Manipulating the host response to treat infections

Guest speakers: R.E.W. (Bob) Hancock, Henk P. Haagsman

Moderator: Neeloffer Mookherjee

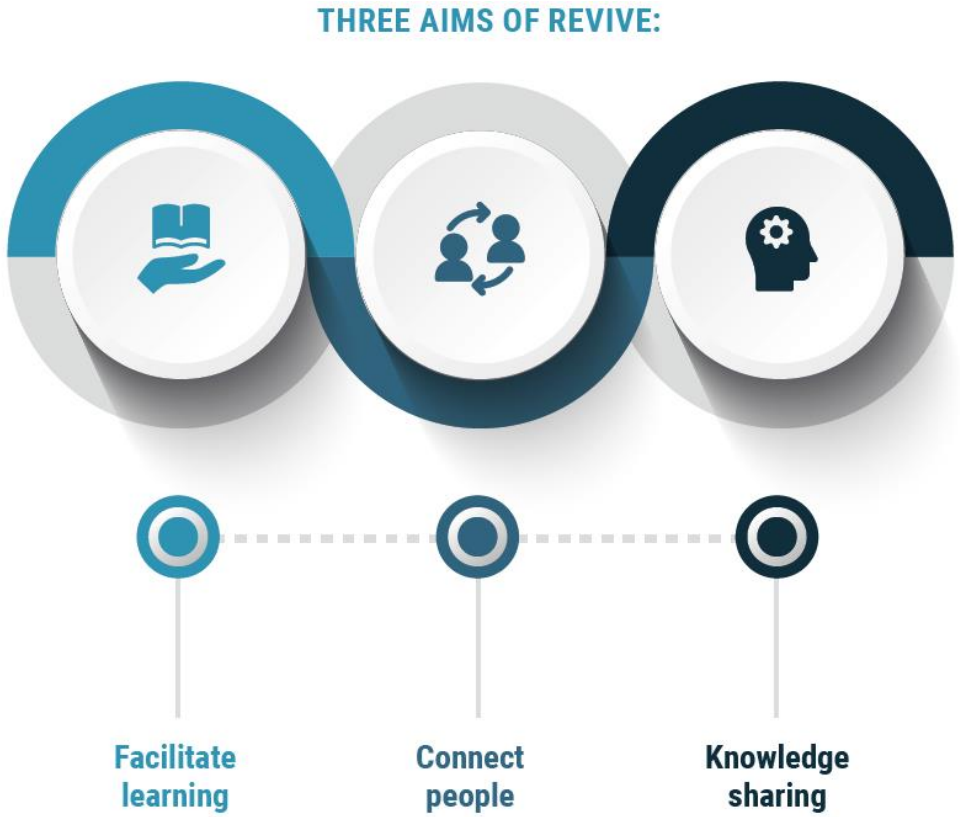
Host: Astrid Pentz-Murr (GARDP)

14 January 2021

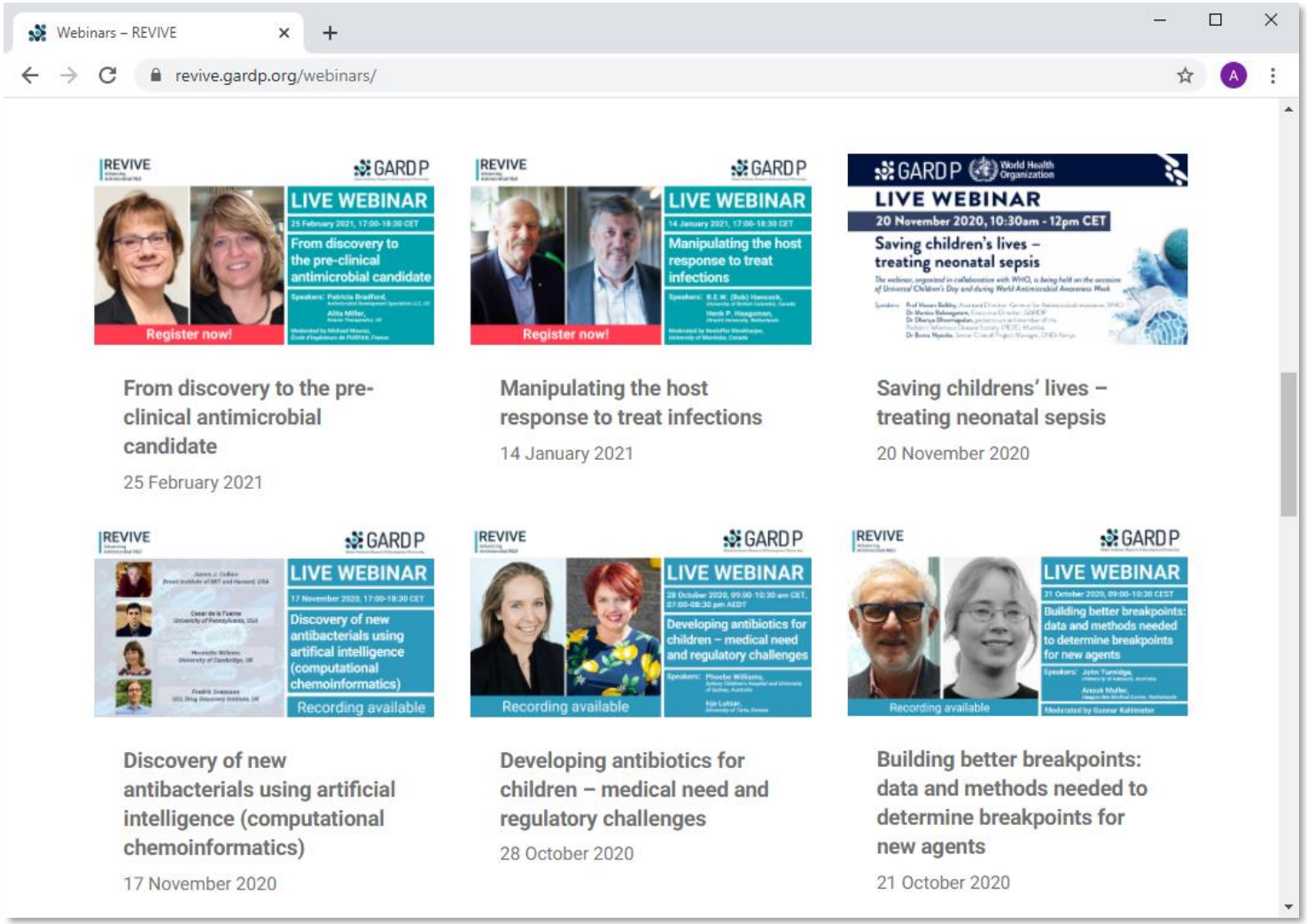
GARDP
Global Antibiotic Research & Development Partnership

REVIVE

An online space to connect and support the antimicrobial discovery, research, and development community

