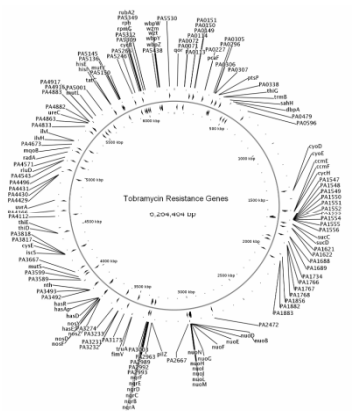
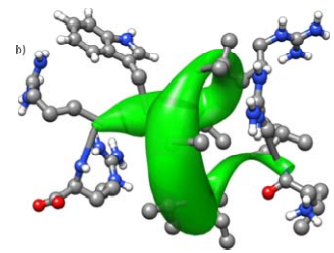
Alternative to Antibiotics: Selective Modulators of Innate Immunity



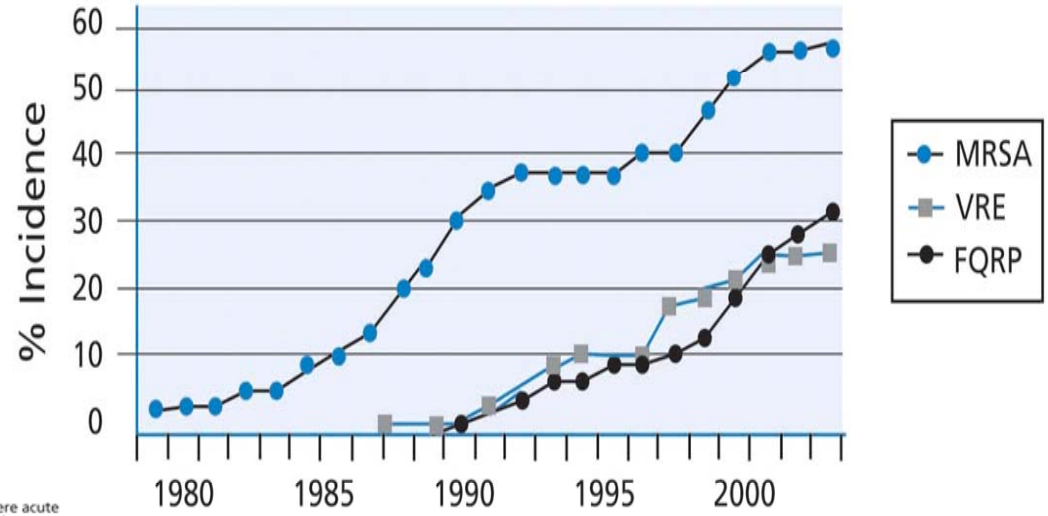
R.E.W. (Bob) Hancock

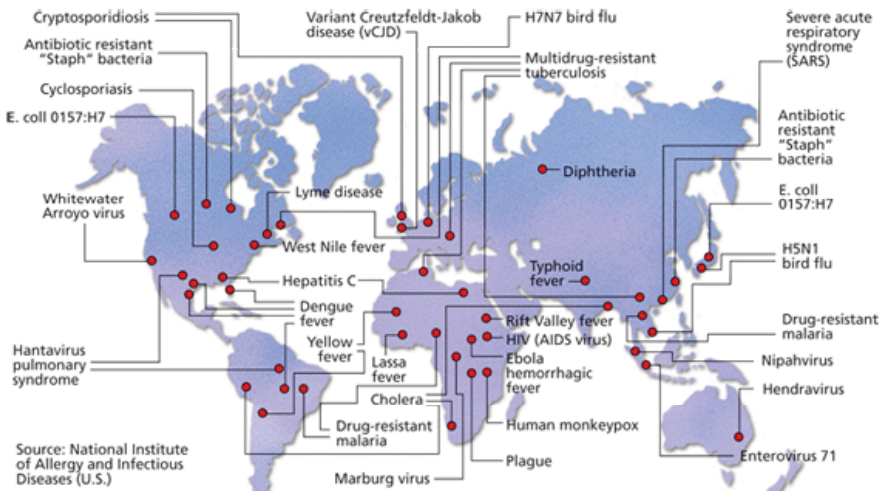
University of British Columbia, Vancouver Canada

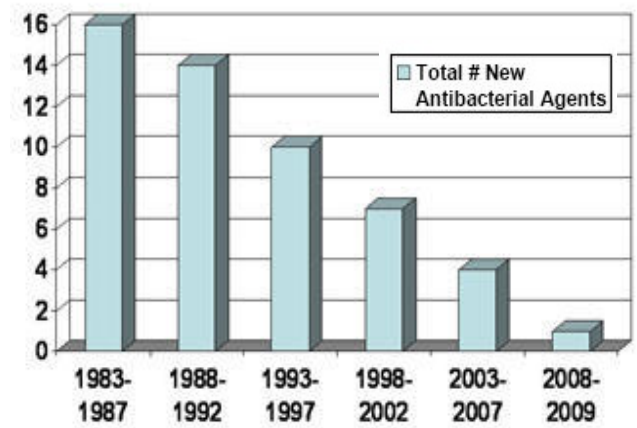


Approaching the Antibiotic Resistance Era

- Multidrug resistance
- Dry pipeline
- Emerging problems
- Animal Uses



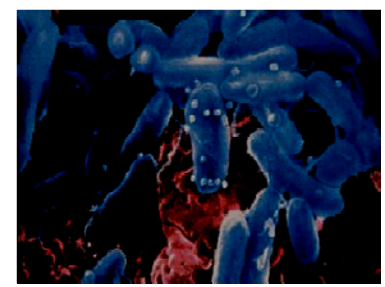




Spellberg, *CID* 2004, Modified

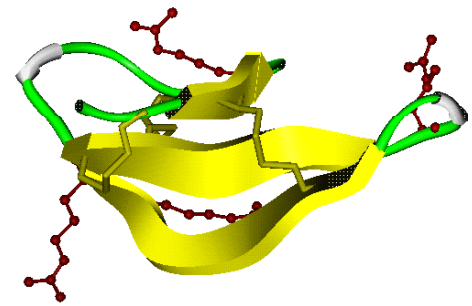
IDSA - "Bad Bugs No Drugs"

There is an urgent need for new strategies to treat infections



Cationic Peptides

Birds do it, bees do it, even educated fleas do it.... *Cole Porter*



- **Important host defense mechanism in all complex species of life.**
- **>1000 peptides known. Diverse amino acid sequences and structures.**

- **12 to 40 (or more) amino acids. Net charge +2 to +9 (Lys; Arg). Amphipathic.**

- **Role in Innate Immunity involves both antimicrobial and immunomodulatory activities; "Host Defence" peptides.**

Peptide Arrays and Machine Learning (QSAR) creates improved 9-mer peptides

- **Relates activity to structure (based on primary sequence and physical and inductive "descriptors"); using Neural Networks.**
- **Trained on tested peptides; predicts activity of virtual peptides**
- **Very effective and quite accurate for 100,000 peptide library**

