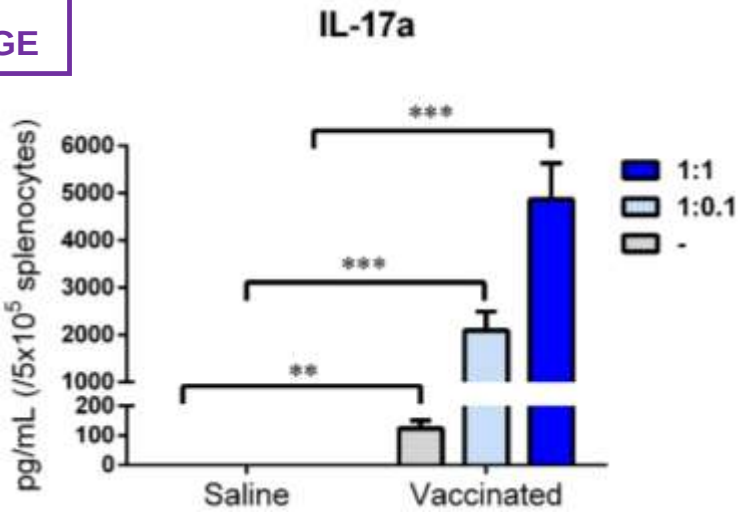
BEFORE CHALLENGE

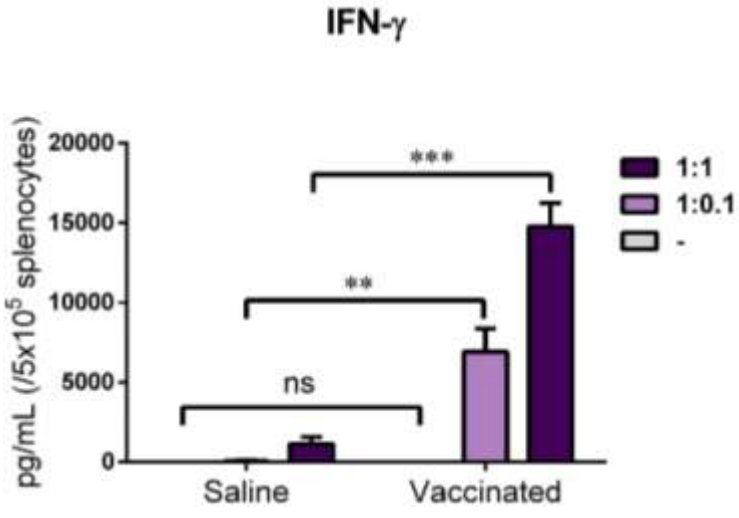


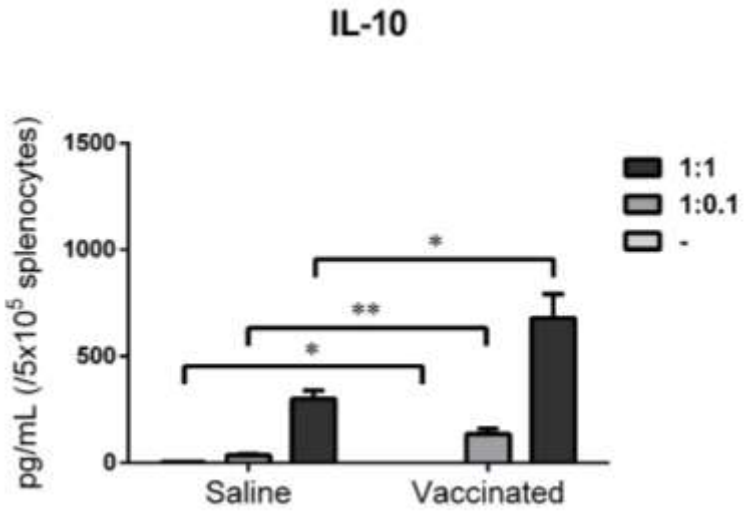




AFTER CHALLENGE







*P<0.05
**P<0.01
***P<0.001 (Student's t test)

α-PA VACCINE ACTIVATES CELL-MEDIATED IMMUNITY THROUGH THE INTRANASAL ROUTE

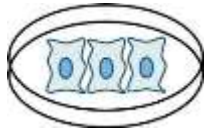
2 WEEKS | 4 WEEKS

D0
IN immunization (2x10^8 CFU)
***n*=6**
Saline *n*=6

D14
IN immunization (2x10^8 CFU)
***n*=6**
Saline *n*=6

D42

BEFORE CHALLENGE



IL-17: Th17, γδT
IFN-γ: Th1
IL-10: Treg?
IL-4: Th2

- Significant **IL-17a** (*P*<0.001) and **IL-10** (*P*<0.05) induction in splenocyte cultures of vaccinated mice
- No **IL-4**

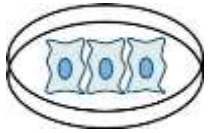
2 WEEKS | 4 WEEKS

D0
IN immunization (2x10^8 CFU)
***n*=7**
Saline *n*=6

D14
IN immunization (2x10^8 CFU)
***n*=7**
Saline *n*=6

D42-49
Challenge

AFTER CHALLENGE



IL-17: Th17, γδT
IFN-γ: Th1
IL-10: Treg?
IL-4: Th2

- Significant **IL-17a** (*P*<0.01), **IFN-γ** (*P*<0.01) and **IL-10** (*P*<0.05) induction in splenocyte cultures of vaccinated mice
- Increased **IL-17a** and **IFN-γ** levels after challenge
- No **IL-4**

α-Pa triggers cytokine-secreting cells through the intranasal route
and an exacerbation of the cellular immune response after challenge

CONCLUSIONS

The intranasal immunization using a D-Glu auxotroph of *Pseudomonas aeruginosa* (PAO1 Δ*murI* - α-Pa vaccine):

- induces systemic antibody production in BALB/c mice, triggering IgM and IgG (IgG1 and IgG2a, mainly) effectively
- elicits IgG's which are **cross-reactive against heterologous strains of *P. aeruginosa***
- confers mice **protection against acute pneumonia** caused by high-virulent and high-risk *P. aeruginosa* strains
- is more effective than the PAO1-inactivated vaccine
- **stimulates cell-mediated immunity**, evident from the boosting of IL-17-, IL-10 and IFN-γ- levels

Thanks to..

- Germán Bou PhD, group leader

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