Bacterial Ghosts as Carrier and Targeting Systems for Mucosal Antigen Delivery

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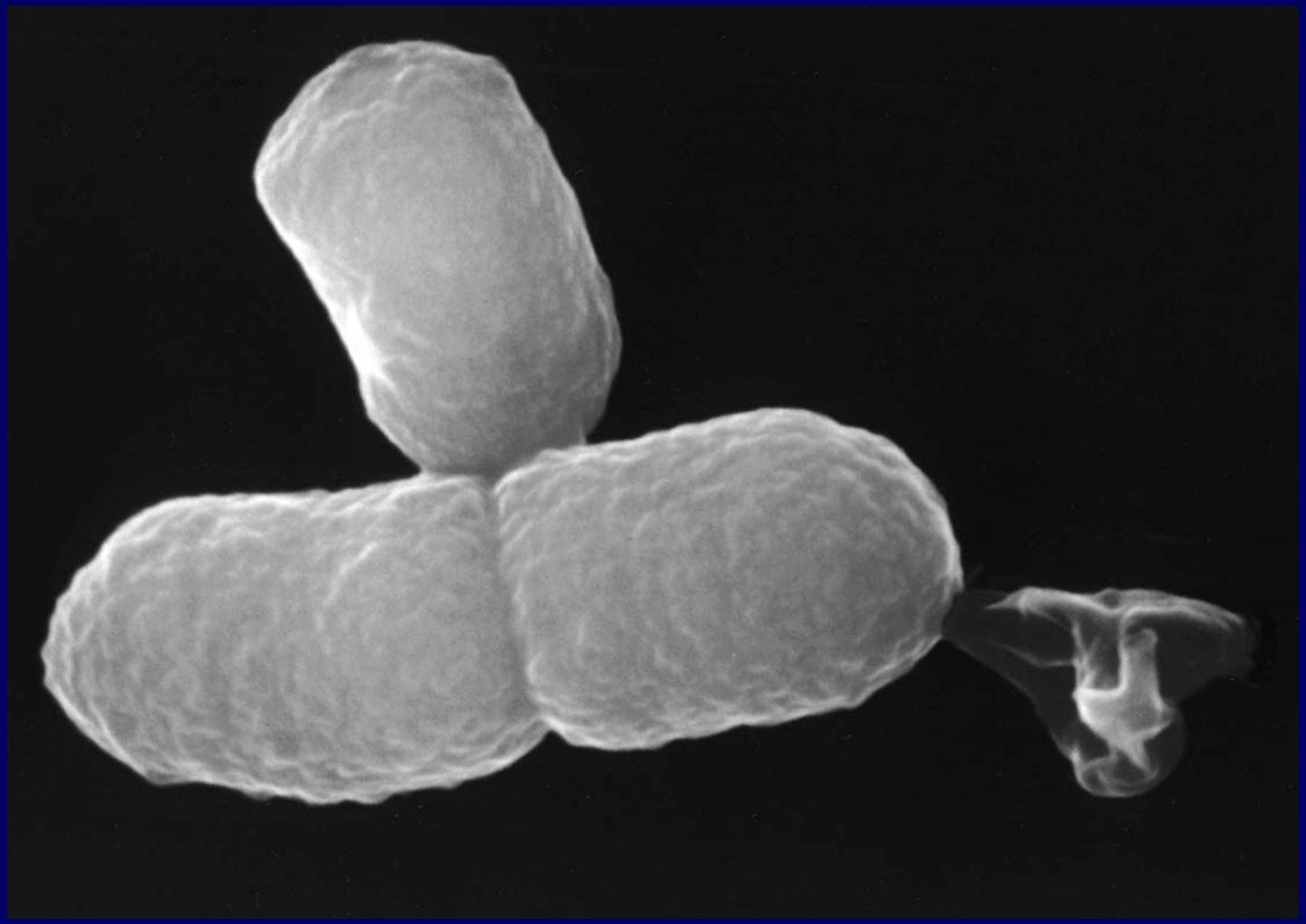
Overview

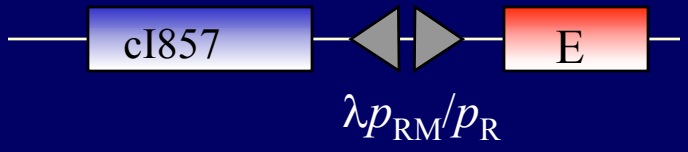
1. Characterization of Bacterial Ghosts
2. Bacterial Ghosts as Antigen Carrier and Targeting Systems

Induction of E-mediated lysis in Gram-negative Bacteria



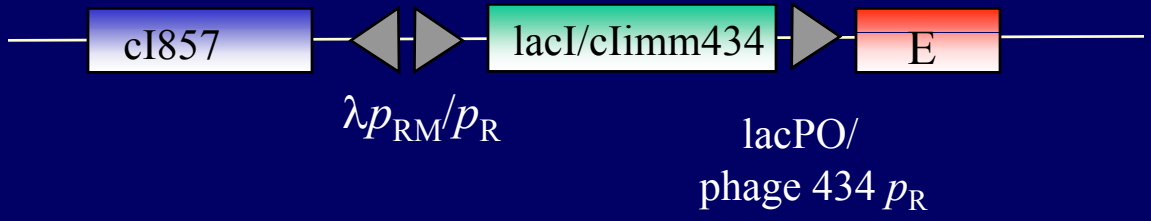
Thermo-Inducible Gene *E* Expression Systems

A: Heat-Inducible



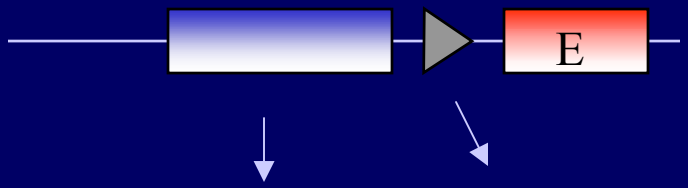
- Lambda wildtype: repression: < 30°C/induction > 30°C
- Lambda WJ mutation: repression: < 36°C/induction > 36°C
- Lambda C10 mutation: repression: < 38°C/induction > 38°C

B: Cold-Sensitive



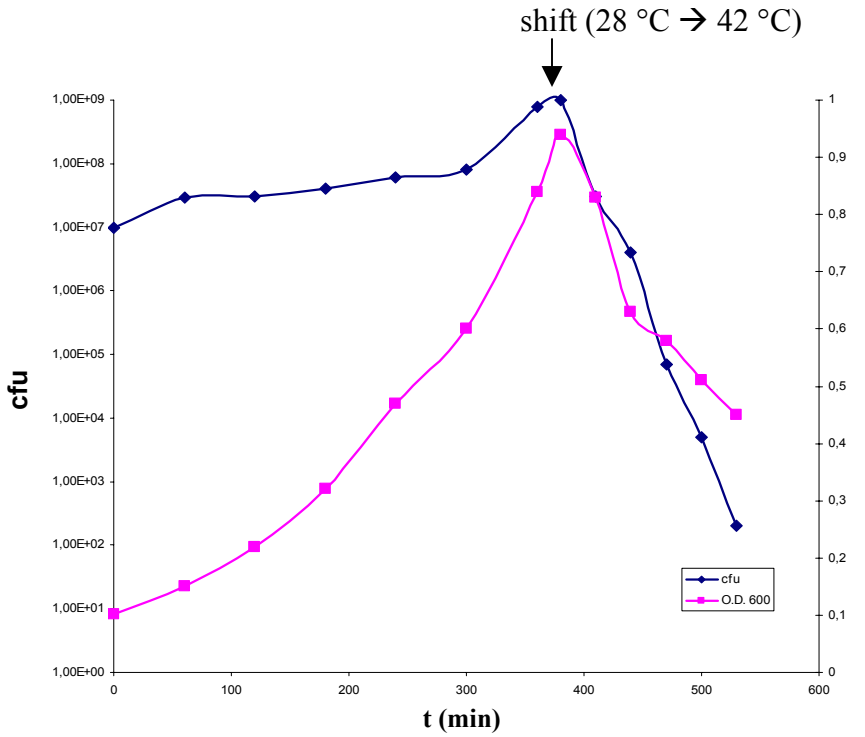
- pCS1: Lambda/lac system
- pCS2: Lambda/phage 434 system

Chemical-Inducible Gene *E* Expression Systems



A: Lac system: inducible with Lactose/IPTG	lacI	lacPO/Ptac
B: Tol system: inducible with 3MBZ	xylS	P_{Tol}
C: Arabinose system: inducible with Arabinose	araC	P_{BAD}

Actinobacillus pleuropneumoniae (App)



High E-lysis efficiency of App9 in 30 l fermention:

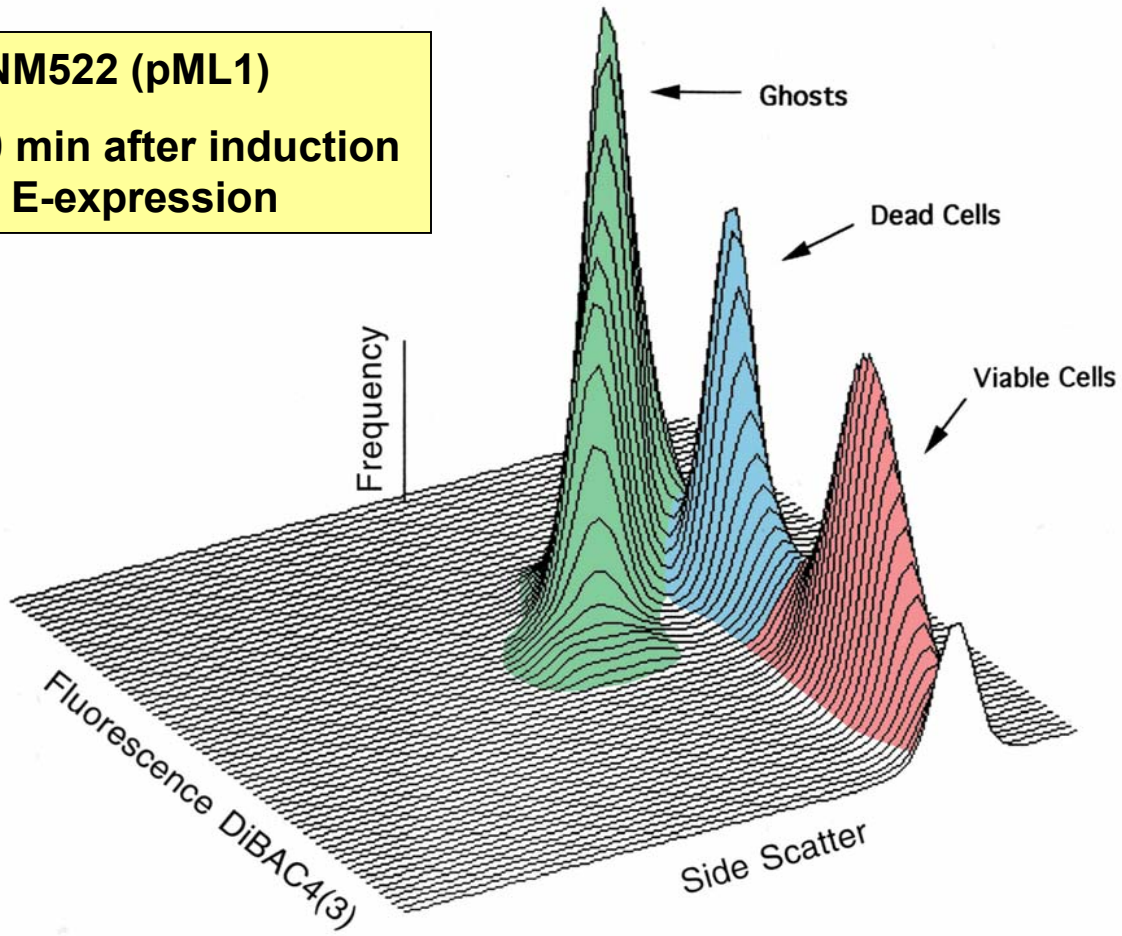
10^9cfu/ml to ~10^2cfu/ml reduction

Total inactivation after additional lyophilization step

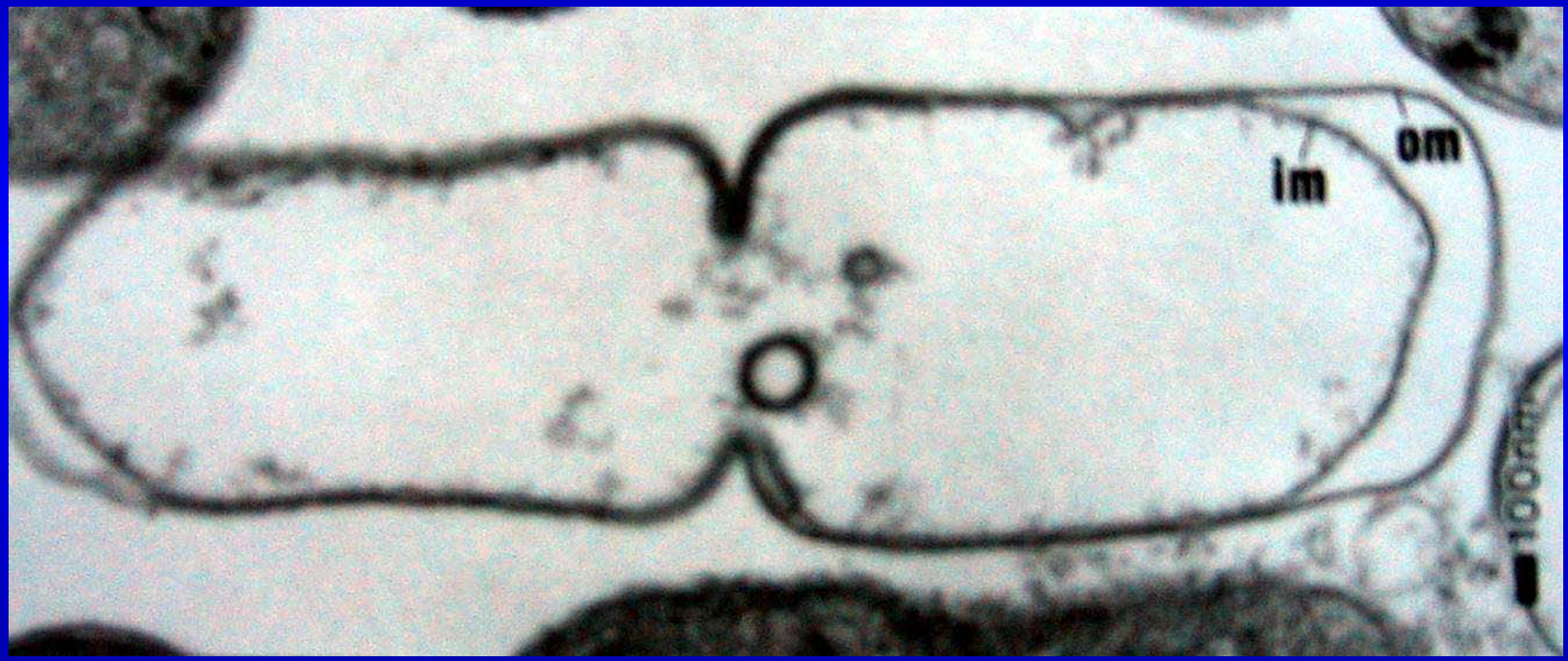
Online-monitoring of bacterial ghost formation by flow cytometry

***E.coli* NM522 (pML1)**

time point: 20 min after induction of gene E-expression



EM Characterization of Bacterial Ghosts



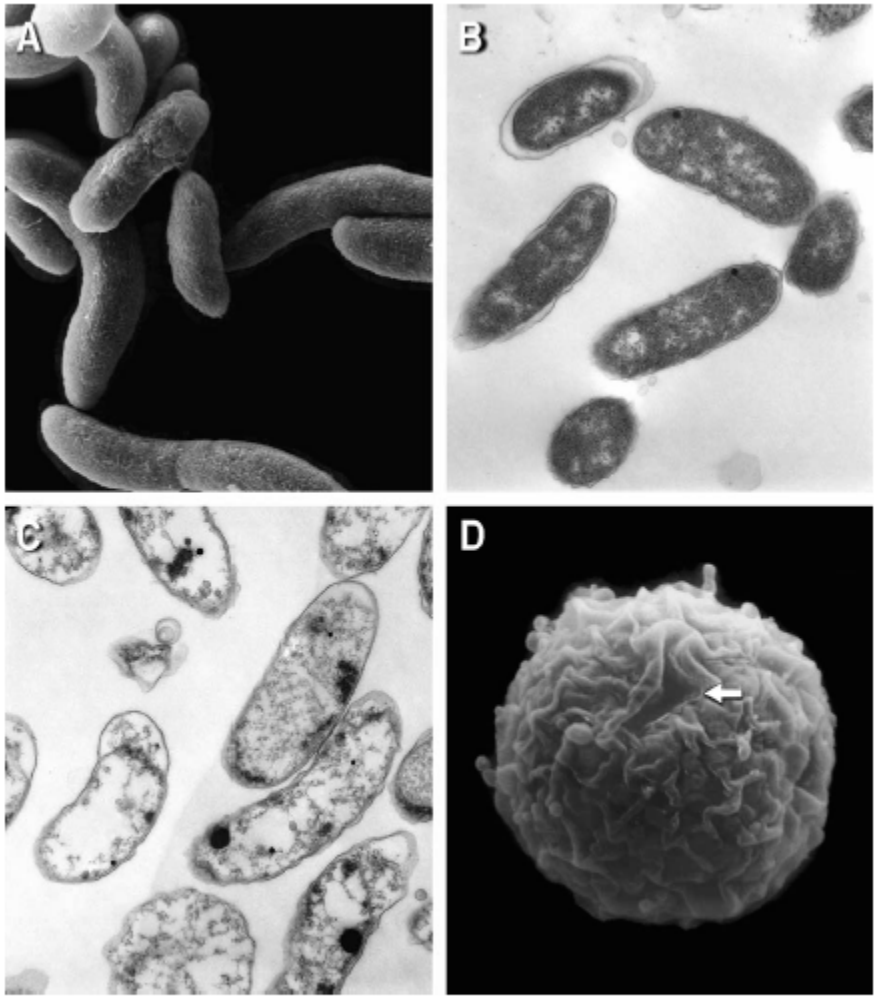
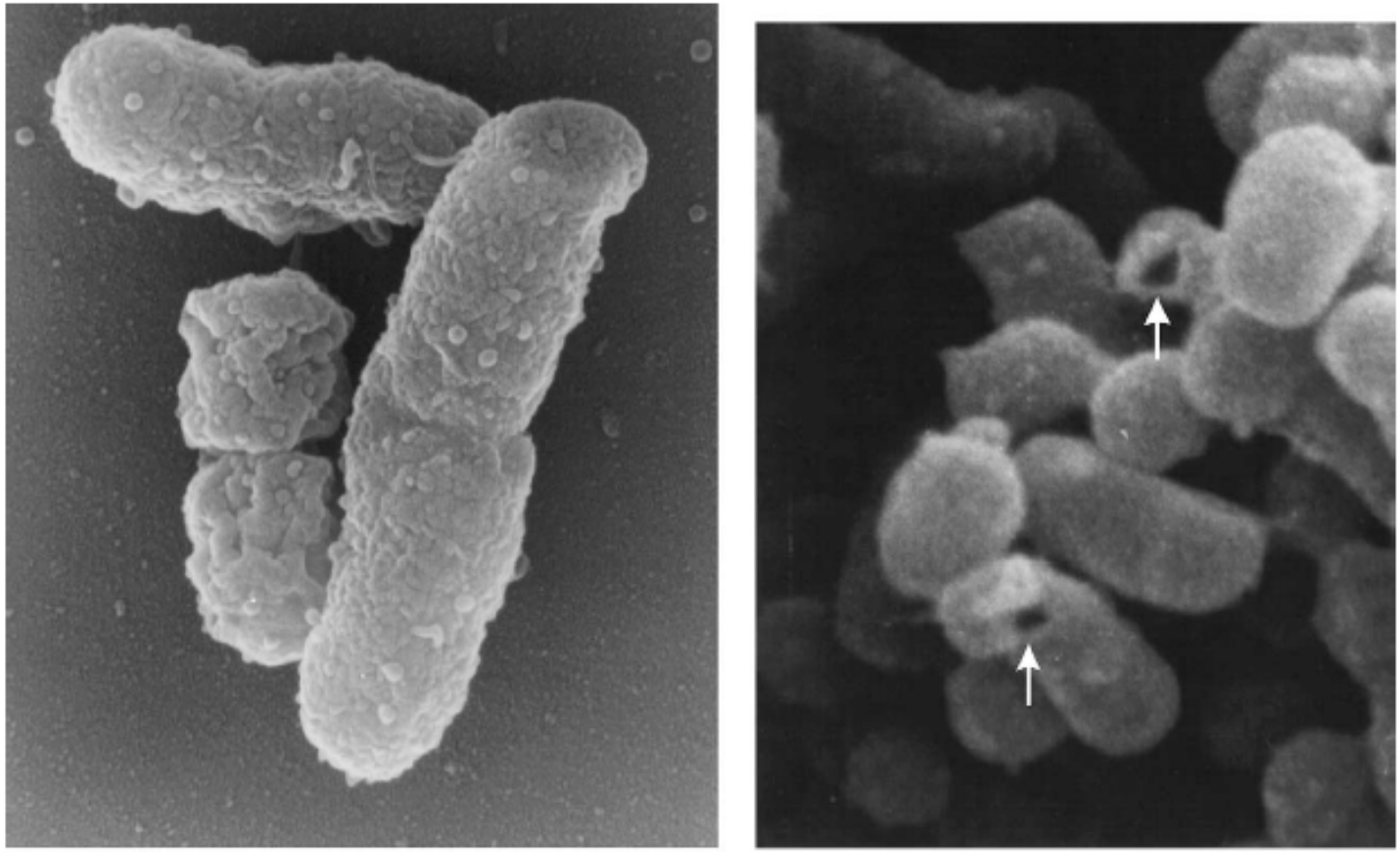


Fig. 3. Characterization of *V. cholerae* and *V. cholerae* ghosts (VCG): (A) field emission scanning electron micrograph (FESEM) of *V. cholerae* 01 HI; (B) transmission electron micrograph (TEM) of ultrathin sections of *V. cholerae* 01 HI (pDKLO1) prior to gene *E* induction; (C) protein E-lysed *V. cholerae* 01 cells (VCG) with intact cellular morphology but devoid of cytoplasmic contents; (D) FESEM of a VCG showing the transmembrane lysis tunnel (arrow) and surface structures.