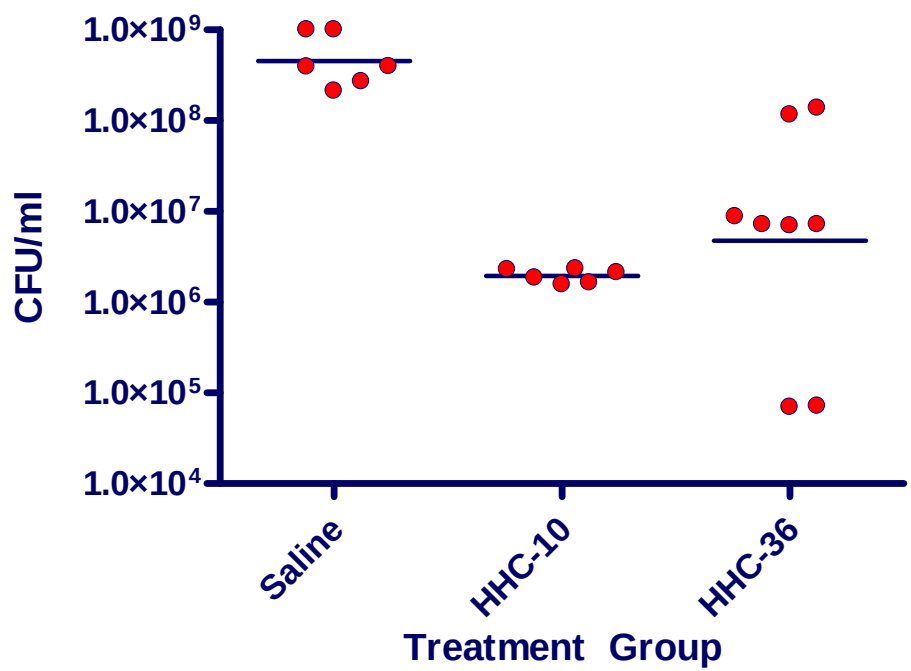
Bacterium	MIC (μM)							
	Bac2A	HHC-10	HHC-36	MX-226	Tobramycin	Imipenem	Ceftazidime	Ciprofloxacin
P. aeruginosa								
H103 (wild type)	48	**0.8**	**0.7**	19	1.1	6.7	3.6	0.4
9 (Brazil MDR)	192	**5.9**	**5.7**	76	>274	428	>231	386
198 (Brazil MDR)	95	**1.5**	**1.4**	153	>274	107	231	97
213 (Brazil MDR)	192	**3**	**5.7**	38	>274	428	>231	193
LES400 (Liverpool MDR)	95	**5.9**	**11**	76	8.6	3.3	58	1.5
H1027 (Liverpool MDR)	95	**0.8**	**1.3**	38	69	27	58	3.0
H1030 (Liverpool MDR)	95	**3**	**1.4**	76	17	27	58	0.8
P. maltophilia								
ATCC13637	[illegible]	[illegible]	1.4	[illegible]	[illegible]	[illegible]	[illegible]	0.8
E. cloacae								
218R Class C β-lactamase	[illegible]	**3**	**11**	9.6	1.1	0.4	58	0.4
E. coli								
63103 (ESBL)	24	**1.5**	**5.4**	38	137	0.4	231	>386
64771 (ESBL)	24	**1.5**	**3.7**	38	274	0.4	>231	>386
K. pneumonia								
61962 (ESBL)	192	**25**	**174**	153	69	0.4	>231	0.4
63575 (ESBL)	192	**6.2**	**22**	76	34	0.4	>231	193
S. aureus								
ATCC25923	24	**5**	**29**	9.6	0.5	0.4	29	0.4
C623 (MRSA)	24	**1.5**	**1.4**	19	274	0.4	115	6.0
E. faecalis								
ATCC29212	12	**12**	**43**	76	34	1.7	231	0.8
W61950 (VRE; VanA)	48	**99**	**>174**	>153	>274	6.7	>231	97
f43559 (VRE;VanB)	12	**3.1**	**10.8**	153	69	6.7	>231	97
E. faecium								
mic80(VRE; VanA)	3	**1.5**	**1.3**	38	274	428	>231	97
t62764 (VRE; VanB)	48	**49**	**174**	76	>274	>428	>231	386

MICs = 0.8 to 6 μg/ml vs. most Superbugs

10X better than MX-226;

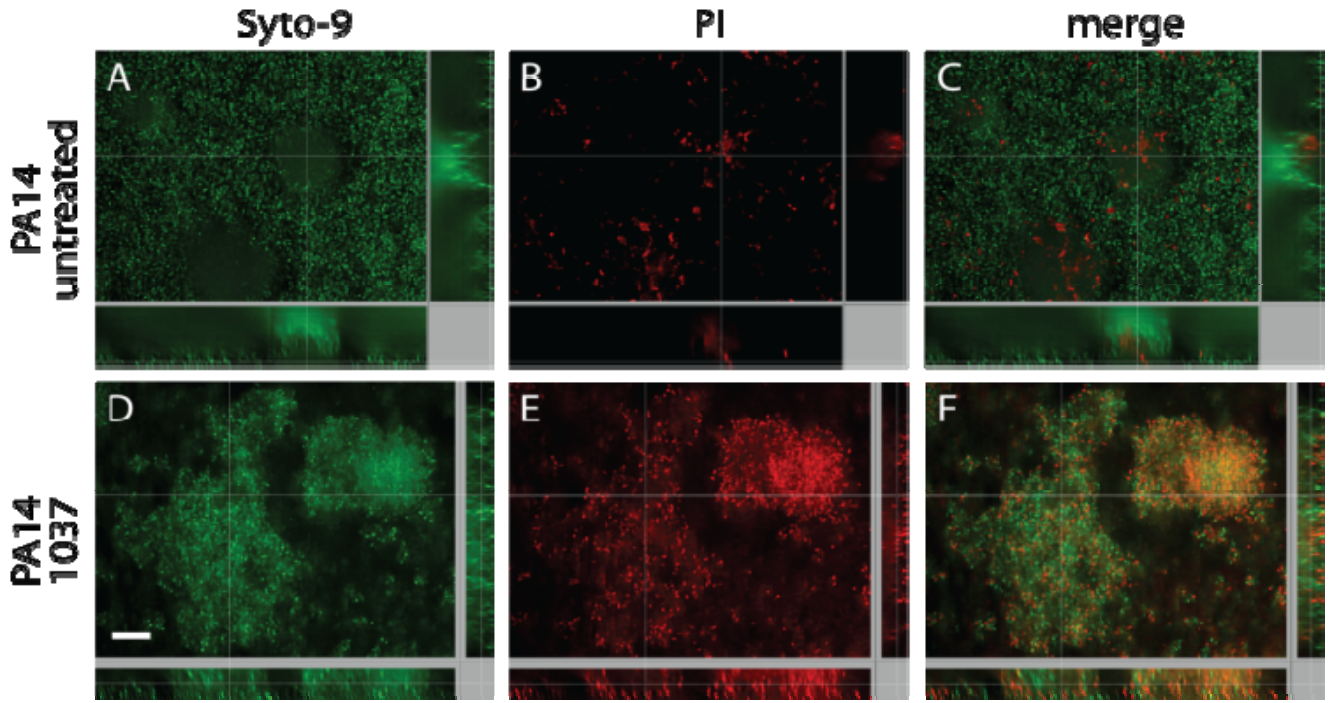
Overall better than 4 leading antibiotics

Protects in mice (IP and IV) vs. *S. aureus*



Cherkasov et al. 2009. ACS Chemical Biol. 4:65-74.

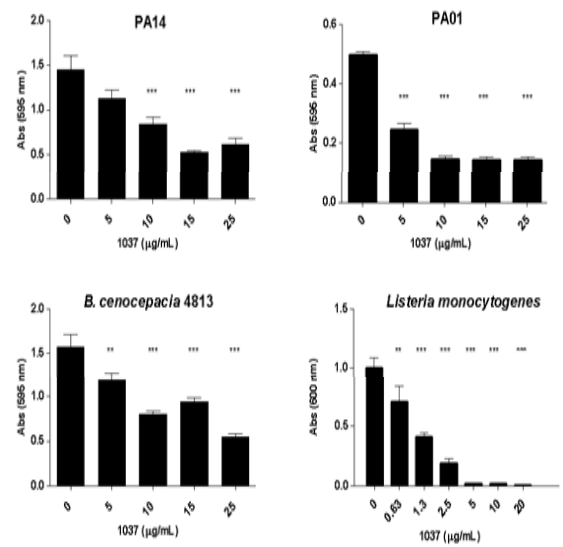
New Therapeutic Possibilities-Anti-Biofilm Action