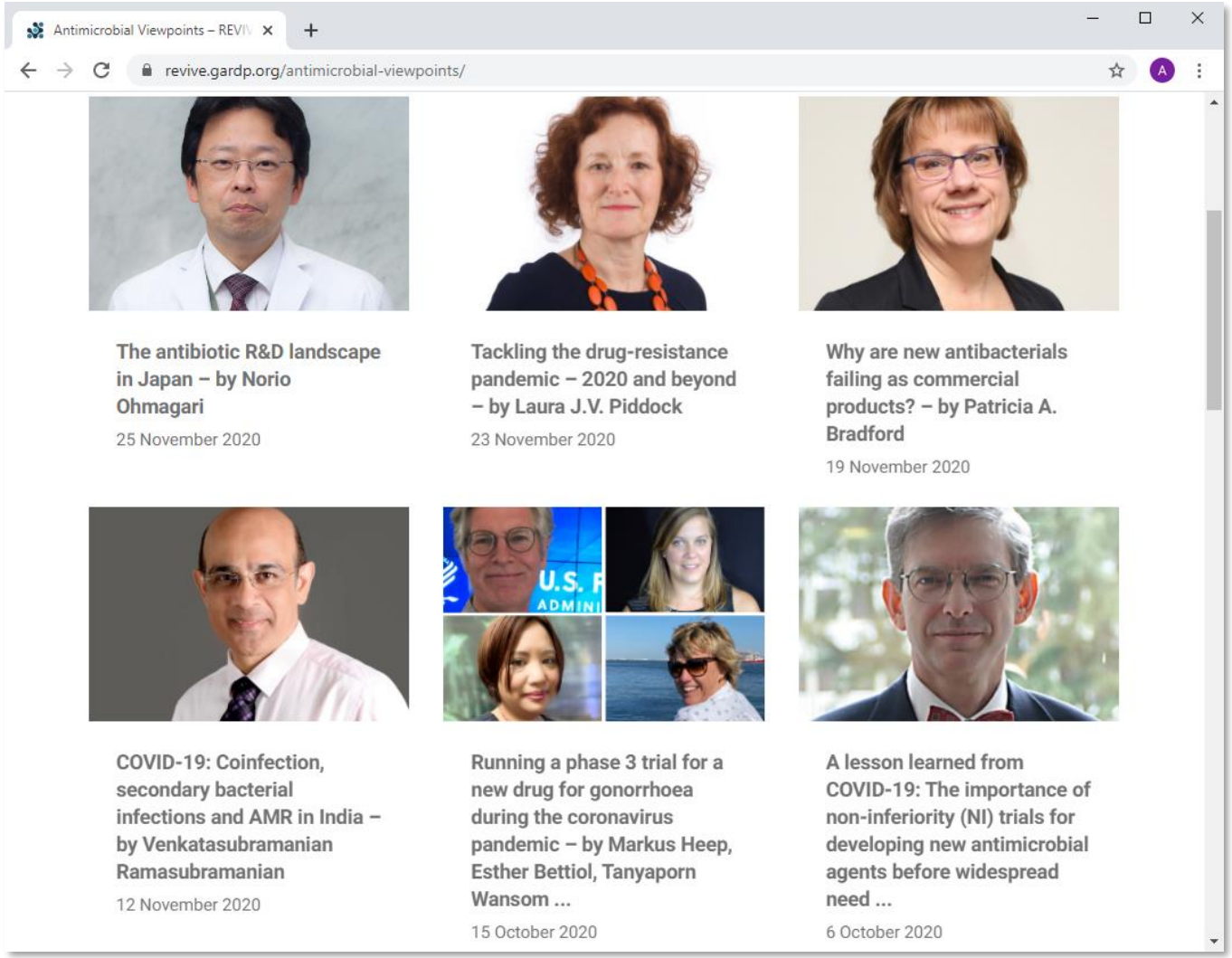


Webinar recordings



revive.gardp.org/webinars

Antimicrobial Viewpoints



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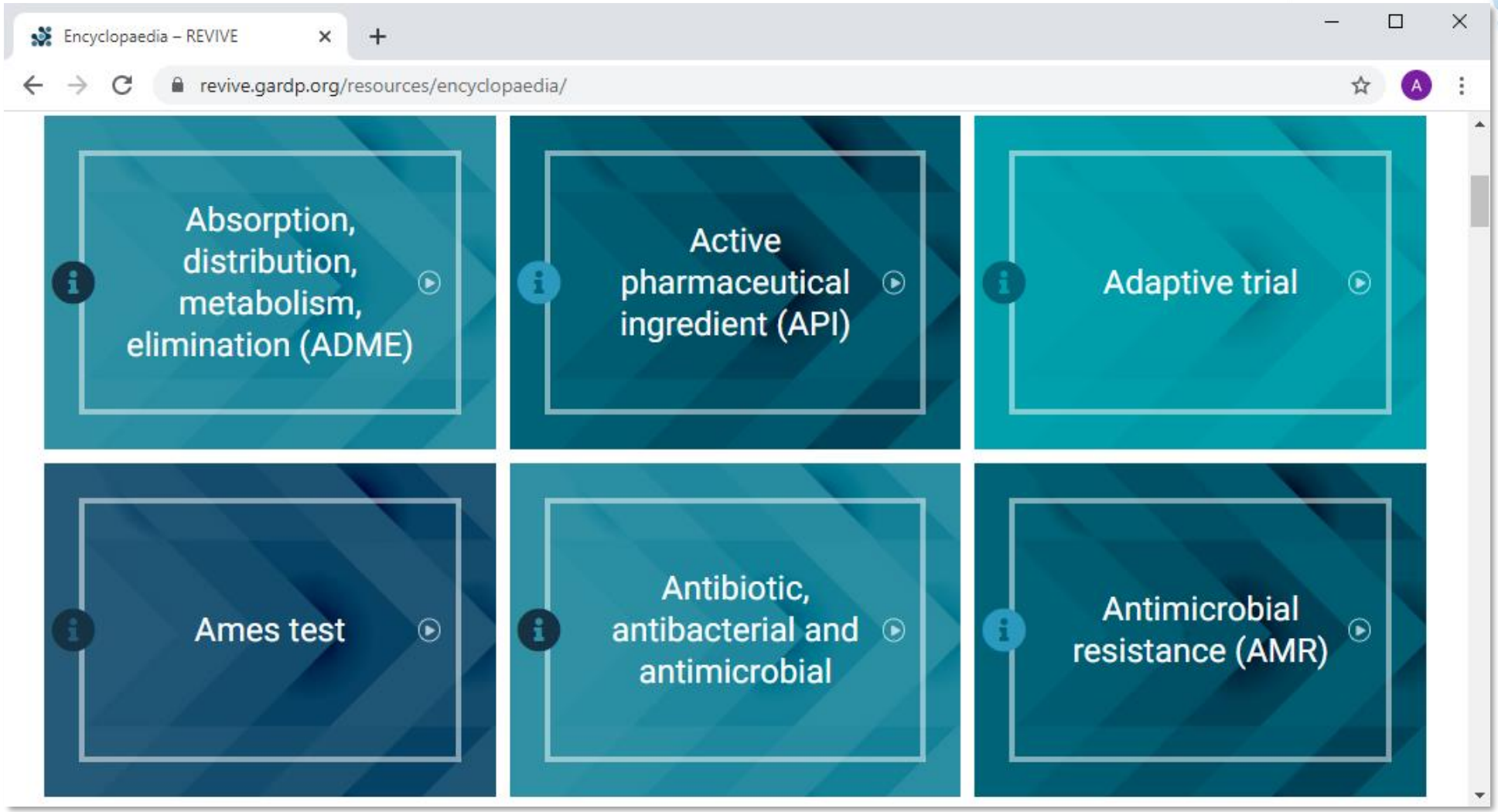
Antimicrobial Encyclopaedia



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Today’s speakers

Manipulating the host response to treat infections

R.E.W. (Bob) Hancock
UBC Killam Professor and Canada Research Chair
University of British Columbia (Canada)

Henk P. Haagsman
Professor of Molecular Host Defence
Utrecht University (Netherlands)

Moderator:
Neeloffer Mookherjee
Associate Professor
Departments of Internal Medicine and Immunology, University of Manitoba (Canada)

Introduction

Manipulating the Host Response to Treat Infections

Moderator:

Dr. Neeloffer Mookherjee

Associate Professor, Departments of Internal Medicine and Immunology
Univerisity of Manitoba, Canada

Chair, Canadian Institutes of Health Research Sex and Gender Science in Respiratory Health

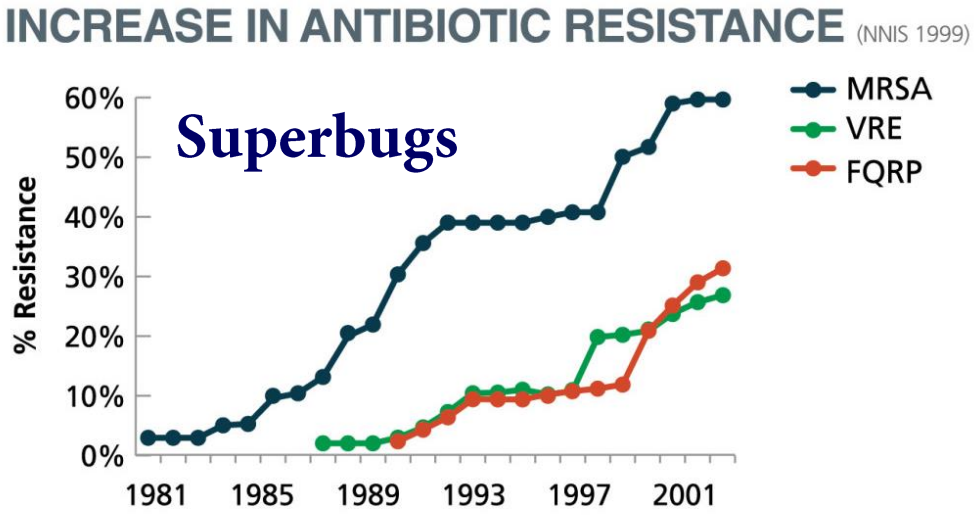
Chair, WISDOM (Women in Science: Development, Outreach and Mentoring) Manitoba, Canada

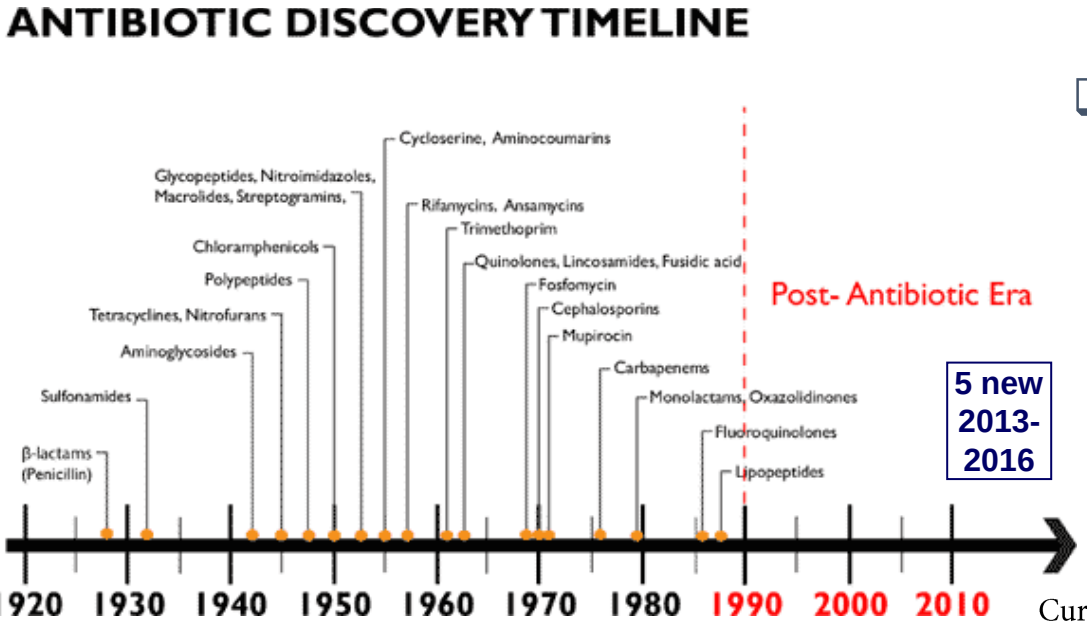
Rady Faculty of Health Sciences | University of Manitoba