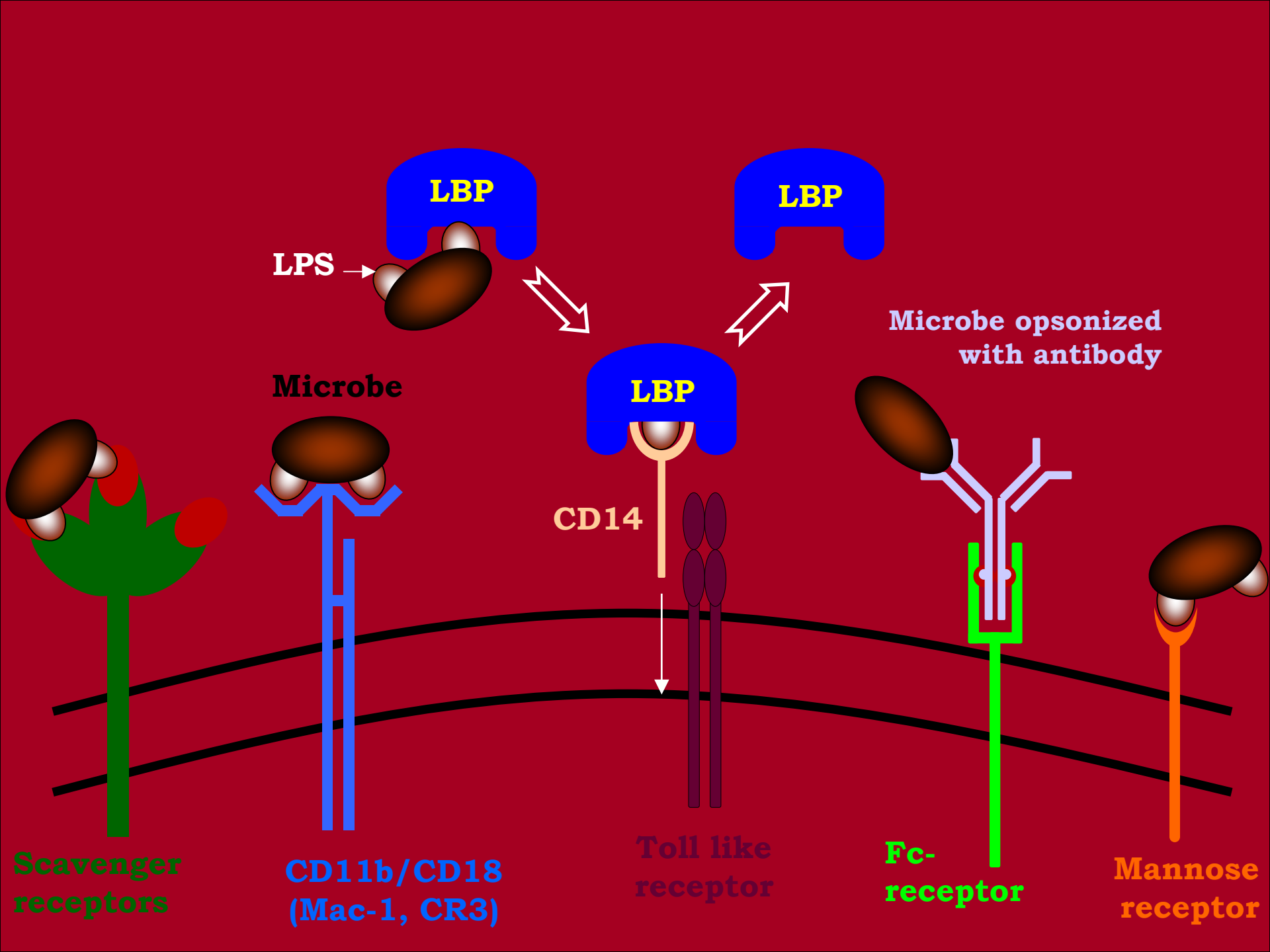


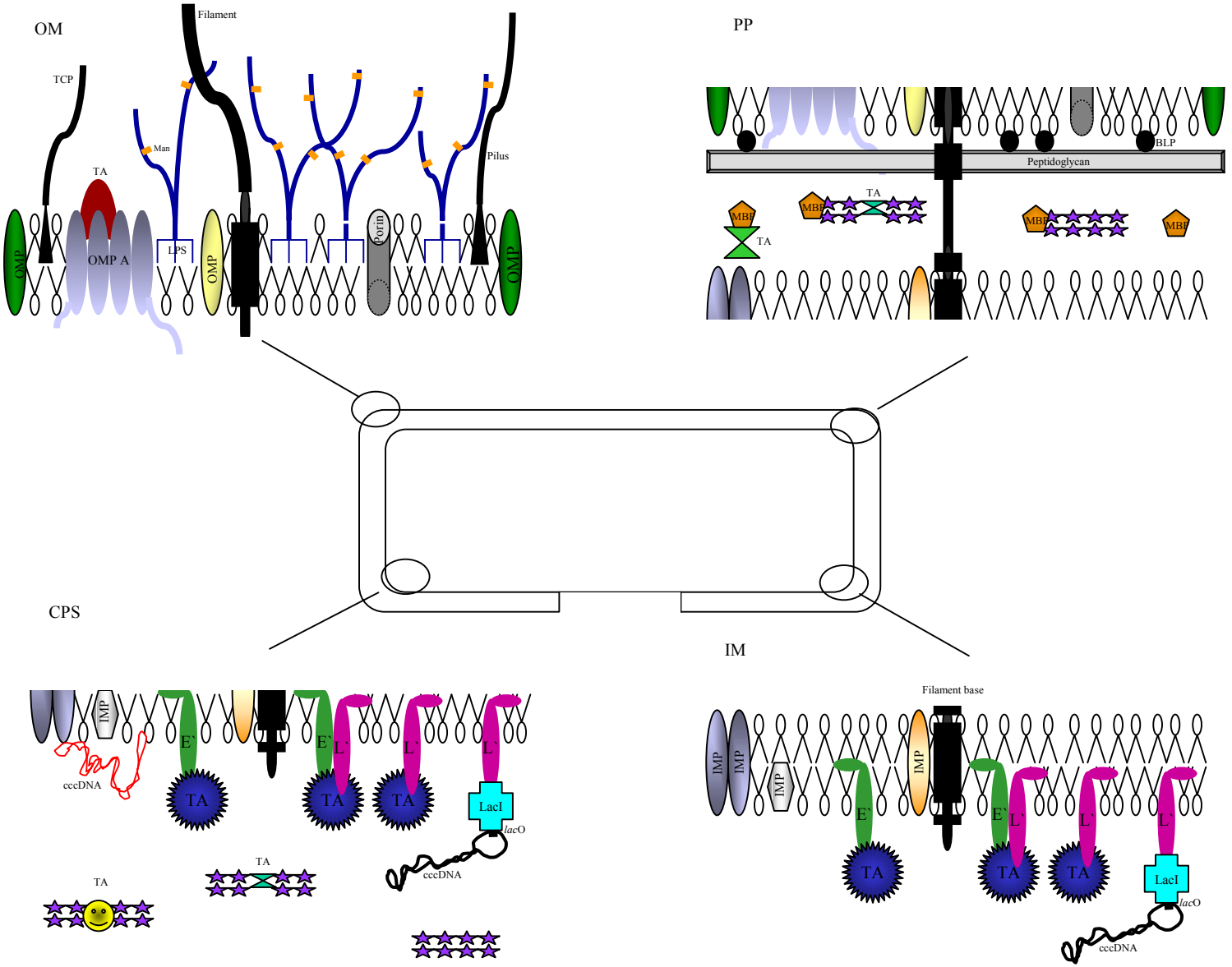
(A) (B)

Fig. 4. (A) Scanning electron-micrograph of *P. haemolytica* (pSON2) grown at 28 °C. (B) Scanning electron-micrograph of *P. haemolytica* (pSON2)-ghosts shifted to 42 °C. Lysis hole is indicated by an arrow.

Bacterial Ghosts as multivalent Antigen Carrier System

- Combination vaccines against bacterial cell envelope and foreign antigens
- Cell envelope as carrier and adjuvans to enhance the immune response against foreign target antigens


LBP
LBP
LPS
LBP
Microbe opsonized with antibody
Microbe
CD14
Scavenger receptors
CD11b/CD18 (Mac-1, CR3)
Toll like receptor
Fc-receptor
Mannose receptor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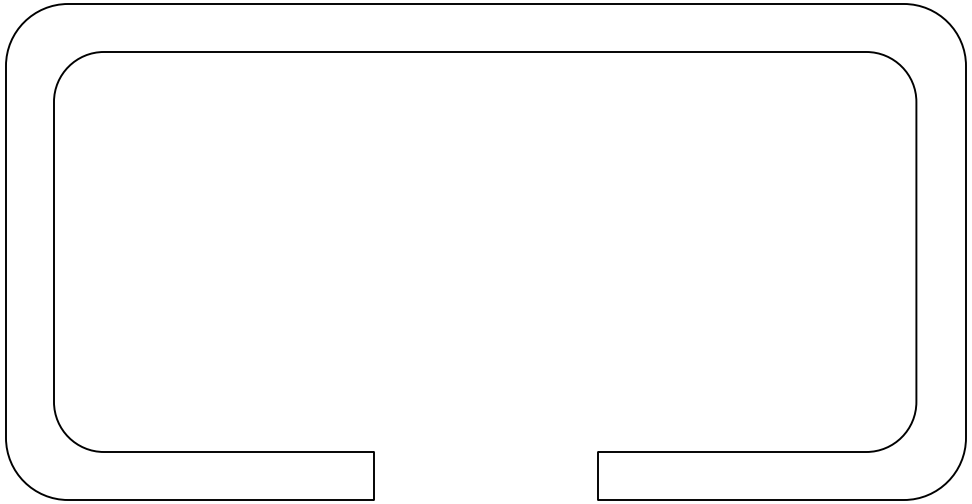


Actinobacillus pleuropneumoniae (App)

Animal Studies

- Immunogenicity and protection in aerosol and endobronchial challenge model (in piglets)
- Mucosal vaccine administration (**aerosol**)
- Parenteral vaccine administration (**i.m.**)

Actinobacillus pleuropneumoniae (App)

Aerosol immunization in swine

challenge: homologous, aerosol

Animals	Immunization (days)	Aerosol challenge (days)	Number of animals with		
			clinical symptoms [%]	pulmonary-lesions [%]	reisolated App [%]
10	$5x10^7$, $5x10^8$, $1x10^9$ cfu **ghosts**, serotype 9; **3x aerosol** (0, 3, 6)	$1x10^9$ cfu serotype 9 **(27)**	0 [0]	0 [0]	0 [0]
10	$5x10^7$, $5x10^8$, $1x10^9$ cfu **inactiv.,** serotype 9; 3x aerosol (0, 3, 6)	$1x10^9$ cfu serotype 9 (27)	0 [0]	0 [0]	0 [0]
5	**Positive control**	$1x10^9$ cfu serotype 9 (27)	**5 [100]**	**4 [80]**	**5 [100]**
5	Negative control	---	0 [0]	0 [0]	0 [0]

Result: Aerosol immunization (3x) with Bacterial Ghosts from *App* serotype 9 protect swine against homologous challenge and prevents charier status.