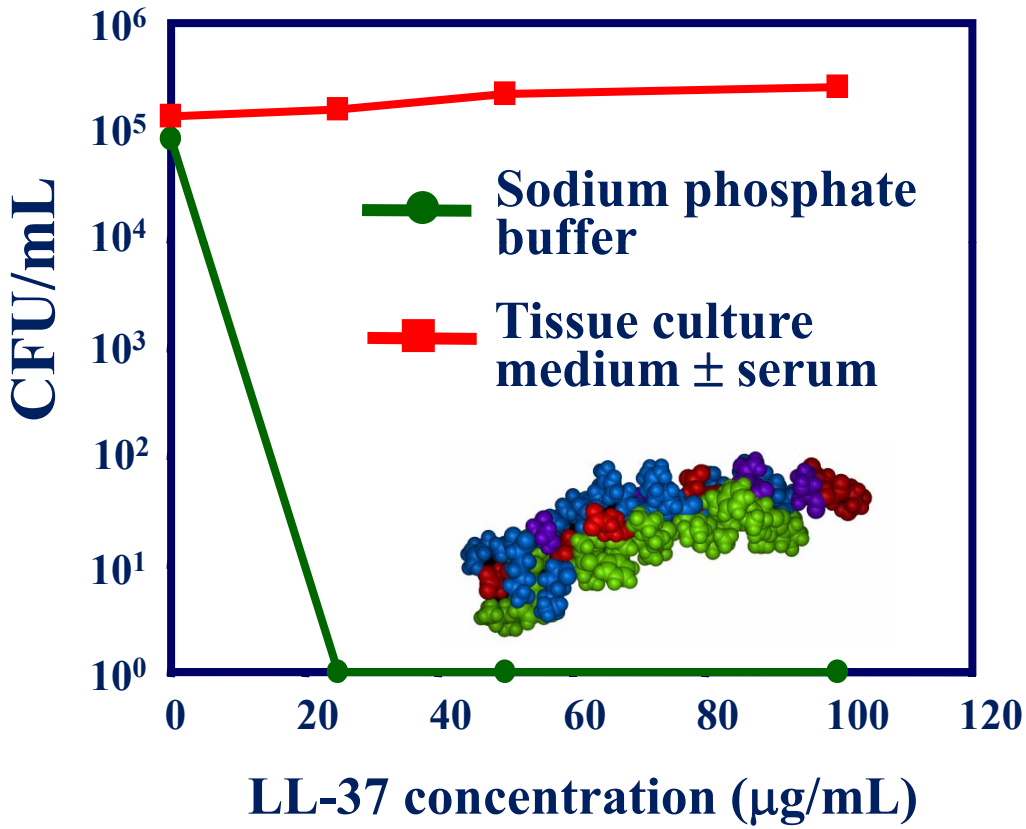
Peptides kill bacteria in biofilms

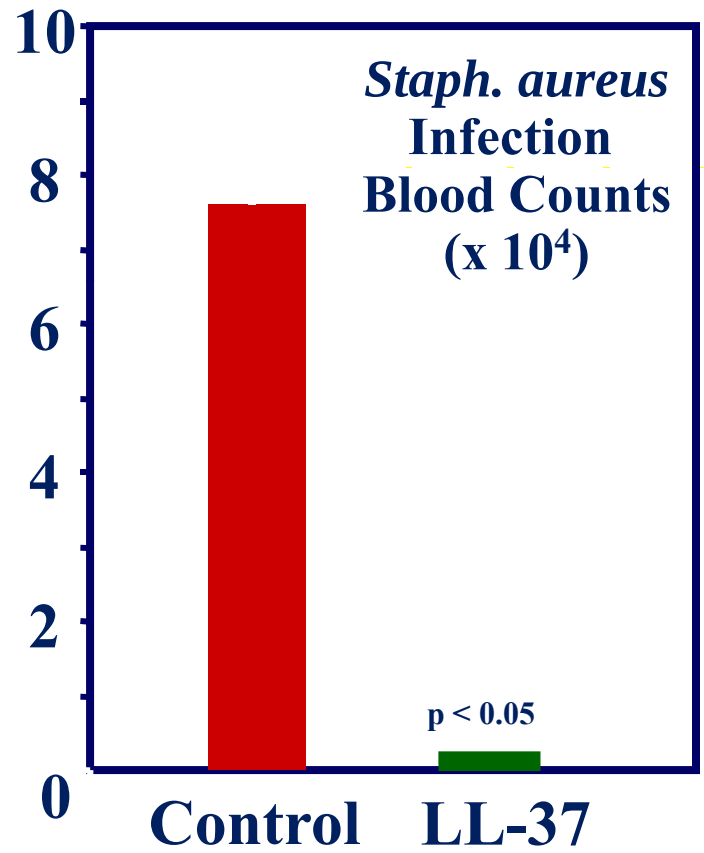
AAC 56:2696-704, 2012

Peptides work vs. MDR Gram positive and Gram negative bacteria & AMP resistant bacteria: IC_{50} < 0.25 μg/ml vs. *Pseudomonas*



Many natural antimicrobial peptides have very weak direct antimicrobial activity e.g. Human LL-37 vs. *S. aureus*



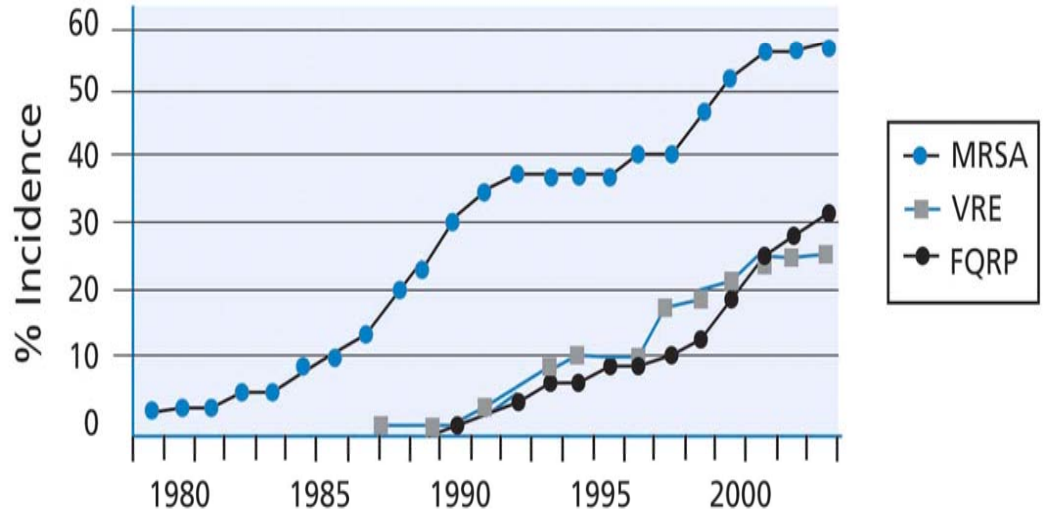


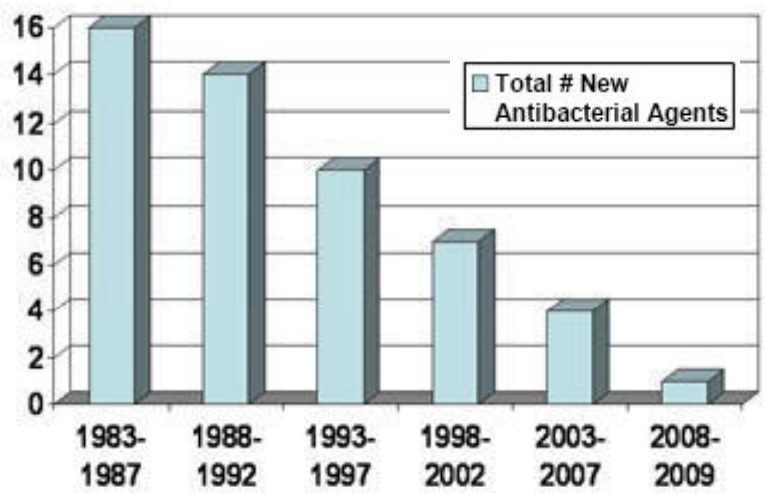
Ability to stimulate innate immunity may be more important

Dawn Bowdish, Monisha Scott

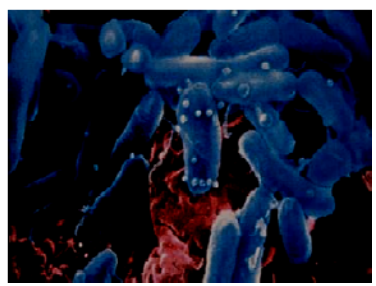
Immune Modulation Approaches

- **Successful antimicrobial therapy requires assistance from the host: immune response**
- **Immune modulation highly used in antiviral and anticancer therapy**