https://www.mookherjeelab.com

@NMfromUManitoba

The Antibiotic Development Paradigm is Fractured



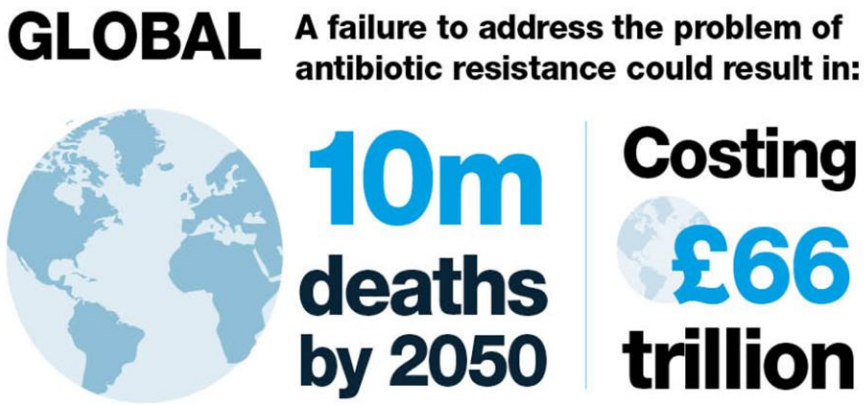


- Antibiotics are the single most successful medical intervention
- Dramatic increase in antibiotic resistance
- Dry pipeline; Traditional and genomics-based approaches to antibiotic development has not worked well.

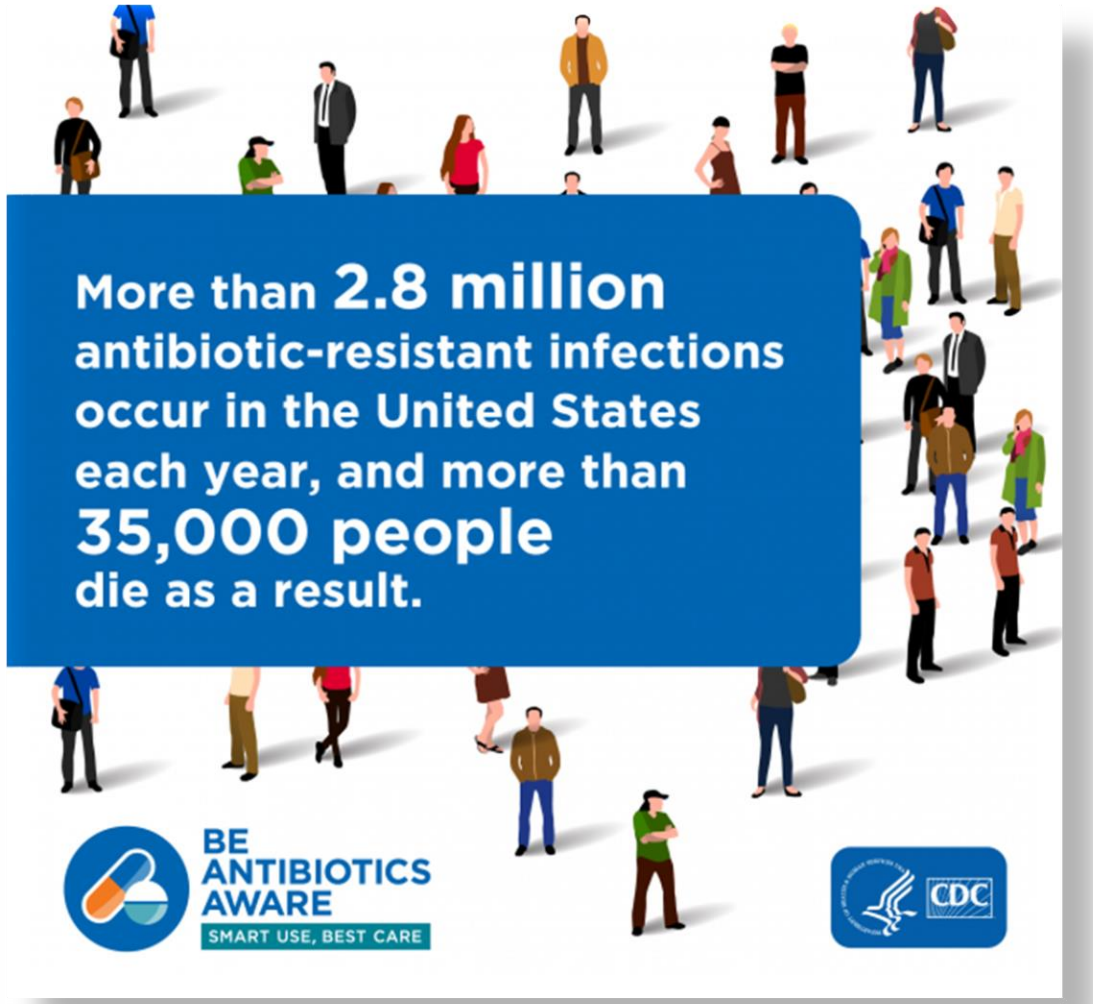
Current Topics in Medicinal Chemistry (2017), 17 , Issue 2

Defeating Antibiotic Resistance:

There is an urgent need for Alternate Strategies



Public Health England (Gov.UK)



https://www.cdc.gov

When do antibiotics fails?

1. Microbial Resistance (mutations/plasmids etc.)
2. Chronic Infections especially biofilms: 65%
   of all infections, highly resistant & associated with harmful inflammation
3. Sepsis: a dysregulated host response to infection (~20-35% death rate; 1 million deaths annually)
4. Individuals with dysregulated immune responses and immunosuppressed:
   chemotherapy, immuno-suppressive disease, genetic diseases, injuries or burns.

Rarely taken into account when developing new anti-infectives

&

are impacted by aberrant host defense response

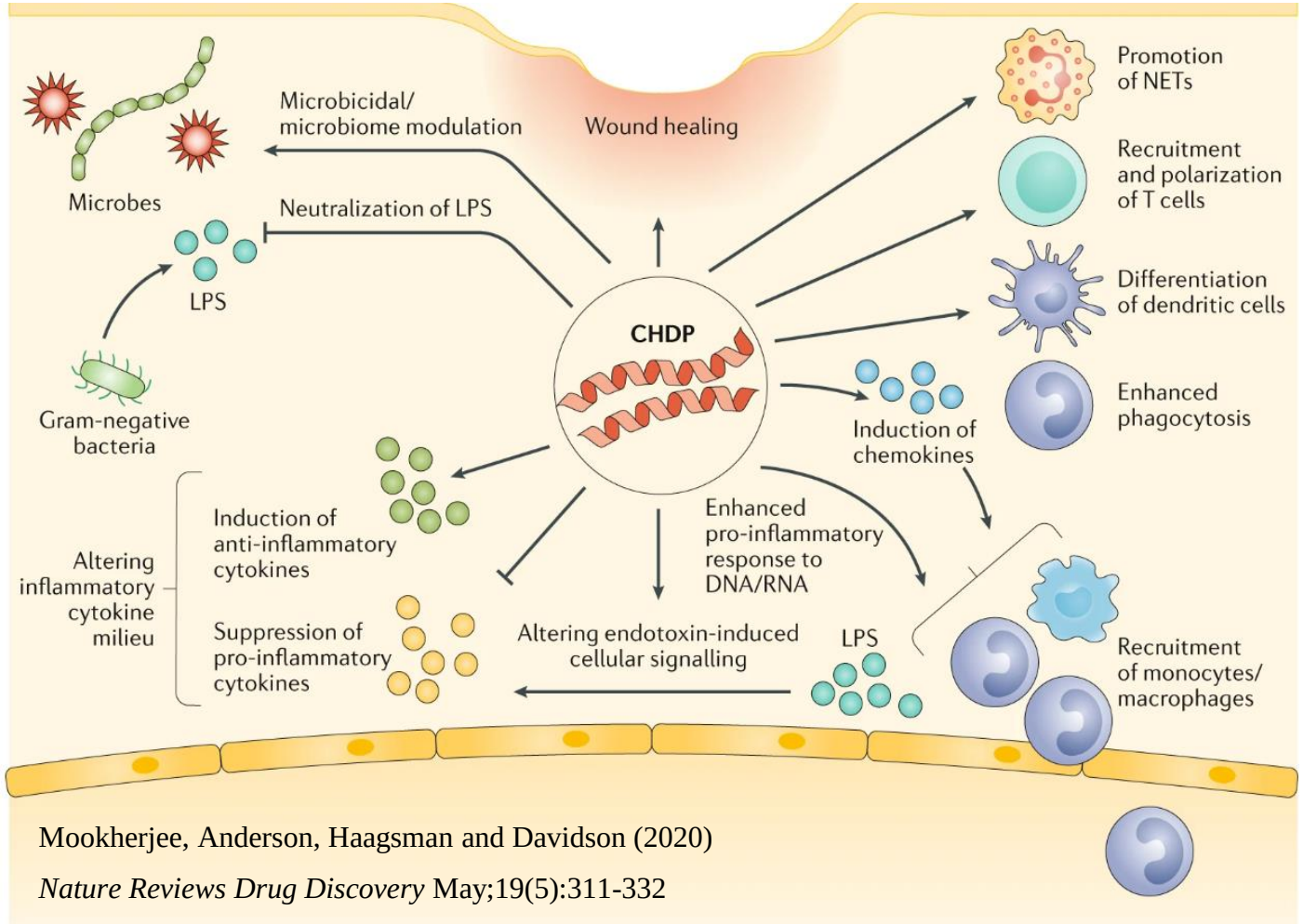
Hancock, R.E.W. 2020. The critical need for alternative approaches to address antibiotic treatment failure.