Actinobacillus pleuropneumoniae (App)

I.m. immunization in swine

challenge: homologous, aerosol

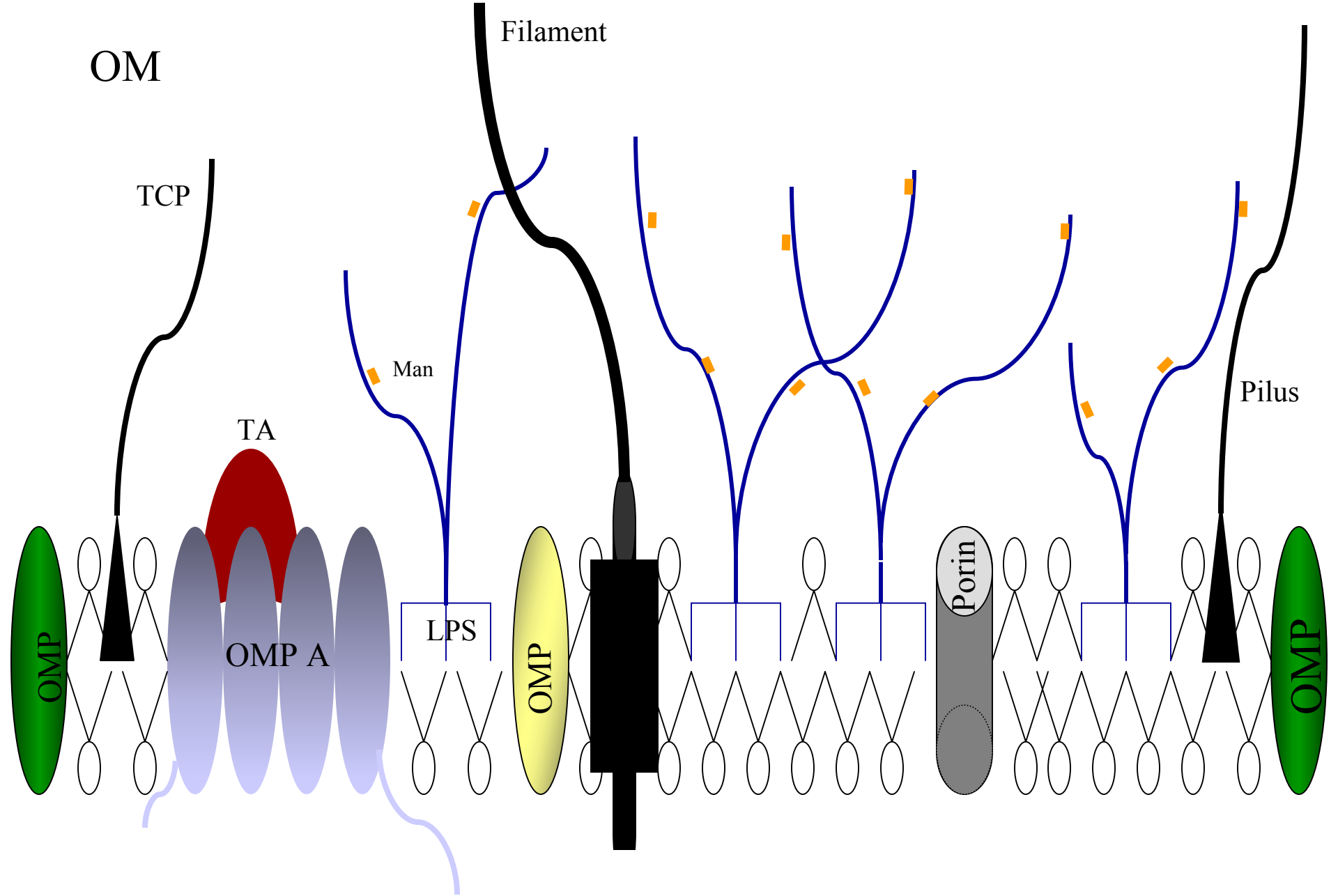
Animals	Immunisation (days)	Aerosol challenge (day)	Number of animals with		
			clinical symptoms[%]	pulmonary-lesiones [%]	reisolated A.pp [%]
10	$5x10^9$ **ghosts**, serotype 9 2x i.m. **(0,14)**	$1x10^9$ cfu serotype 9 **(32)**	0 [0]	0 [0]	0 [0]
9	$5x10^9$ cfu; inactiv., serotype 2 2x i.m. (0,14)	$1x10^9$ cfu serotype 9 (32)	0 [0]	0 [0]	5 [56]
5	**Positive control**	**$1x10^9$ cfu serotype 9 (32)**	**4 [80]**	**3 [60]**	**5 [100]**
5	Negative control	---	0 [0]	0 [0]	0 [0]

Result: I.m. immunization (2x) with bacterial ghosts from *App* serotype 9 protect swine against homologous challenge and prevents charier status.

Actinobacillus pleuropneumoniae (App)

***Benefits* of *App*-Ghost-System**

- Retain morphology and structure of live bacteria
- Preserve surface components (antigenicity)
- Reduced cytoplasmic and genetic material (safety)
- Low cost - Large scale production
- E-mediated lysis is patent-protected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Pilus
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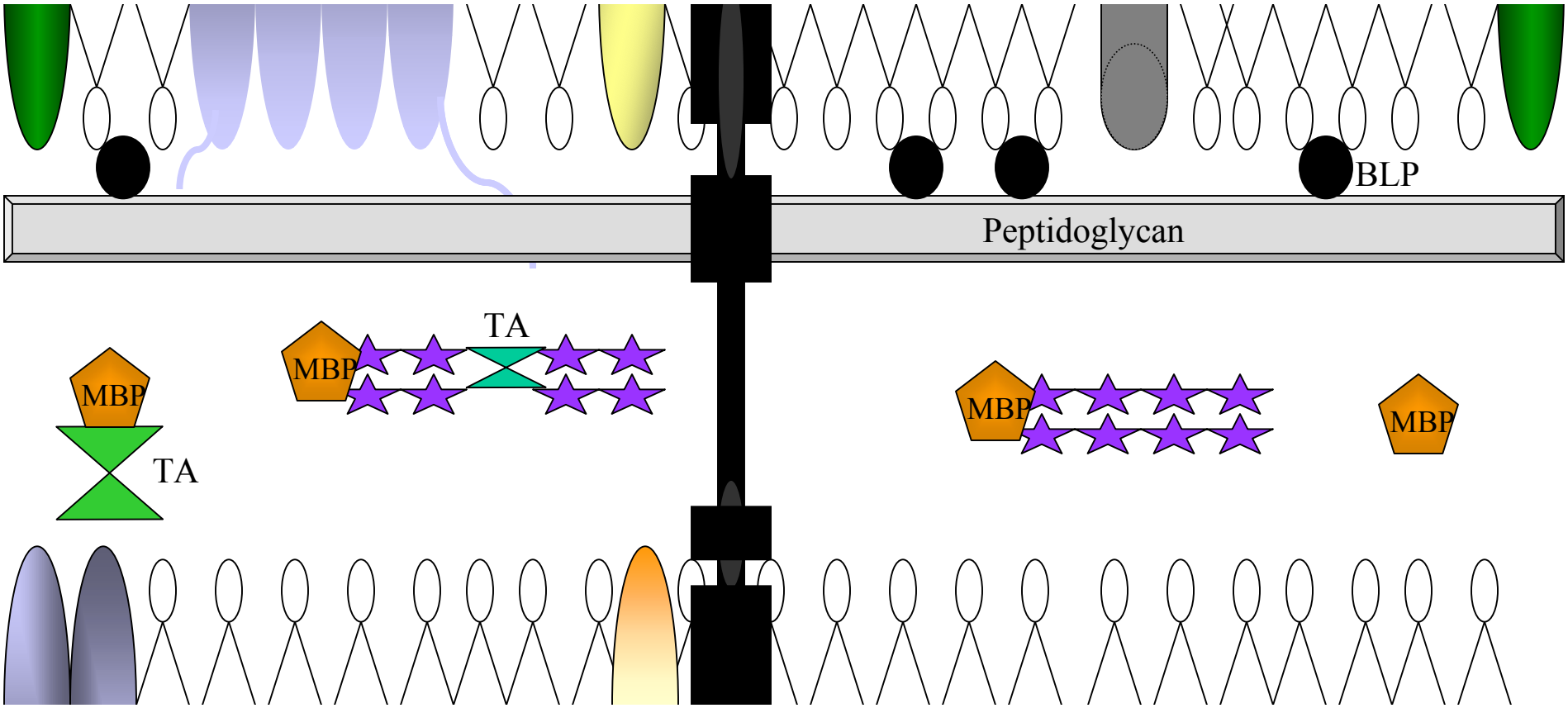
OMP A
LPS
OMP
Porin
OMP

PP



Candidate vaccine against NTHi infections, based on a combination of recombinant S-Layer and bacterial ghosts

Gut immunization regime of DA rats

Ghosts/SbsA/Omp26 or Ghosts/MBP/Omp26

- 10μg Omp26 in ghosts
- intestinal Peyer`s patches (IPP), duodenum (ID)