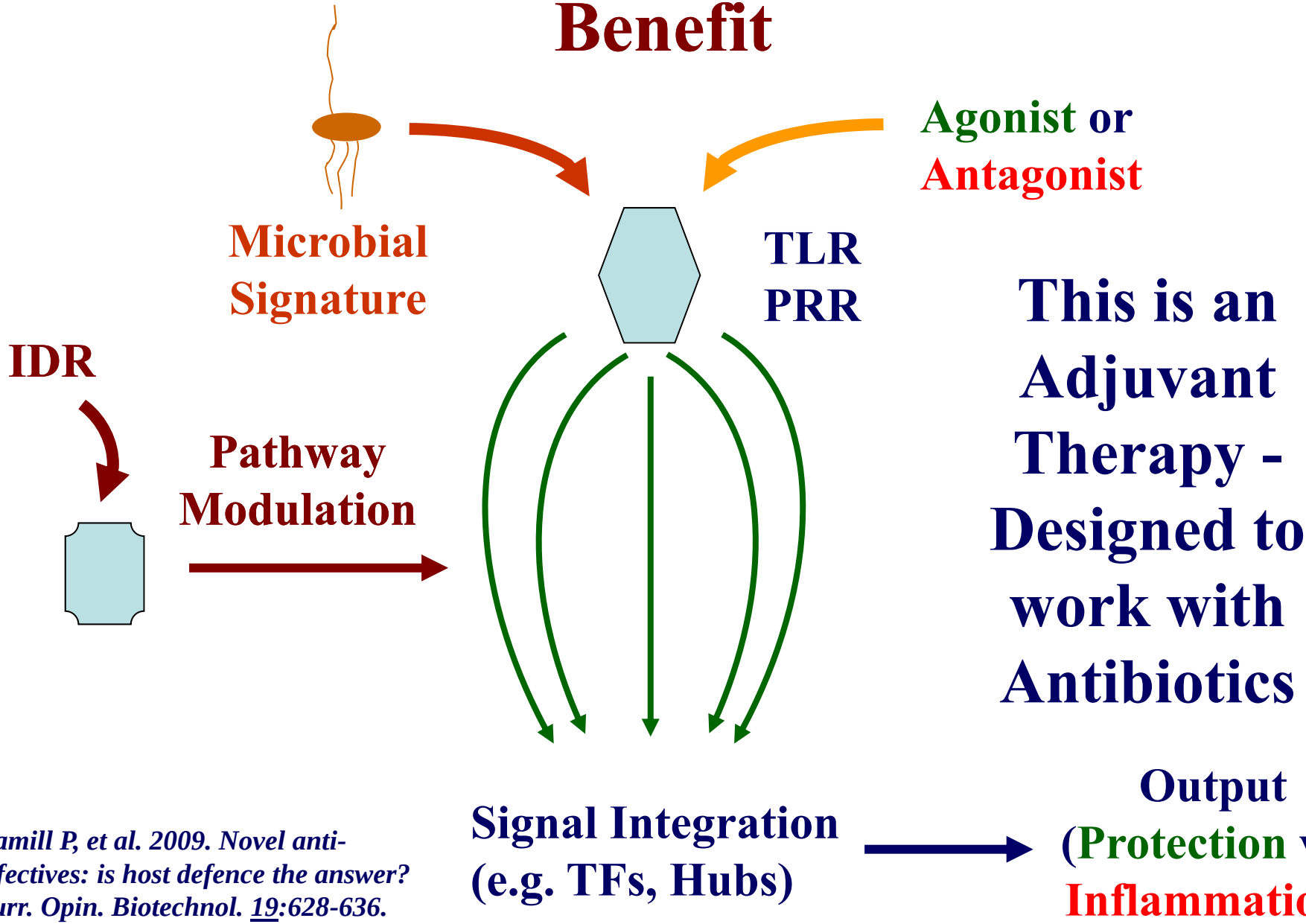
Spellberg, *CID* 2004, Modified



Hamill, P., K. Brown, H. Jenssen and R.E.W. Hancock. 2009. Novel anti-infectives: is host defence the answer? Curr. Opin. Biotechnol. 19:628-636.

IDSA "Bad Bugs No Drugs"

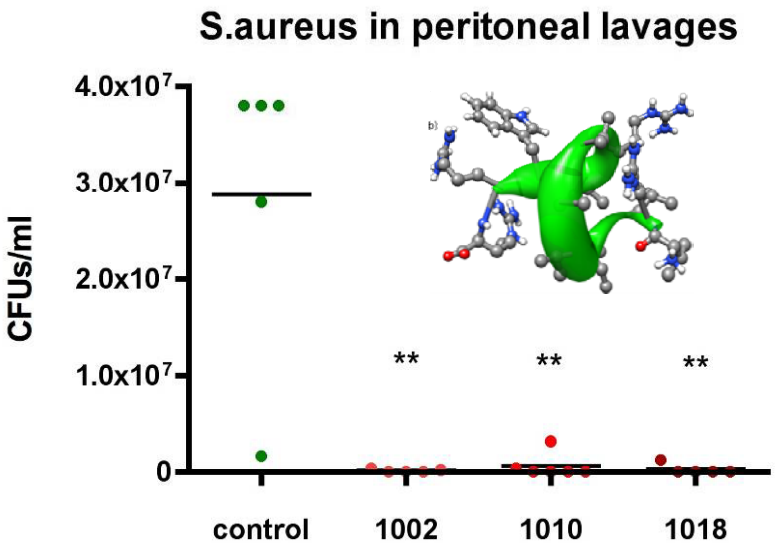
Modulating Immunity for Therapeutic Benefit

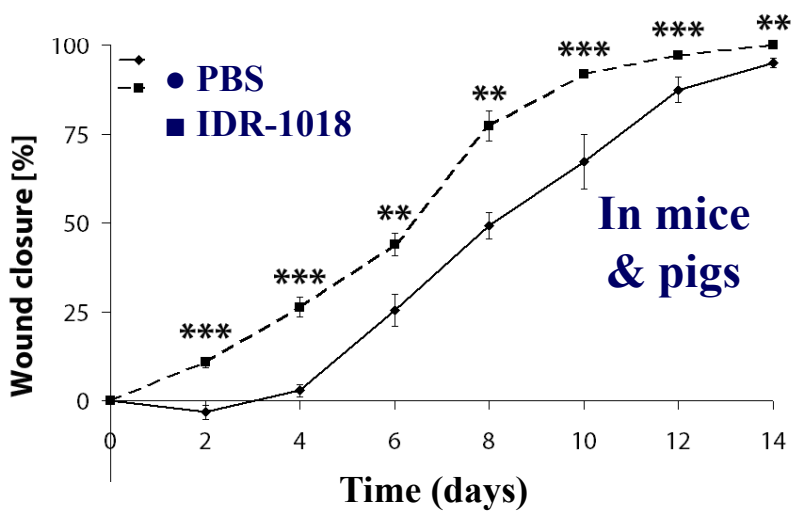


This is an Adjuvant Therapy - Designed to work with Antibiotics

Hamill P, et al. 2009. Novel anti-infectives: is host defence the answer? Curr. Opin. Biotechnol. 19:628-636.

New immunomodulatory peptides show broad protection in Mouse Model Infections





In pig model no effect on infectious load

Also protects vs. TB, *E. coli*, *Salmonella*, MRSA, VRE, *P. aeruginosa*, IBD, CF, Cerebral malaria, Sterile inflammation, etc.

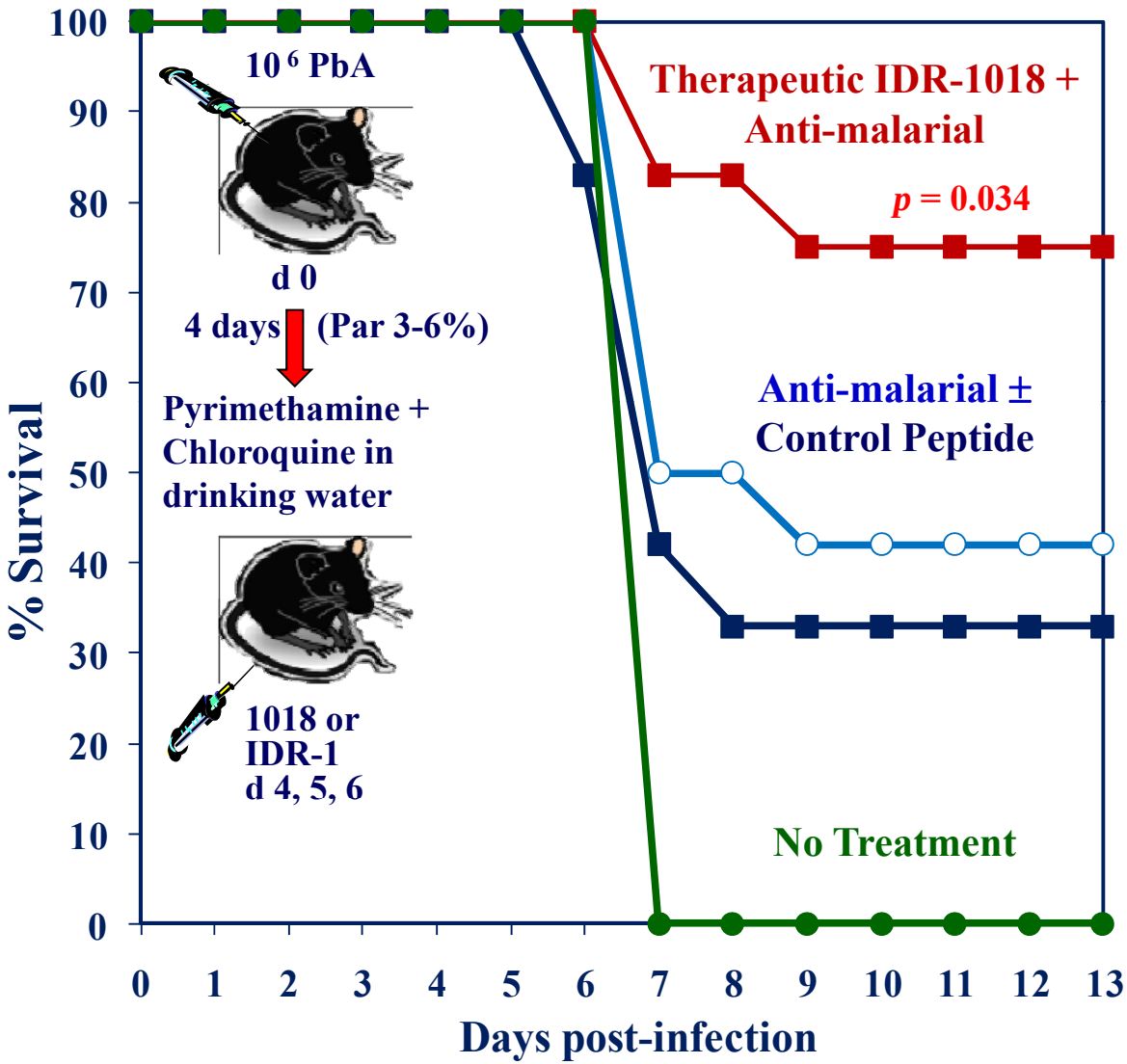
Safety demonstrated in Phase I trials

These peptides developed independently for Grand Challenges Program.