https://revive.gardp.org/the-critical-need-for-alternative-approaches-to-address-antibiotic-treatment-failure/

Harnessing Host Defense: Immune Modulation

- Successful therapy with help from the host's immune response
- Immune Modulation & Host Directed Therapies
  - used in antiviral and anticancer therapies

Cationic Host Defence Peptides



Mookherjee, Anderson, Haagsman and Davidson (2020)

Nature Reviews Drug Discovery May;19(5):311-332

Utrecht University

Veterinary Medicine

25-01-2021

REGULATION AND REPROGRAMMING OF THE INNATE IMMUNE SYSTEM AS ALTERNATIVE TO ANTIBIOTICS

Henk P. Haagsman, Maaike R. Scheenstra, Edwin J. A. Veldhuizen & Albert van Dijk

Division of Infectious Diseases & Immunology

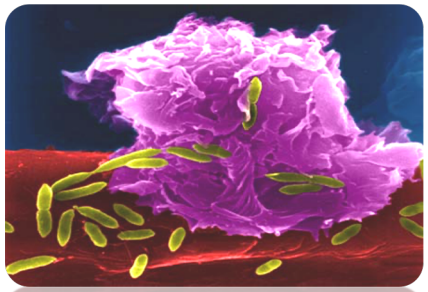
Organisation of the presentation

- Introduction: Host Defence Peptides (HDPs)
- Cathelicidin-derived peptides: paradigms for anti-inflammatory antimicrobials
- Cathelicidin-derived peptides: immunomodulatory activities
- Antimicrobial strategies involving HDPs
- Host-directed therapy by innate immune training via epigenetic reprogramming?
- Conclusions

Innate Host Defence

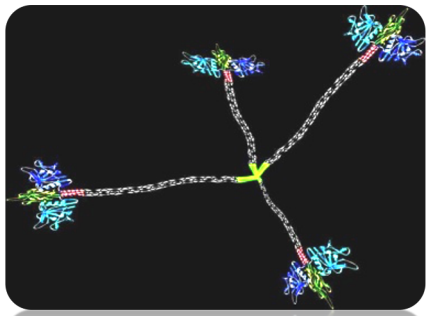
- Present in all organisms
- Limited repertoire of molecules
- Rapid
- Broad specificity
- Ancient

The first line of defence against infections



Cellular defences

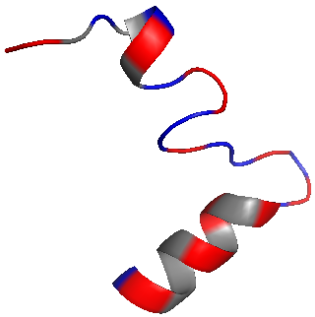
Neutrophils (heterophils)
Macrophages, Innate Lymphoid Cells, Epithelial cells



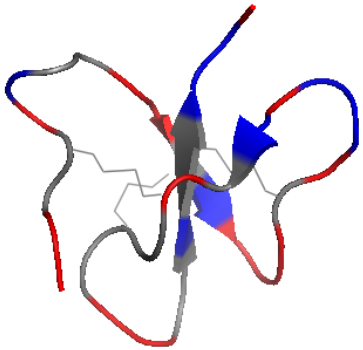
Effector molecules

Enzymes, Host Defence Peptides, Collectins, etc.

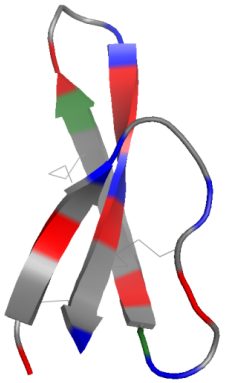
Examples of vertebrate Host Defence Peptides



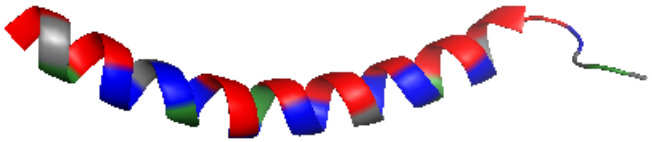
CATH-2



HBD-2



HD-5



LL-37

Magainin-2

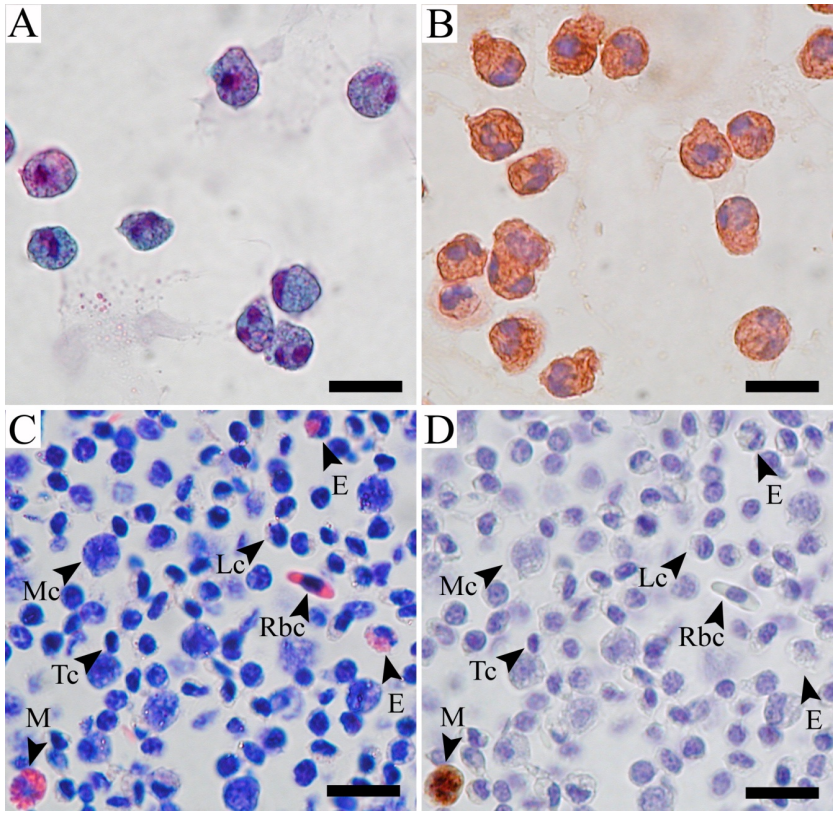
Amino acid side chains: red, hydrophobic; blue, basic; green, acidic

Mookherjee et al. Nat. Rev. Drug Discov., 2020

CATH-2 is produced by chicken heterophils

Heterophils

Mononuclear cells

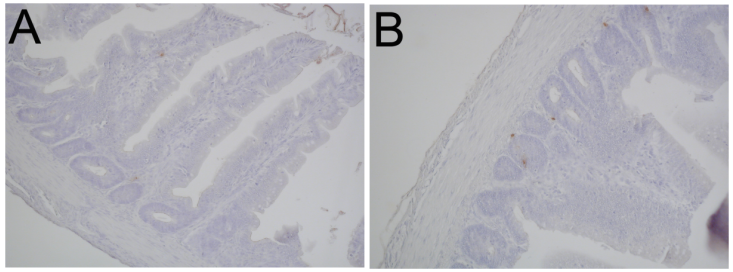


Giemsa Anti-CATH-2

Van Dijk et al. Mol. Immunol., 2009

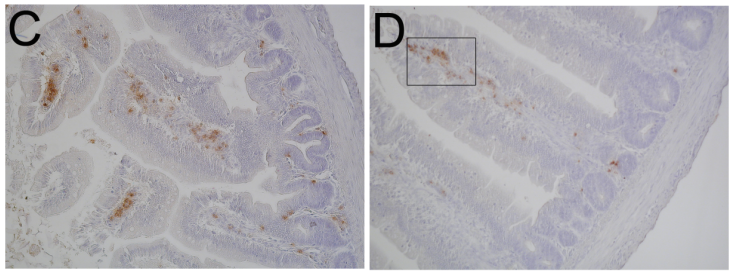
Salmonella enteritidis challenge of chickens results in recruitment of CATH-2 containing heterophils

Control
8h p.i.



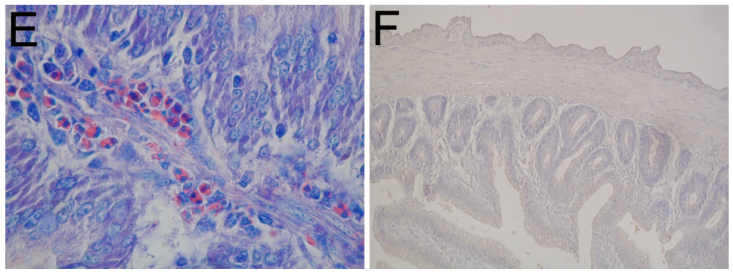
Control
48h p.i.