- IgG, IgG1, IgG2a, IgA, Bacterial clearance

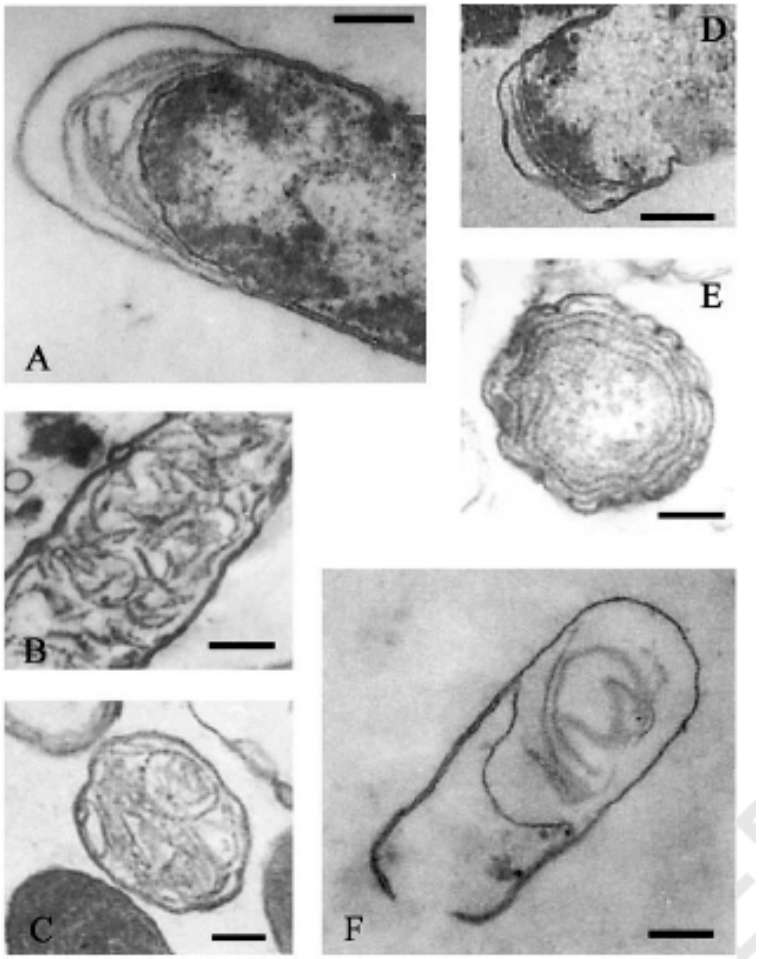
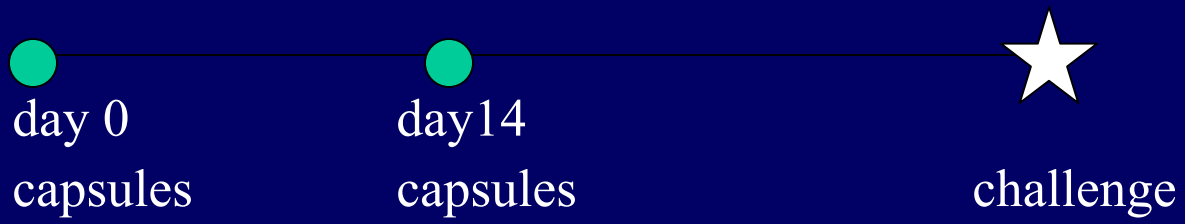


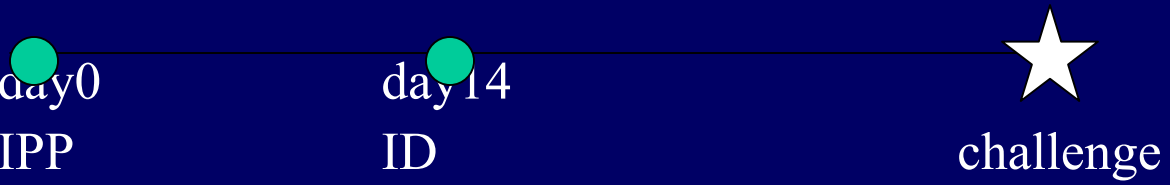
Fig. 4. Electron micrographs of *E. coli* pop2135 cell envelope preparations harboring recombinant SbsA self-assembly products in the cytoplasm and periplasm. Ultrathin sections of a partially lysed (A) and a fully lysed cell (F), both harboring SbsA/MBP self-assembly products in the periplasm. Ultrathin sections showing self-assembly product formation of either SbsA/Omp26$_{3112}$ in the periplasm (D) or SbsA/Omp26$_{878}$ in the cytoplasm (B, C) of bacterial ghosts. Cross-section of a cell harboring cylindrical SbsA self-assembly products in the cytoplasm (E). Bars 200 nm.

Mucosal immunization in DA rats

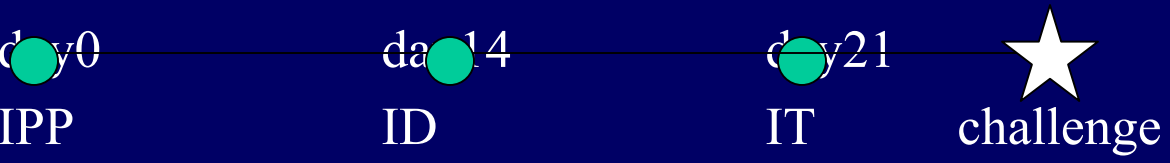
- Oral



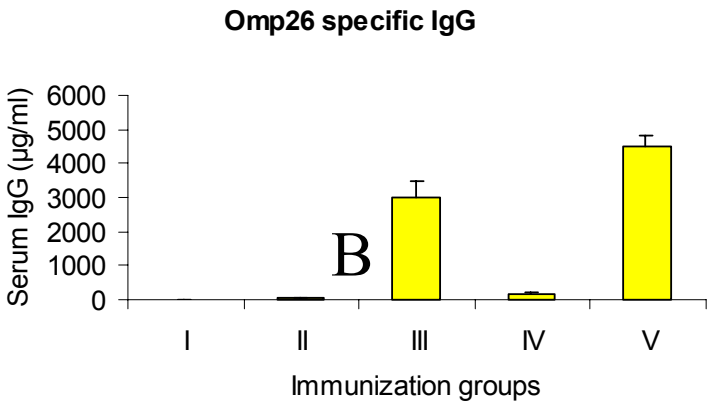
- Gut

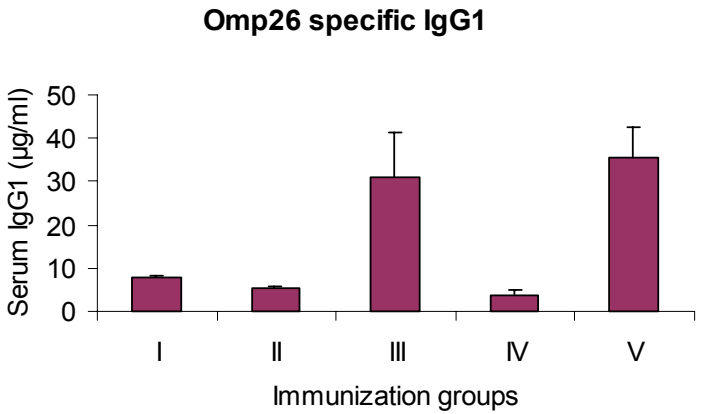


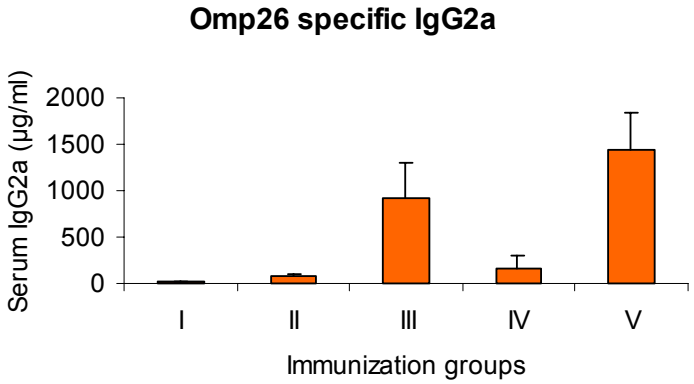
- Gut/Lung



Omp26 specific Antibodies







Bacterial clearance

IMMUNIZATION GROUPS				BACTERIAL RECOVERY (%) ± SEM	
Group	Antigen	Route	No	Lung	BAL
Gut immunization regime					
I	Nonimmune	untreated	4	100 ± 13.2	100 ± 23.2
II	Ghosts/SbsA	IPP/ID	5	90.6 ± 14.3	91.2 ± 14.4
III	Ghosts/SbsA/Omp26	IPP/ID	4	52.7 ± 3.9**	49.6 ± 15.3
IV	Ghosts/MBP	IPP/ID	4	105.1 ± 53.5	53.5 ± 18.5
V	Ghosts/MBP/Omp26	IPP/ID	4	42.0 ± 8.6*	77.6 ± 58.7

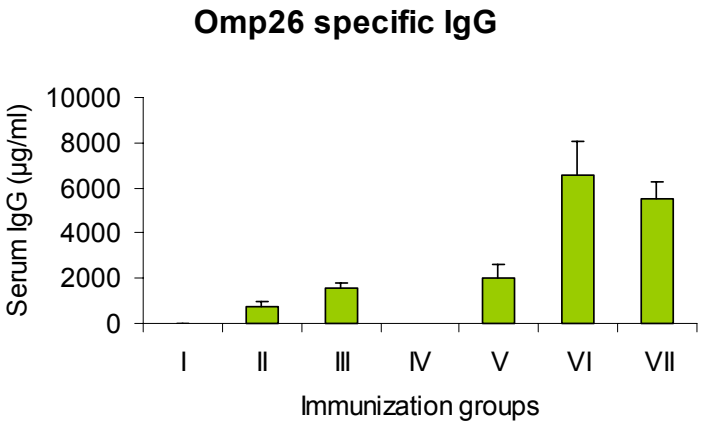
Gut/Lung immunization regime

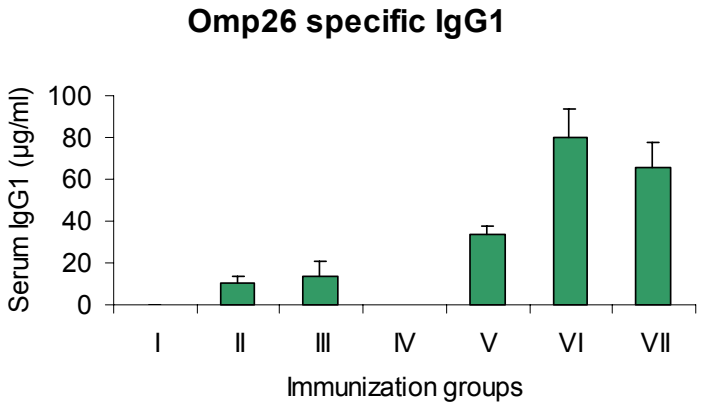
Ghosts/SbsA/Omp26

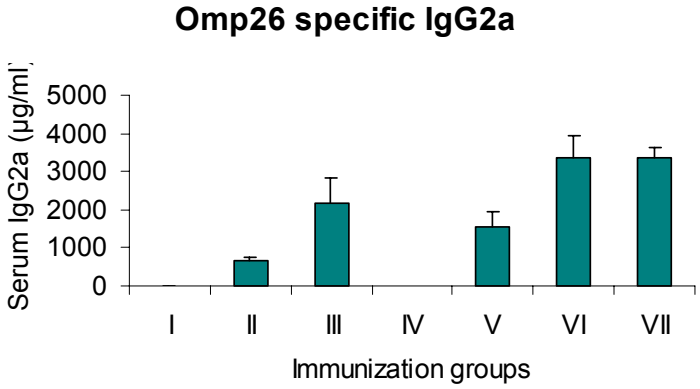
- 20μg Omp26 in ghosts — IPP, ID
- 20μg purified Omp26 — IT