Grand Challenges in Global Health

Lars Steinstraesser, Louis Schofield, Sandra Pillat, Lisa Thorsen, Sarah Mullaly, Bruce Vallance, Brett Finlay

Therapeutic IDR-1018 protects in conjunction with Anti-Malarial Therapy



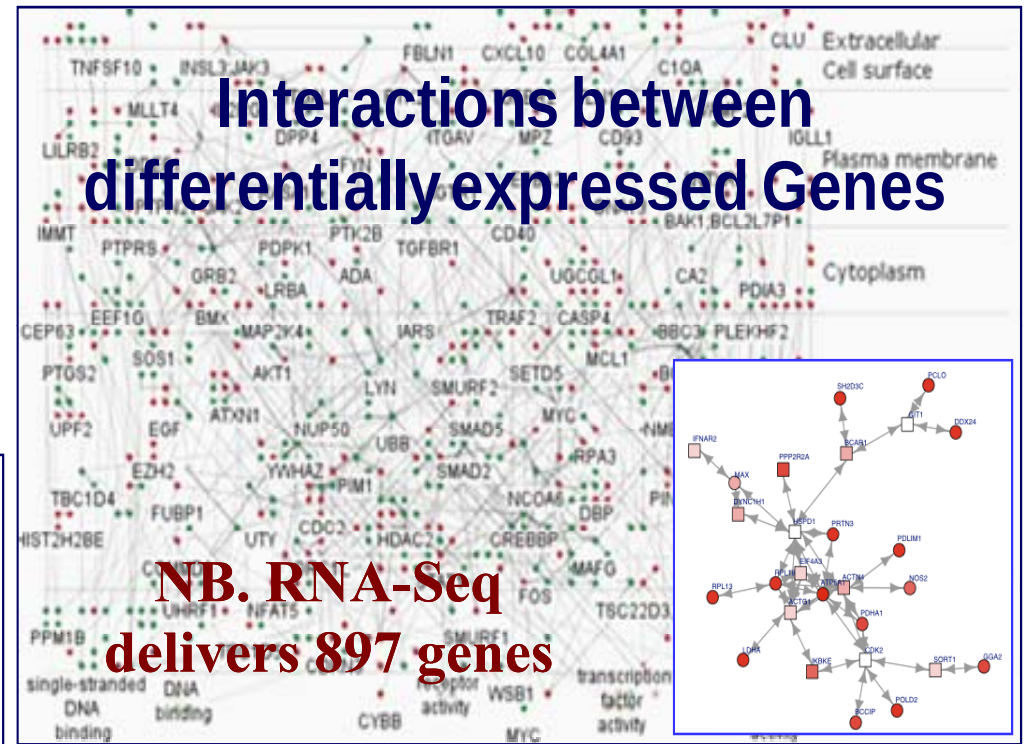
- Only via IV
- No effect on Parasite load
- Anti-inflammatory

Louis Schofield, Ariel Achtman, Sandra Pilat, WEHI. Science Transl. Med,. 2012. 4:135ra64

Systems Biology
Network analysis – IDR peptides

InnateDB
Network
Construction

IDR1 treatment of
Human Monocytes



688 nodes
2895 Edges

Hubba-Hubba
Hub objects analyser

Highly connected
Nodes (Hubs)
& Bottlenecks

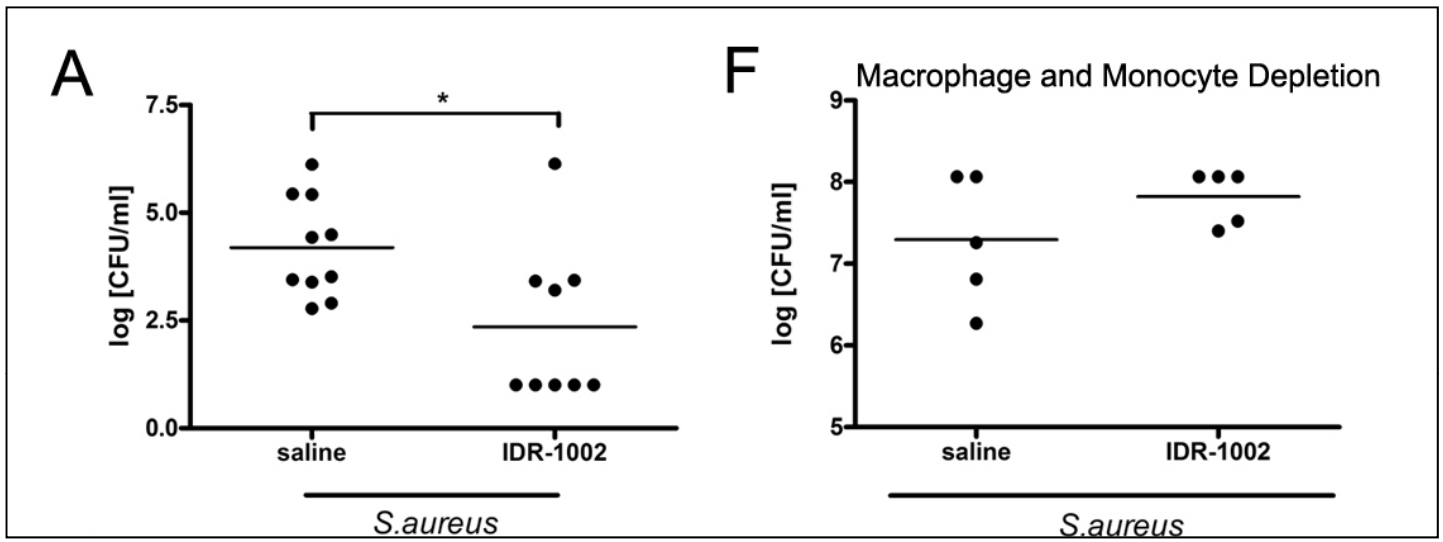
Top Hubs

TRAF2; CRK; HSPA1B; RELA
(MAPK & Chemokine Induction)

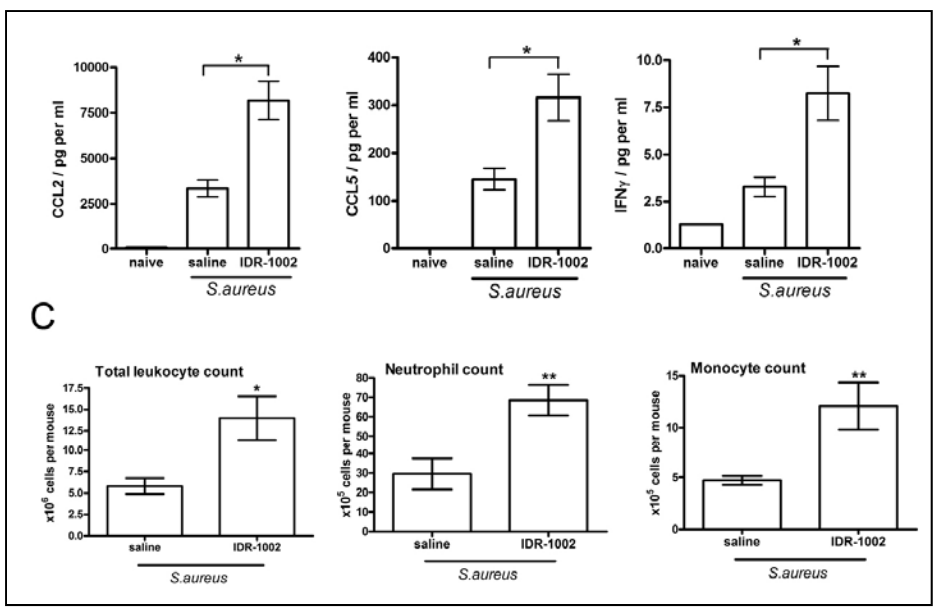
TNFAIP3; SMAD3; CUL-1
(Anti-Inflammatory & TGFβ)