S. enteritidis
8h p.i.



S. enteritidis
48h p.i.

S. enteritidis
48h p.i.
(Giemsa)



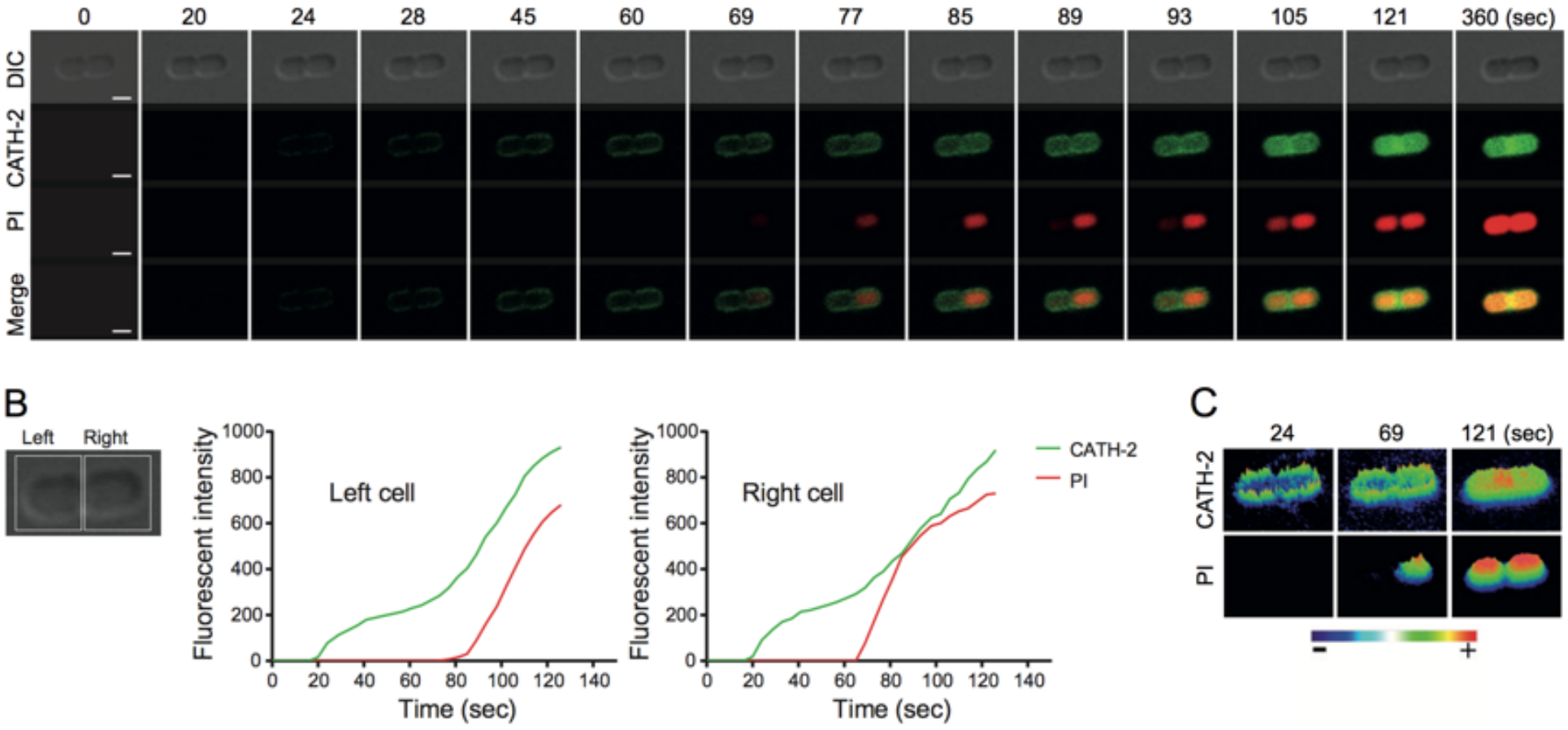
S. enteritidis
48h p.i.
(preimmune)

Van Dijk et al. Mol. Immunol., 2009

Antimicrobial activities of HDPs

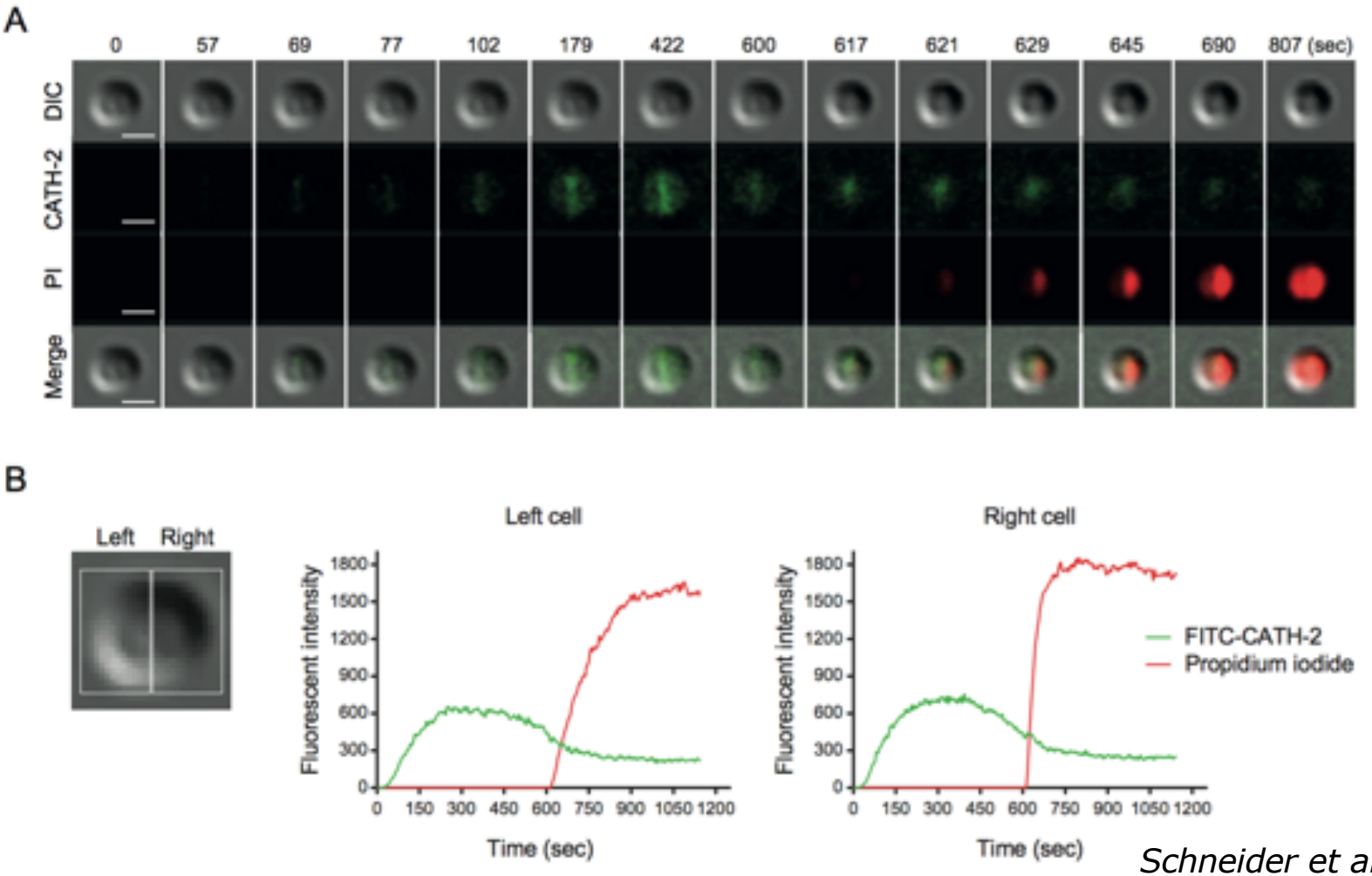
Visualisation of interactions of CATH-2 with bacteria