- IgG, IgG1, IgG2a, IgA
- Bacterial clearance
- Lymphocyte Proliferation

Omp26 specific antibodies



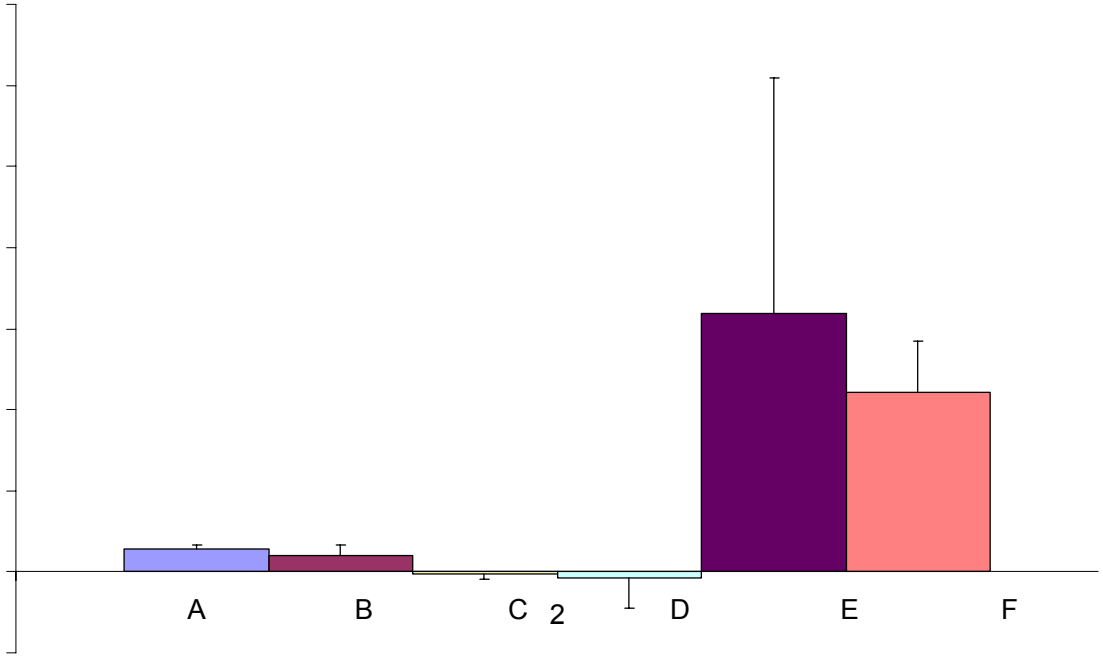




Bacterial clearance

IMMUNIZATION GROUPS				BACTERIAL RECOVERY (%) ± SEM	
Group	Antigen	Route	No	Lung	BAL
Gut/Lung immunization regime					
VI	rOmp26	IT	5	33.8 ±5.0**	21.0 ± 5.1*
VII	WCK rOmp26	IPP/ID IT	5	11.7 ± 6.5**	9.0 ± 2.8**
VIII	Ghosts/SbsA PBS	IPP/ID IT	5	X	X
IX	Ghosts/SbsA rOmp26	IPP/ID IT	4	20.6 ± 7.6**	20.5 ± 6.5*
X	Ghosts/SbsA/Omp26 rOmp26	IPP/ID IT	5	7.9 ± 1.7**	5.8 ± 1.5**
XI	Ghosts/SbsA/Omp26 rOmp26	ID/ID IT	5	4.2 ± 0.8**	5.6 ± 2.4**

Omp26 specific lymphocyte proliferation



SI= cpm experimental 10µg/ cpm experimental 1µg

Results

- Both regimes induce an Omp26 specific antibody response
  - higher in Gut/Lung immunized animals
- Gut immunization results in limited clearance
- Gut/Lung immunization results in very efficient clearance
- Gut/Lung immunization induces Omp26 specific lymphocyte proliferation

Conclusions

- Omp26 delivered in bacterial ghosts is immunogenic
  - Amount of Omp26 rather than location/ immobilzation
- Gut immunization (oral) induces Omp26 specific humoral response
  - Th1 dominant
- Boost with purified Omp26 enhances bacterial clearance
  - IT immunization important
- Bacterial ghosts unspecifically enhance bacterial clearance

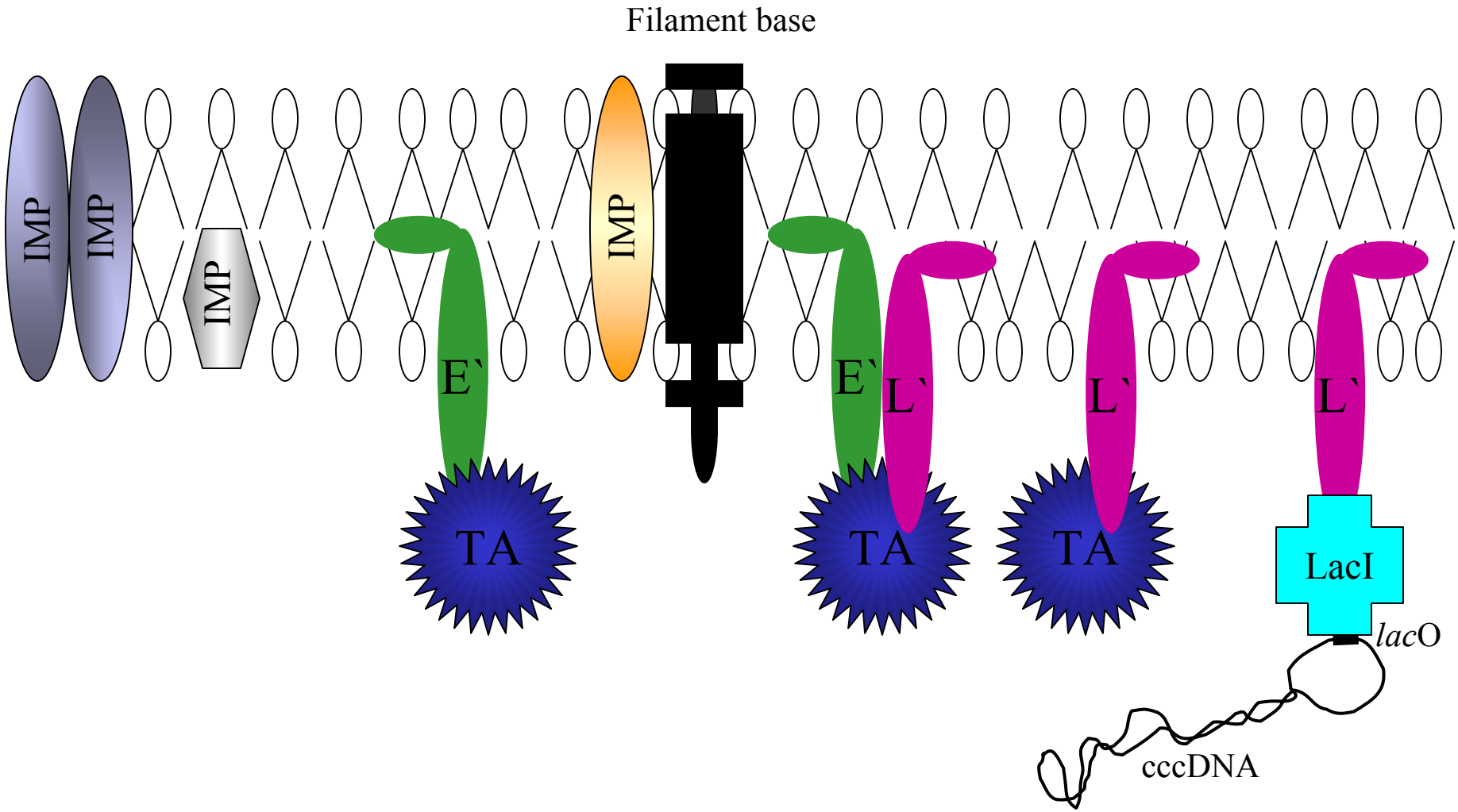
Bacterial Ghost Delivery of Possum Immunocontraceptive Vaccine

Serumtiter

Oral route	**Intraduodenal route**	**Conjunctival/ Nasal route**
2/8 1:128- 1:1024 nonresponders <1:32	6/8 1:256- 1:4096 nonresponders <1:32	8/8 1:4096- 1:8192

Antibodies against the target antigen ZP3 were detected in the serum, follicular fluid and oviduct secretions of immunised animals. It was demonstrated that possums can mount an immune response to a contraceptive protein expressed by bacterial ghosts.