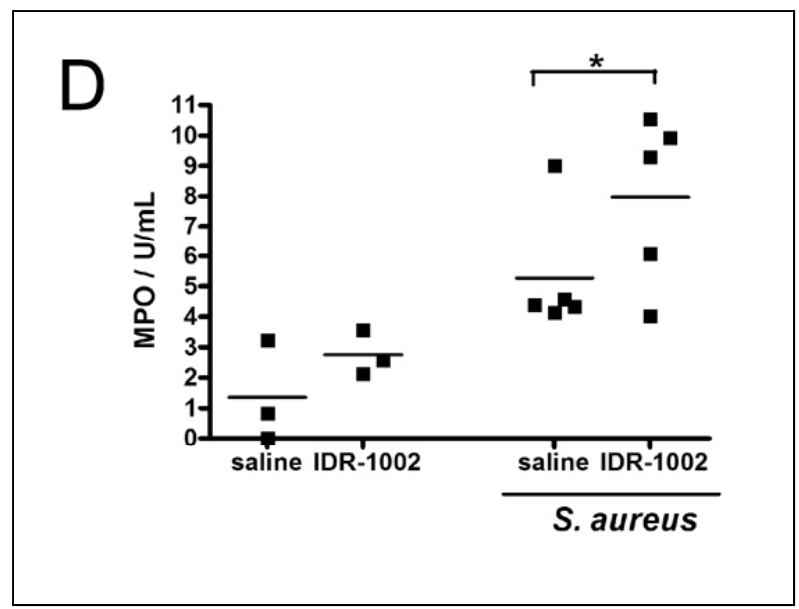
CREBBP1; IRF7; AKT2; GRB2
(JAK-STAT & IFN)

Monocytes/Macrophages are essential and recruitment is enhanced in infection model

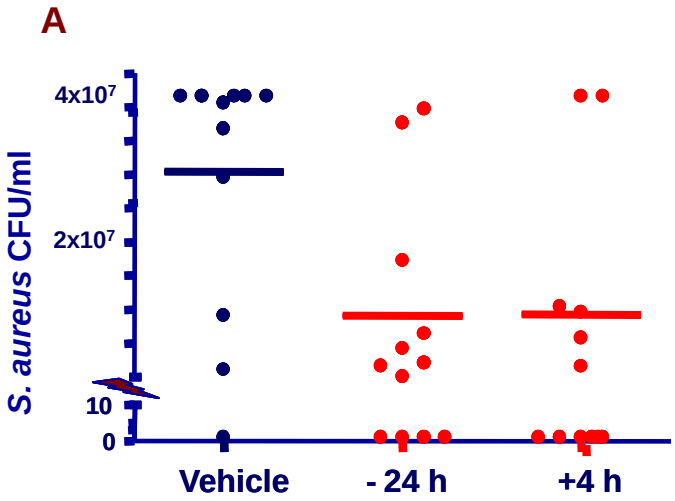


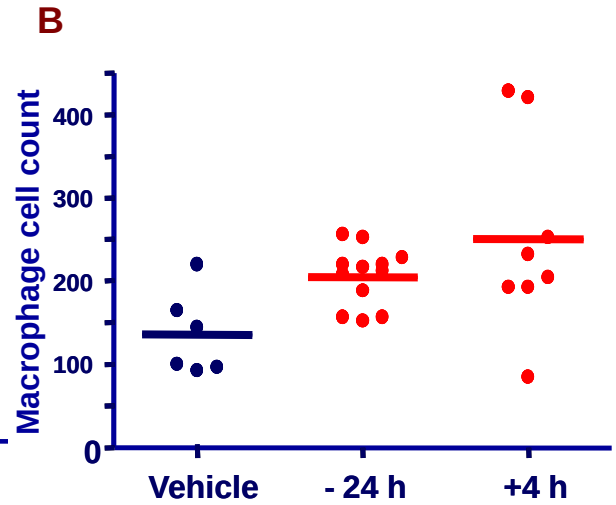
Nijnik et al. 2010. J. Immunol. 184:2539-50.