Fast membrane binding and permeabilisation of *E. coli* by CATH-2



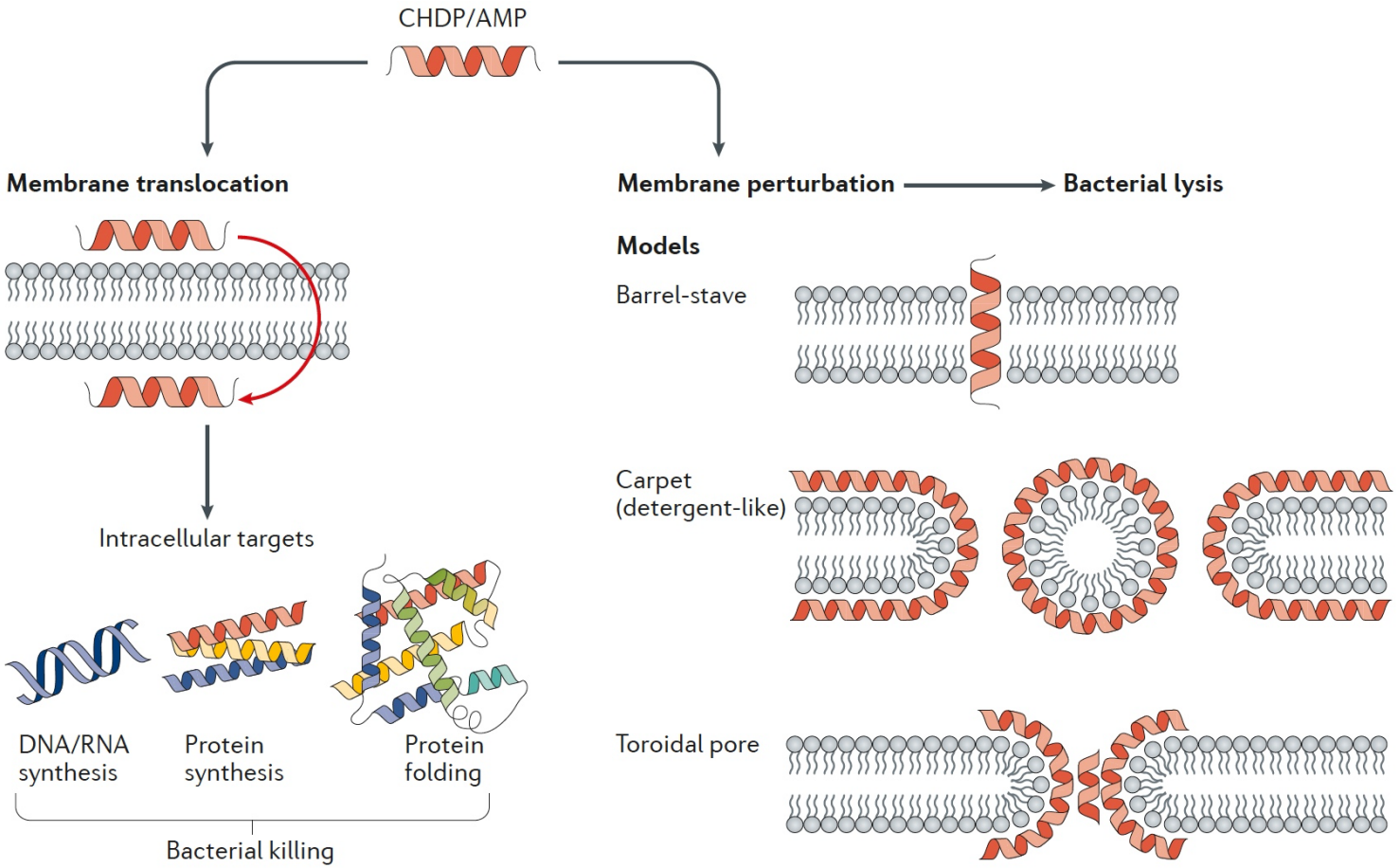
Schneider et al. Sci. Rep., 2016

Fast membrane binding and permeabilisation of *S. aureus* by CATH-2



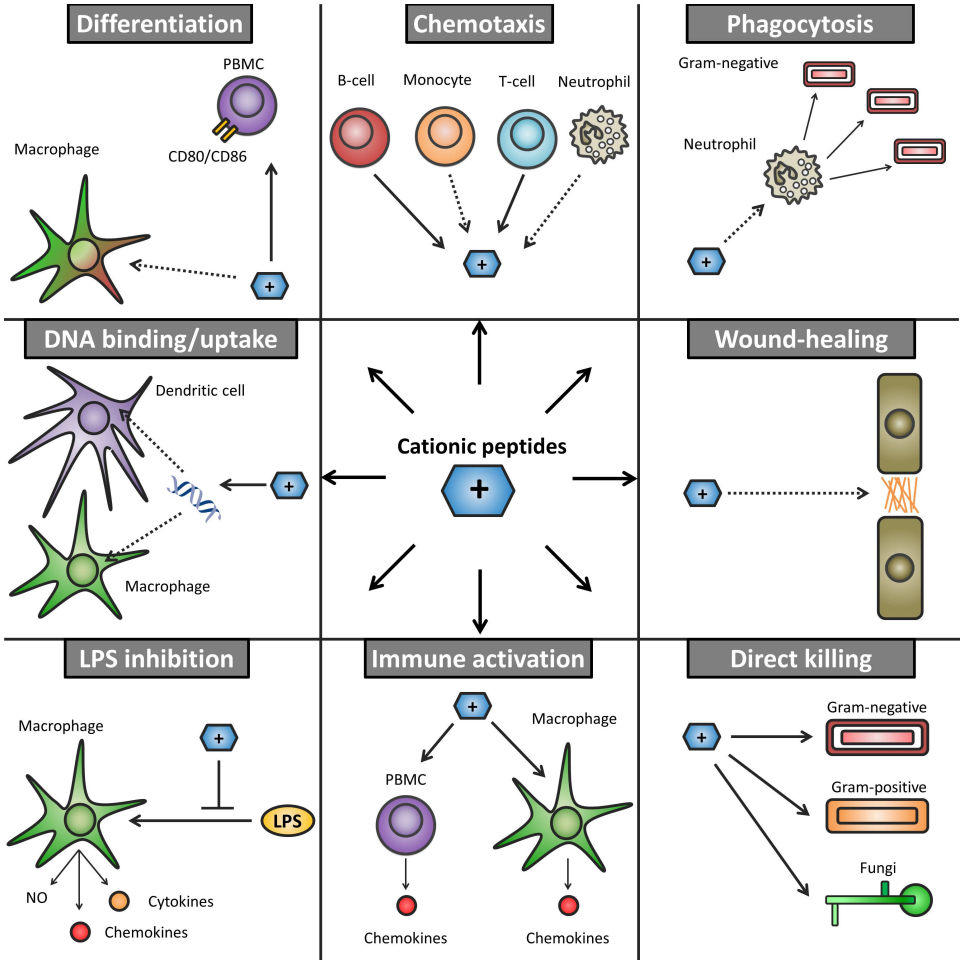
Schneider et al. mSphere, 2017

Antibacterial mechanisms of Cationic Host Defence Peptides



Mookherjee et al. Nat. Rev. Drug Discov., 2020

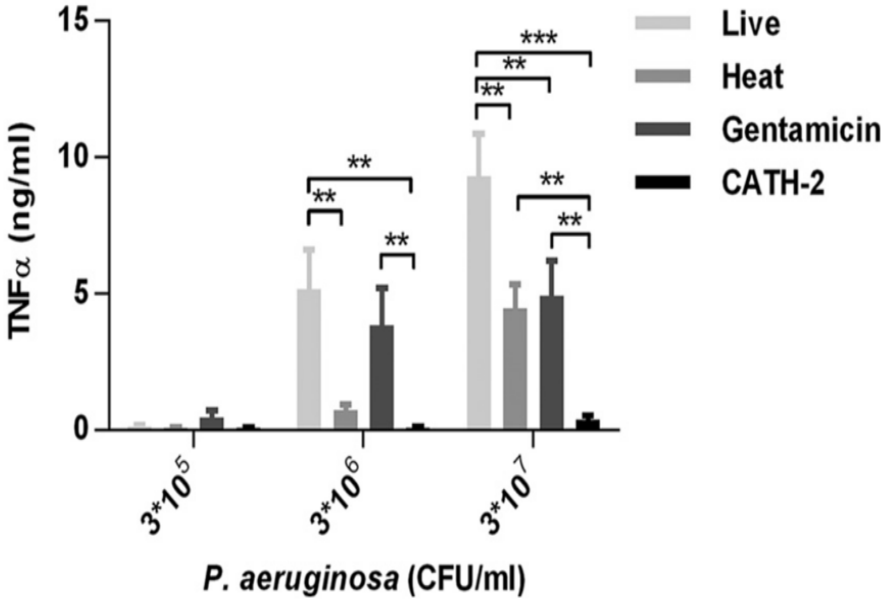
Functions of host defence peptides



Cuperus et al. Dev. Comp. Immunol., 2013

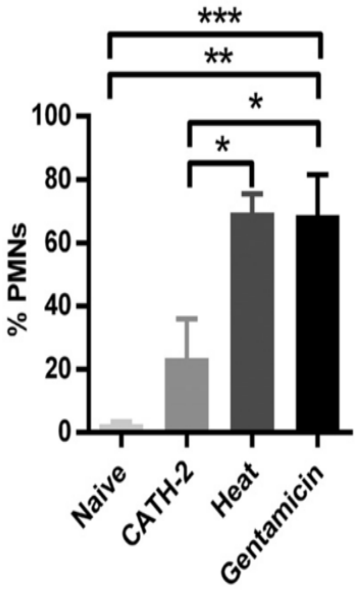
HDPs reduce the inflammatory response

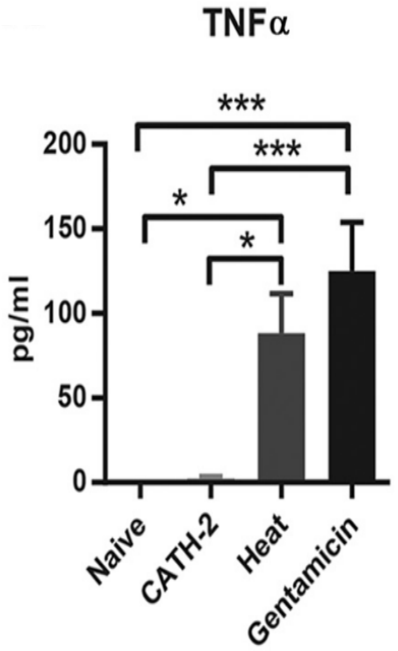
Example:
CATH-2 “silently” kills *P. aeruginosa in vitro* and *in vivo*