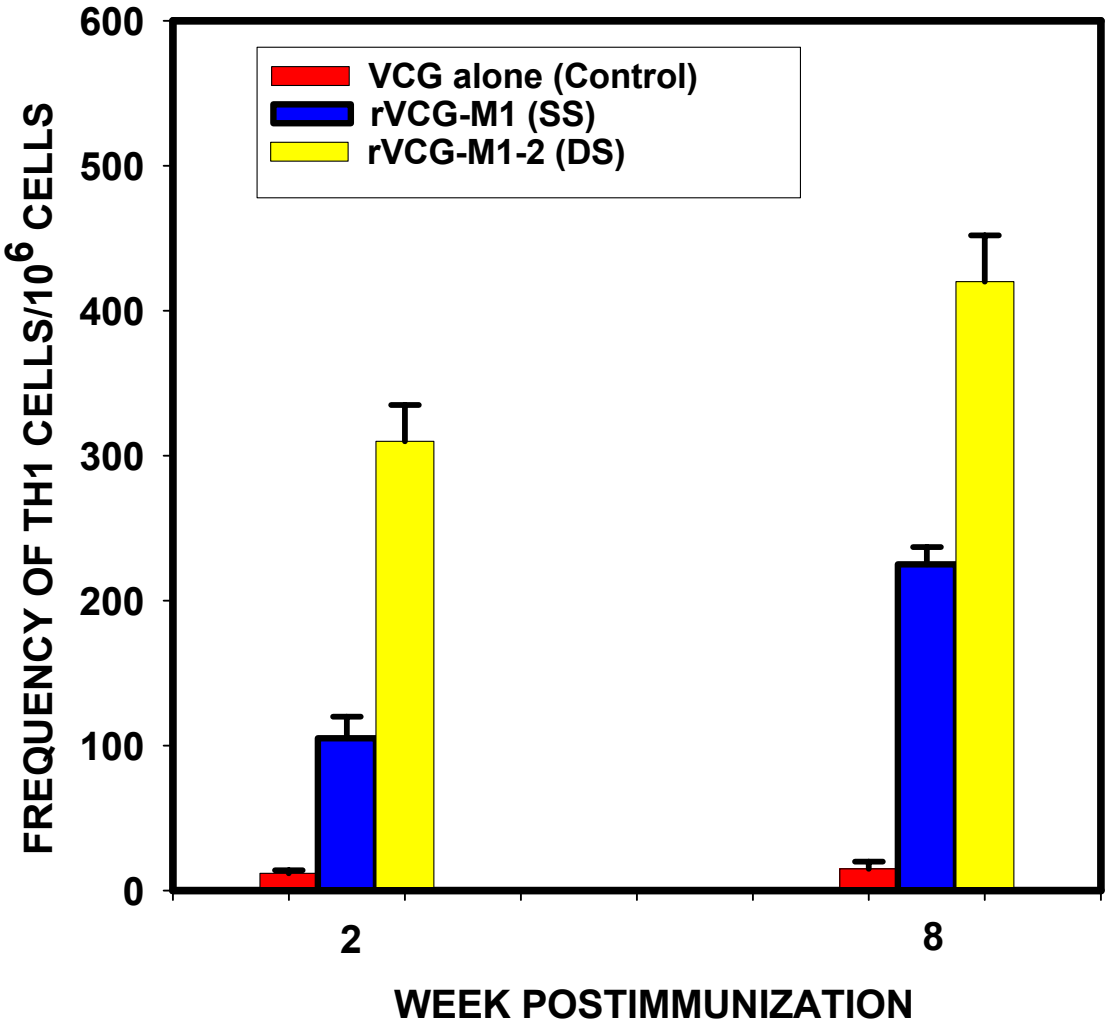
IM



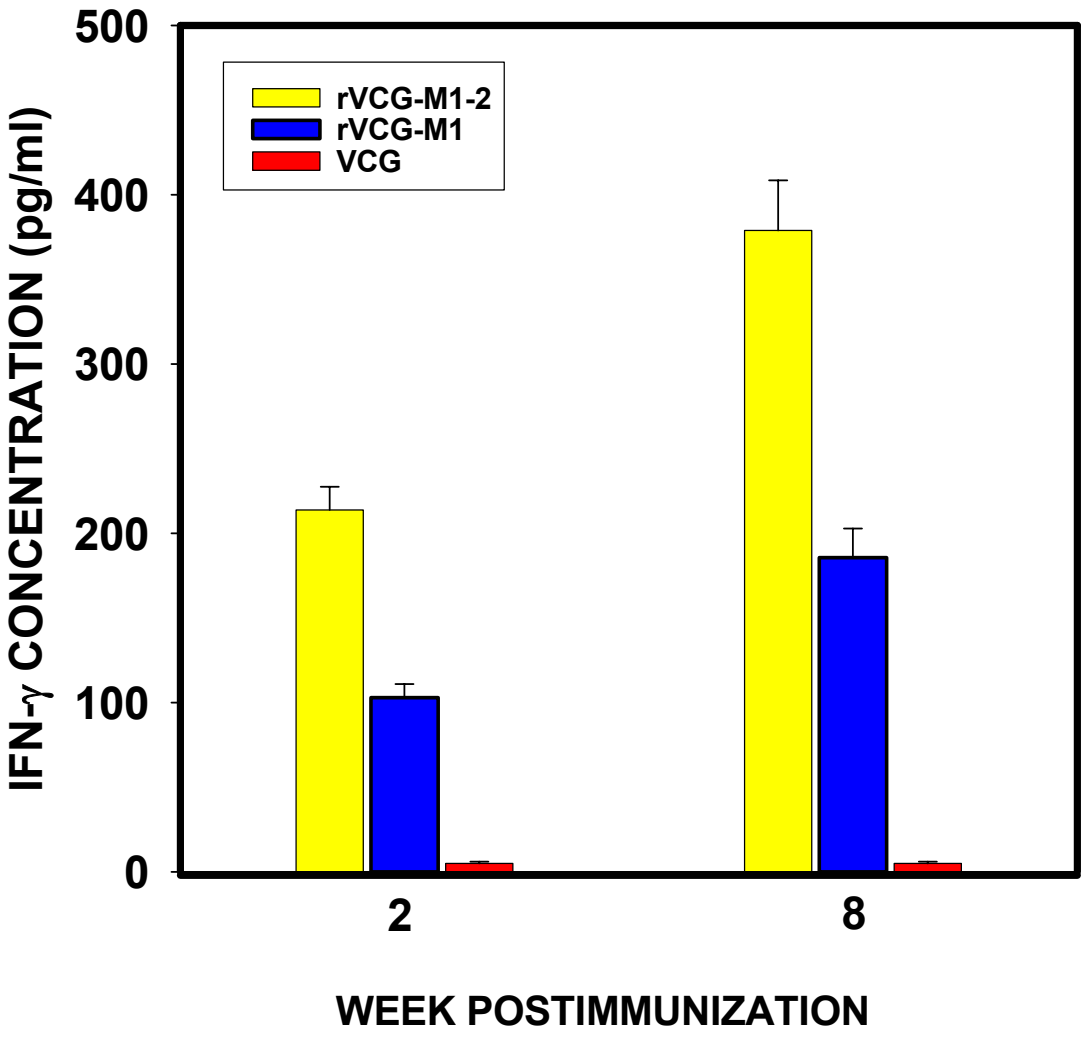
The surrogate mouse model for evaluating vaccine efficacy against Clamydia trachomatis

- **Step 1. Immunization with rVCGs**
- **Step 2. Immunogenicity**
  - **Th1 response by antigen-specific IFN-γ**
  - **Assessment of antibody response – IgA, IgG2a**
- **Step 3. Preparation of immune T cells**
  - **Isolation of splenic/ILN T cells**
- **Step 4. Adoptive transfer of immune T cells into naïve mice & assessment of protective immunity**
  - **Swabbing of mice and isolation of chlamydia in tissue culture**

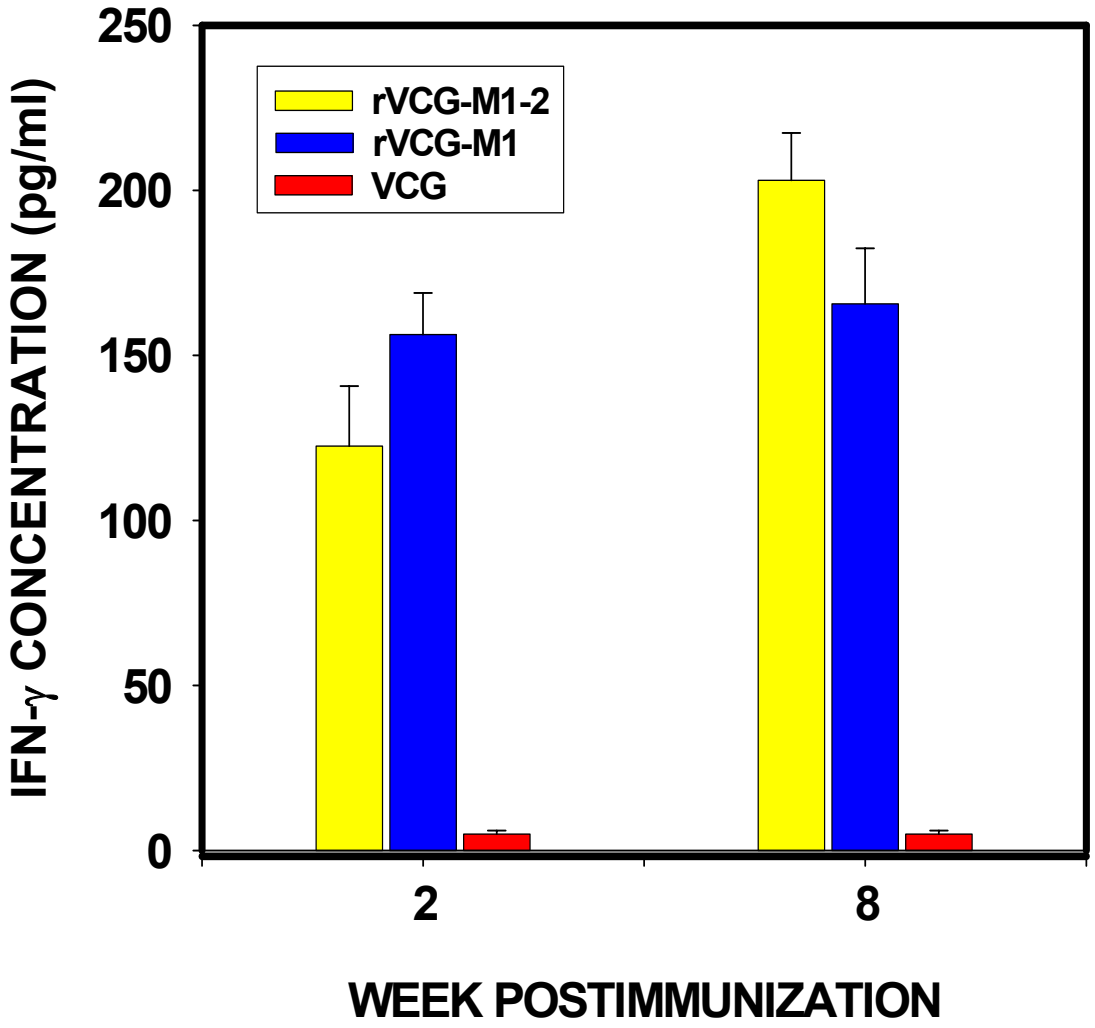
Relative Frequency of Specific TH1 Cells