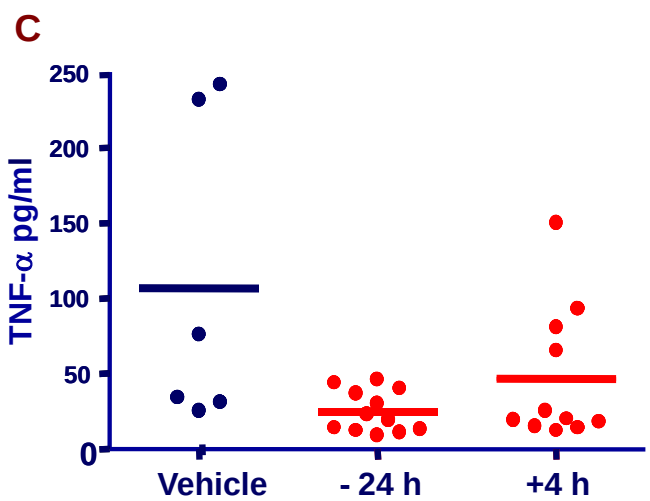


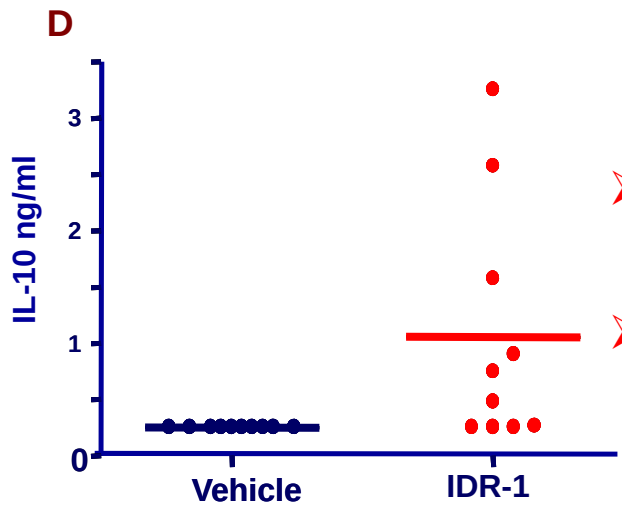


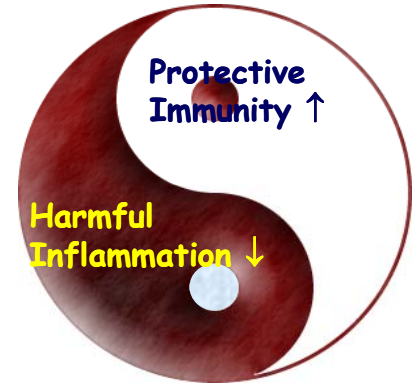
Mechanism in animal models









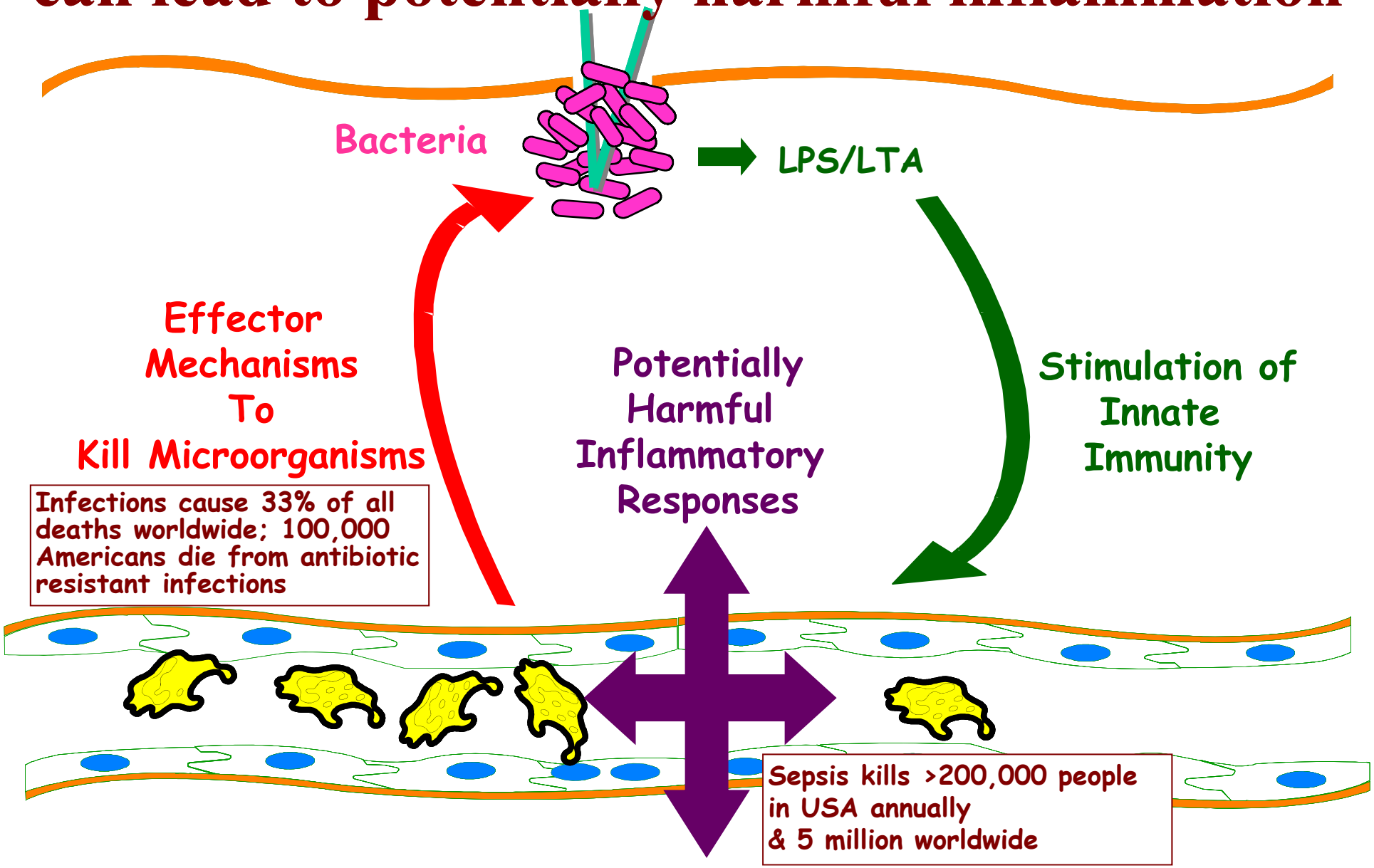


- Peptide protects with pre and post treatment (A)
- Pro-inflammatory cytokines TNFα (C) and IL6 reduced. Anti-inflammatory cytokine IL-10 increased (D)
- Macrophages increase at the site of infection (B)
- Macrophages (liposomal chlodronate) but not neutrophils or lymphocytes are essential for protection

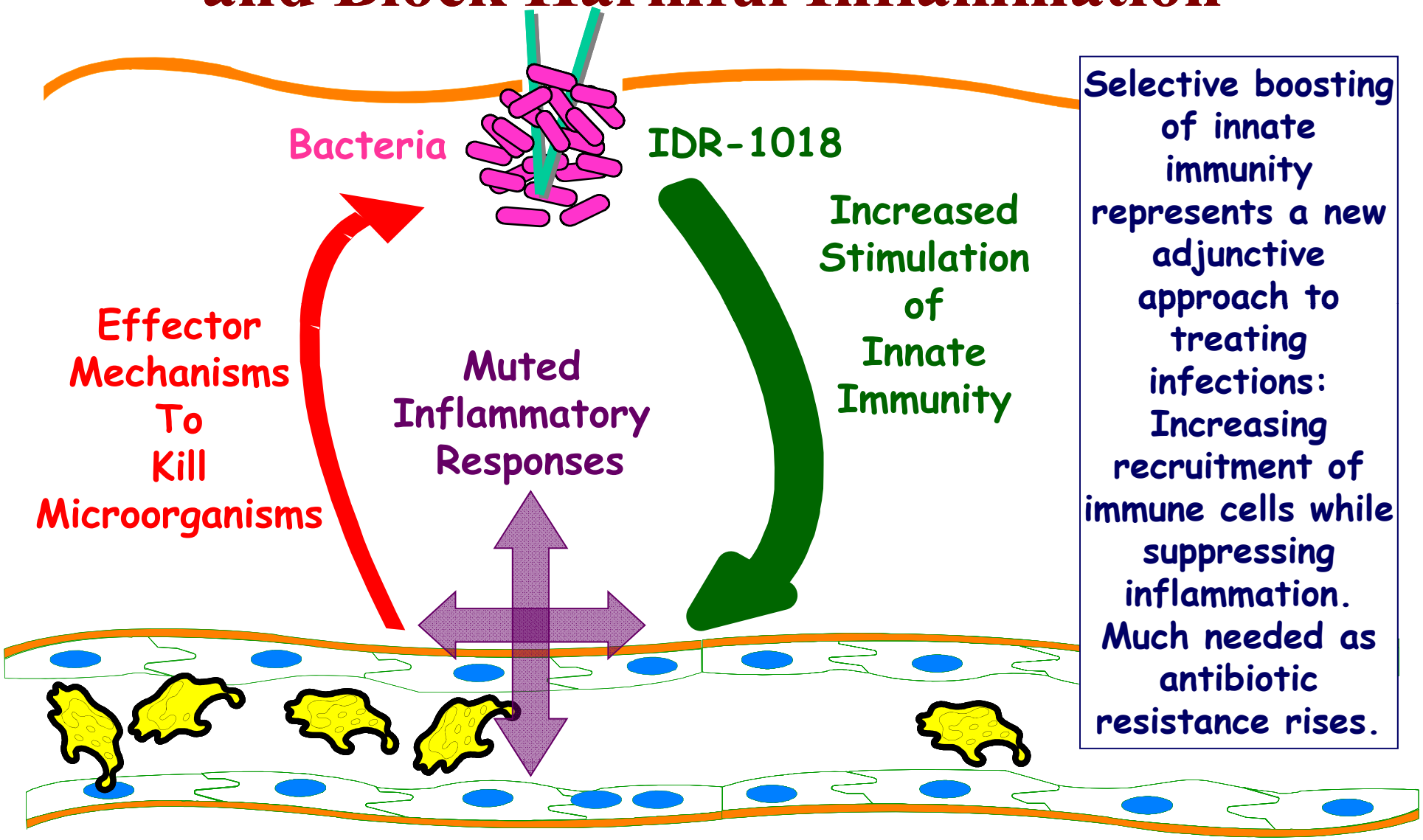
Balancing of Inflammation in vivo

Scott et al. Nature Biotech 25:465-472, 2007

Summary: Normal immunity protects but can lead to potentially harmful inflammation



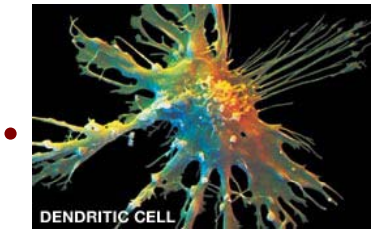
Summary: Peptides Boost Innate Immunity and Block Harmful Inflammation



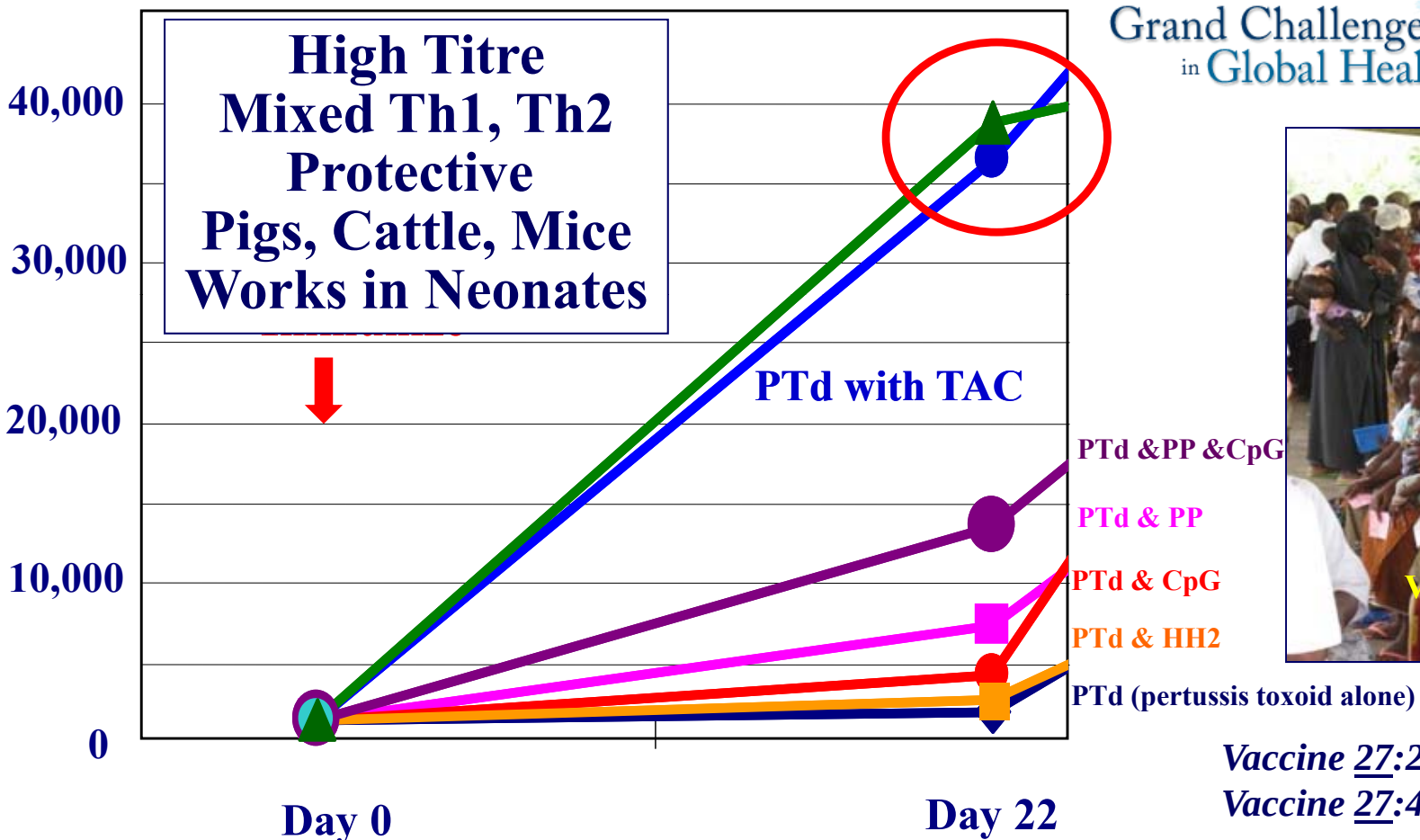
Selective boosting of innate immunity represents a new adjunctive approach to treating infections: Increasing recruitment of immune cells while suppressing inflammation. Much needed as antibiotic resistance rises.