2h incubation

J774A.1





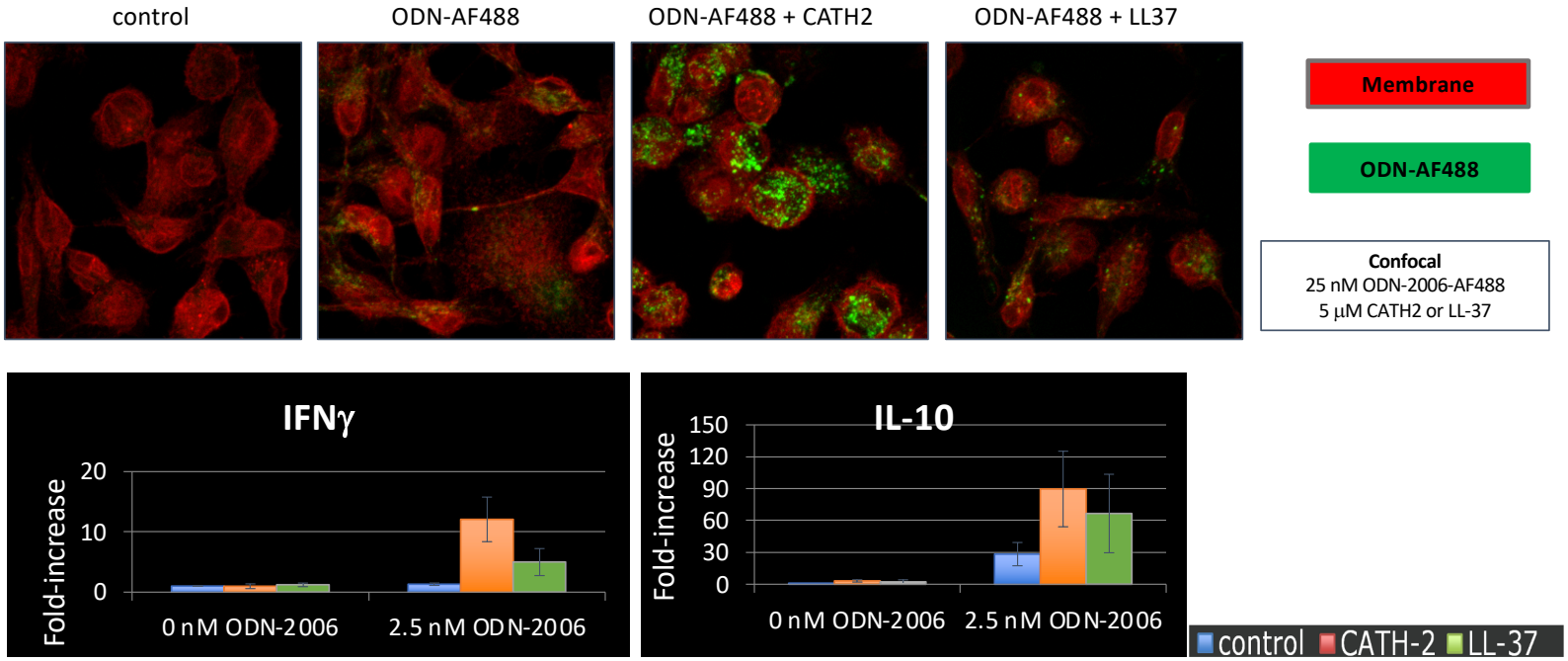
6h after instillation

Mouse lung

Coorens et al. Infect. Immun., 2017

HDPs increase the inflammatory response

Example:
CATH-2 enhances DNA uptake by macrophages



Enhancement of TLR9 (mammals) or TLR21 (birds) activation by CpG in vaccinations

Coorens et al. J. Immunol., 2015

HDP-derived products as alternatives to antibiotics

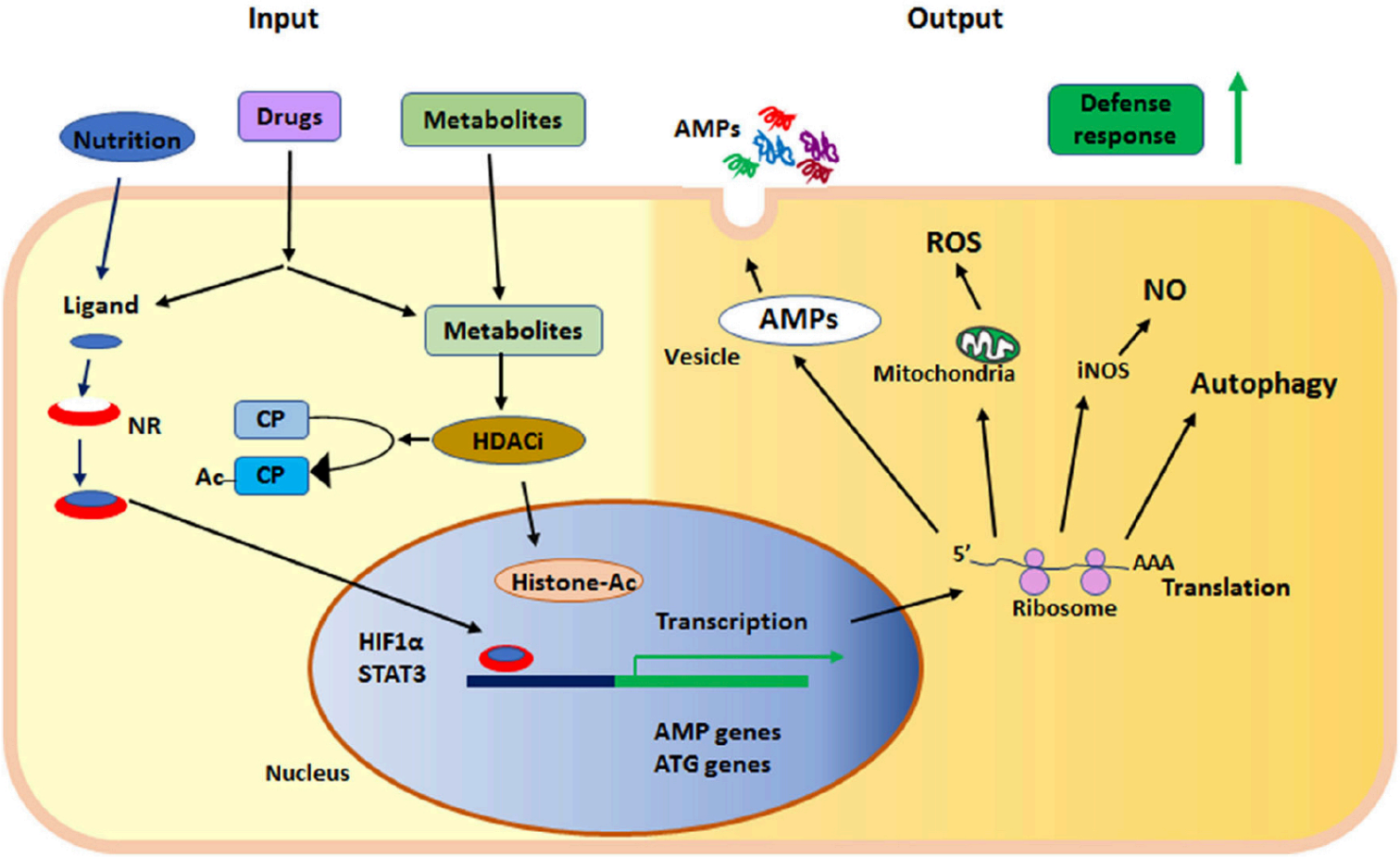
Strategies:

- Direct administration:
  - Antimicrobial activity
  - Immunomodulatory activities
- Stimulation of endogenous production of HDPs