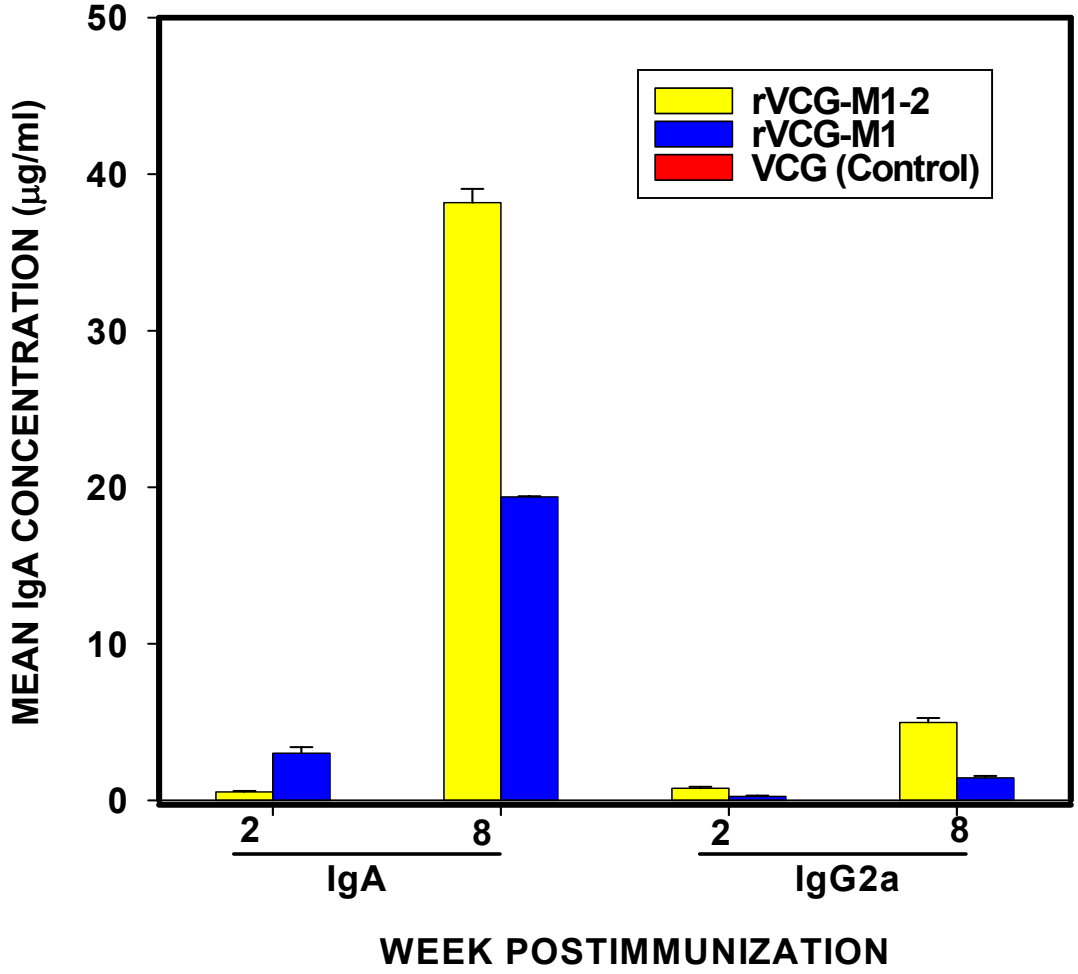
IFN-γ Secreted by Genital Tract T Cells



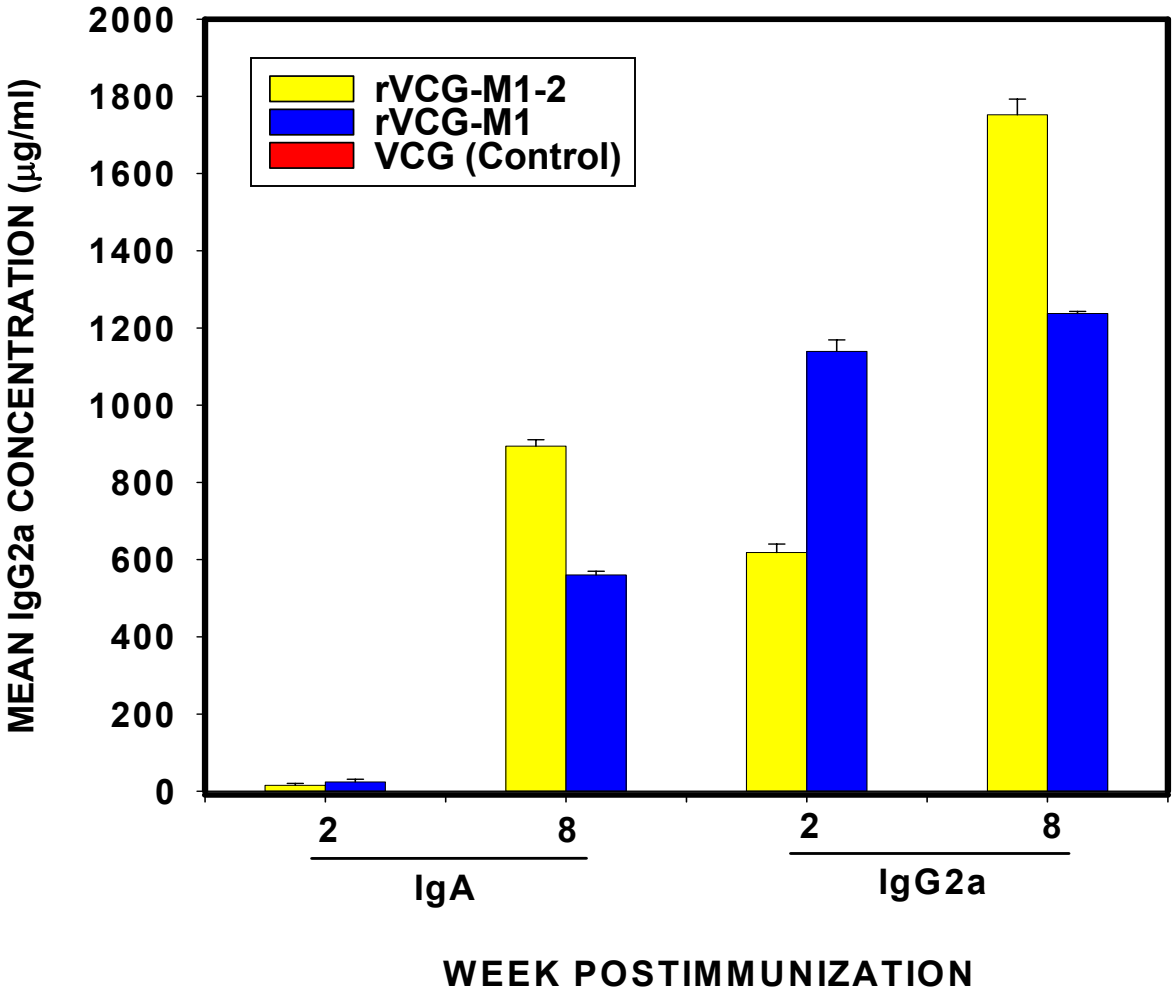
IFN-γ Secreted by Splenic T Cells



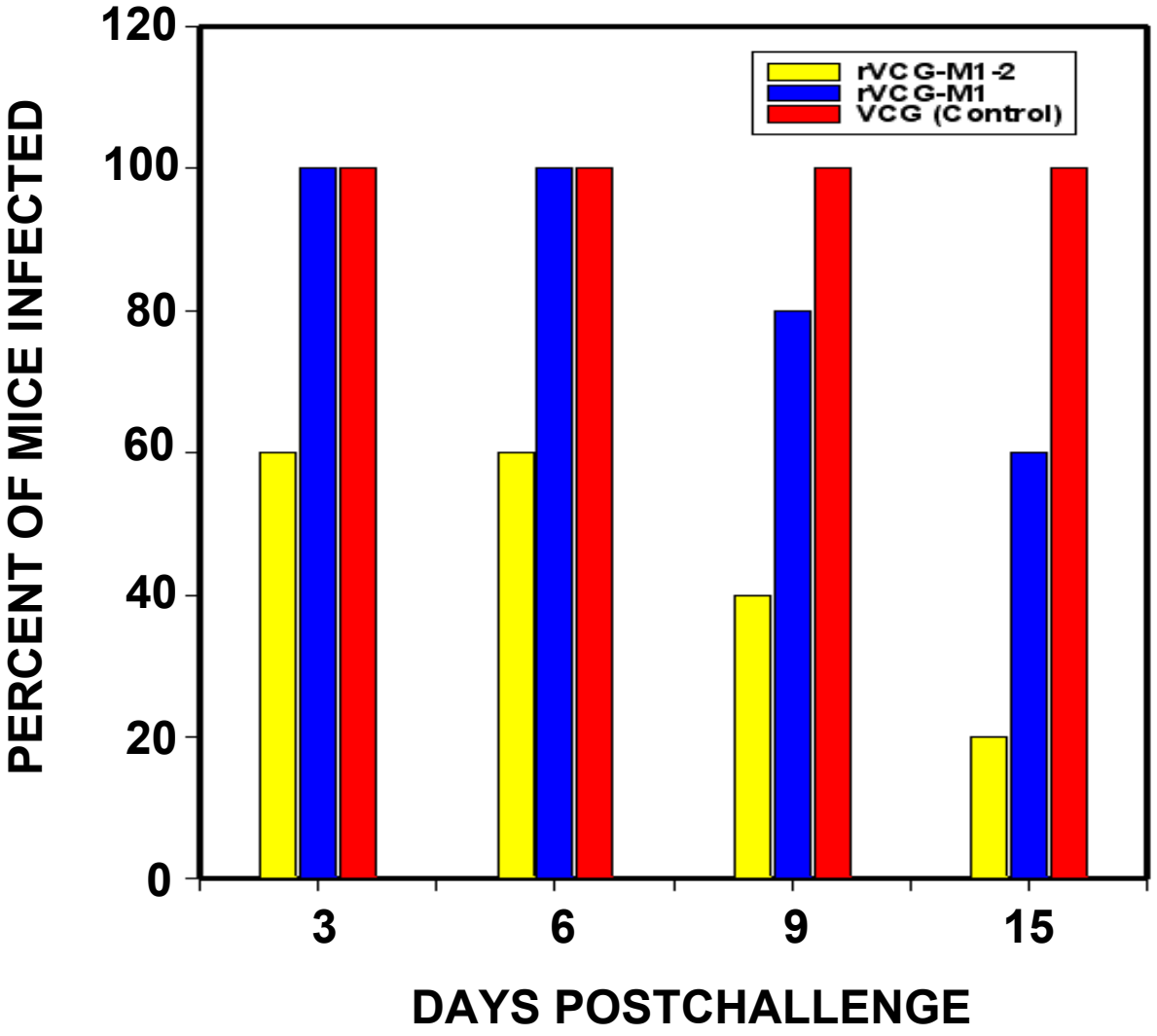
Secretory Antibodies in Genital Mucosa



Secretory Antibodies in Serum



Induction of Protective Immunity



Summary of Results

- TH1-associated Ab (sIgA & IgG2a) response higher in DS versus SS vaccine
- SS vaccine is partially protective against a genital chlamydial challenge in mice
- The DS vaccine more efficacious than the SS vaccine

Bacterial ghosts as candidate vaccines

•bacterial ghosts are non living and represent an advantagous alternative to heat, chemical or irradiation inactivated bacteria

• the production process (E-mediated lysis) does not denature bacterial ghost envelopes

•bacterial ghosts can be produced in large quantities by fermentation

•bacterial ghosts are stable as freeze dried material for long periods of time and do not require a cold chain for storage

•bacterial ghosts can be applied by oral, rectal, aerogen, sc or im routes for induction of mucosal immunity

International collaborations

NTHi Vaccine
Jennelle Kyd
Centre for Forensic Studies
University of Canberra

C.trachomatis/V.cholerae
Francis O. Eko
Morehouse School of Medicine
Atlanta

Immunocontraception
Janine Duckworth
Manaaki Whenua Landcare Research
Lincoln, NZ