Triple adjuvant formulation (TAF) gives the potential for single dose protection vs. pertussis.

Overcoming the Roadblock of Multiple Dosing

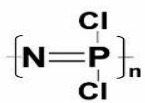


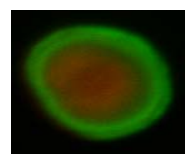
Grand Challenges in Global Health



Vaccine 27:2055-2064, 2009
Vaccine 27:4662-4671, 2009

CpG ODN





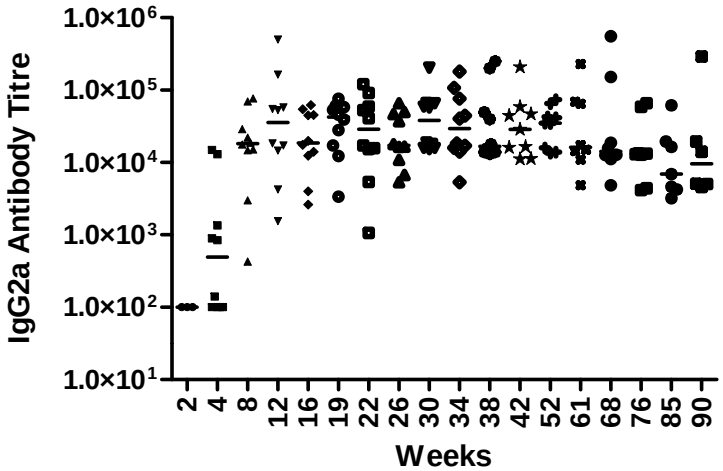
Polyphosphazene; PP

Host Defence Peptide (HH2)

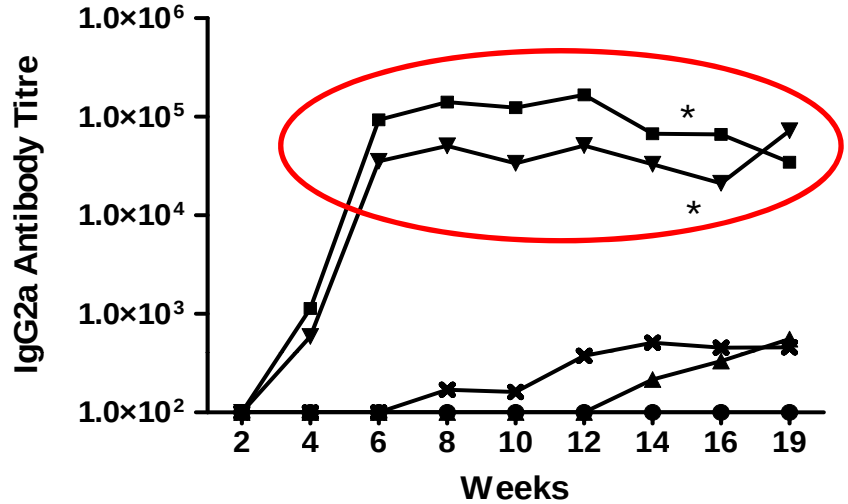
Volker Gerdts, Lorne Babiuk, Bob Hancock and many others

Features of the Adjuvant Formulation

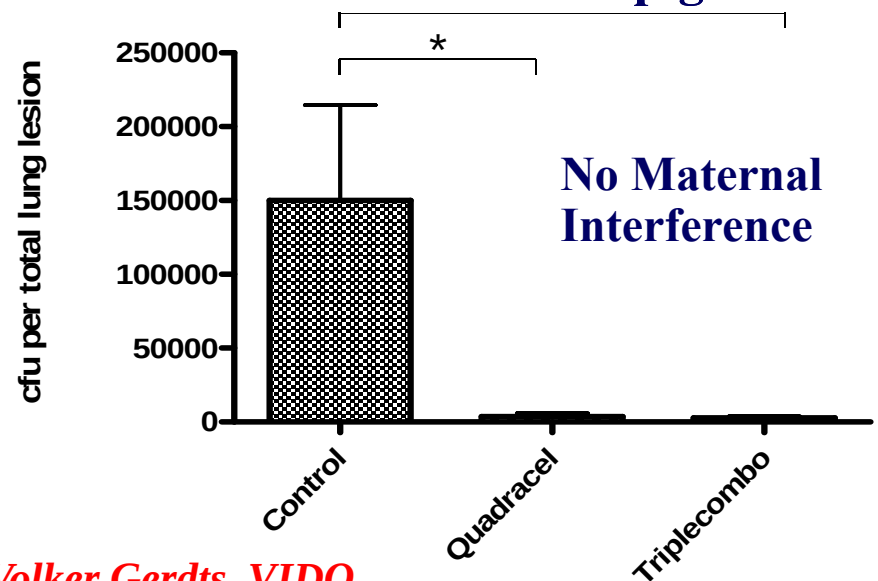
Duration of immunity > 2 years (mice) >10 months in pigs



Comparison to Alum (2 different doses)



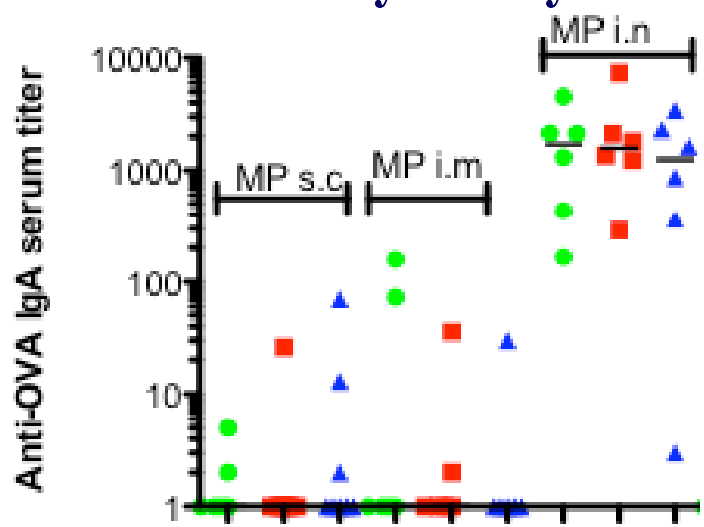
Protective – Neonatal pigs



No Maternal Interference

Volker Gerdts, VIDO

Works mucosally at very low doses



OVA + 0.6 µg CpG, 1.2 µg HDP, 0.6 µg Polyphosphazene

Acknowledgements

Lab: Laure Janot, Matt Mayer, Chris Fjell, Ana Nijnik, Neeloffer Mookherjee, Jason Kindrachuck, Reza Falsafi,

Collaborators: Volker Gerdts; Bruno Rivas, Rogelio Hernandez; Edie Dullaghan and CDRD; Louis Schofield; Brett Finlay

Grand Challenges in Global Health

Pathogenomics of Innate Immunity

A GenomeBritishColumbia Project