Host-directed therapy by increasing production of HDPs

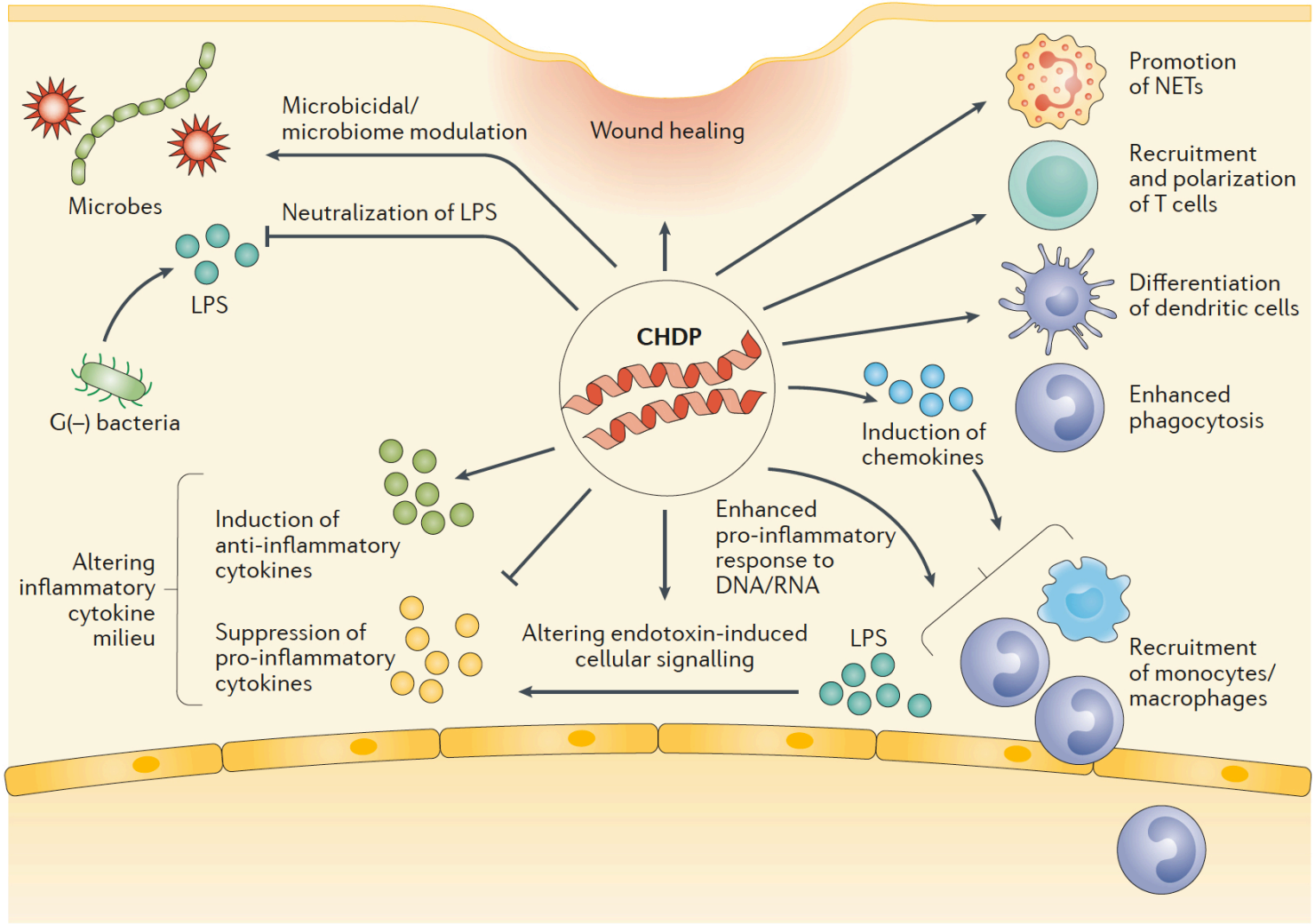
Example:
Application of histone deacetylase (HDAC) inhibitors

Induction of innate effectors in phagocytes and epithelium



Bergman et al. Front. Immunol., 2020

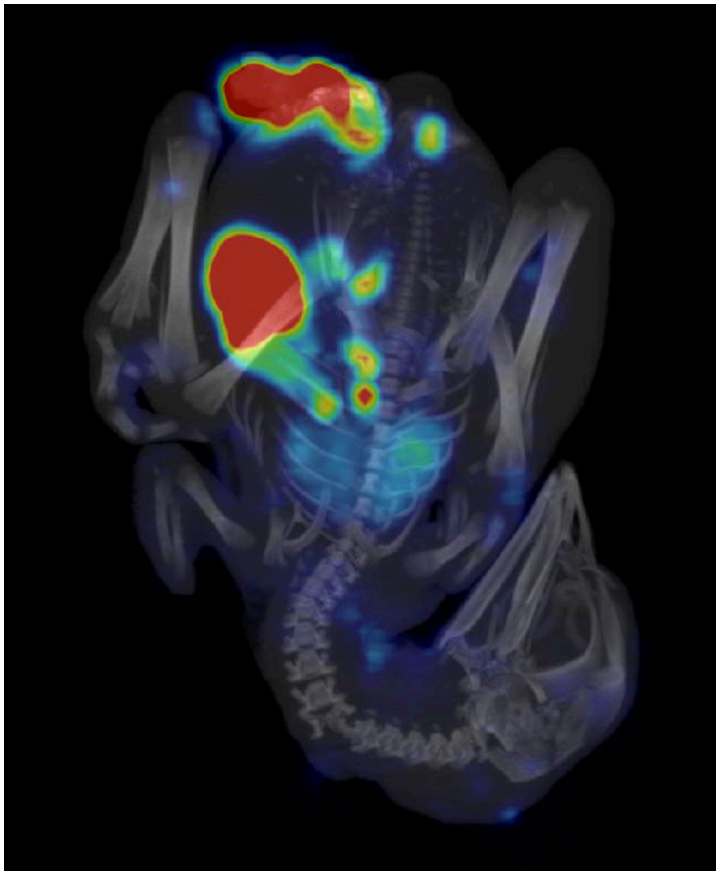
Immunomodulation by Cationic Host Defence Peptides



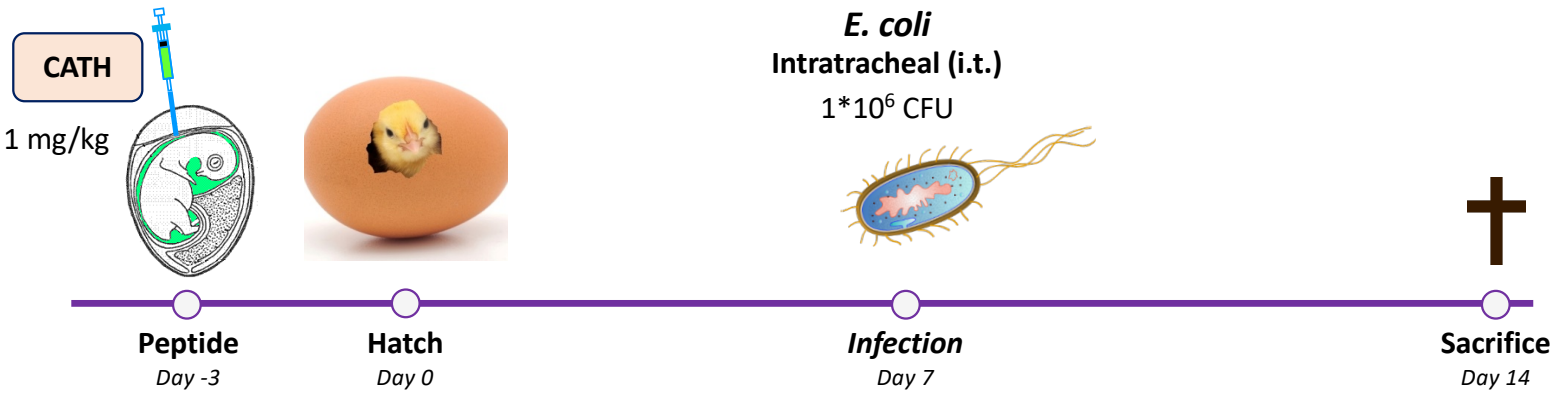
Mookherjee et al. Nat. Rev. Drug Discov., 2020

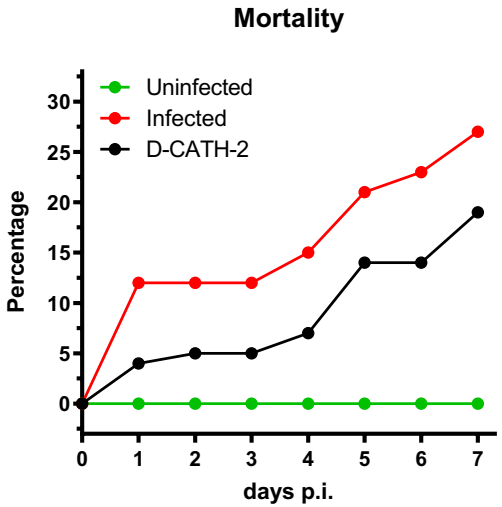
Prophylactic potency of CATH-2 and derivatives in veterinary medicine

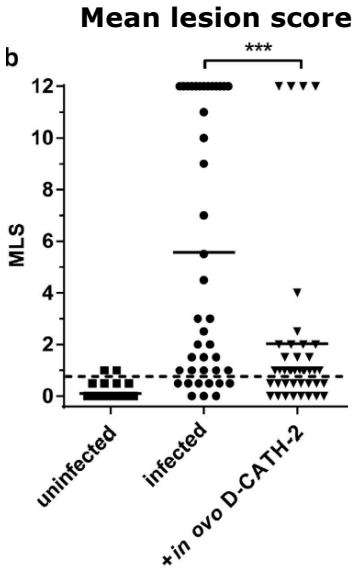
In ovo administration (chicken embryos)

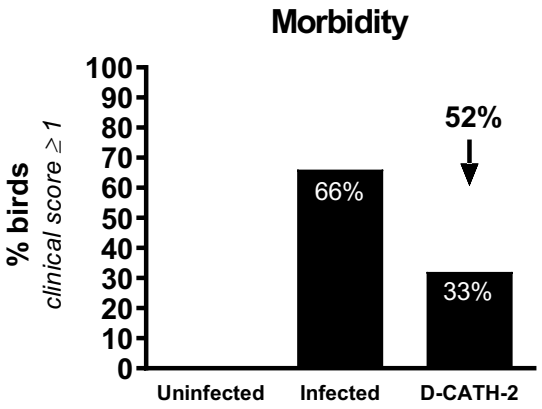


In ovo D-CATH-2 administration protects from colibacillosis



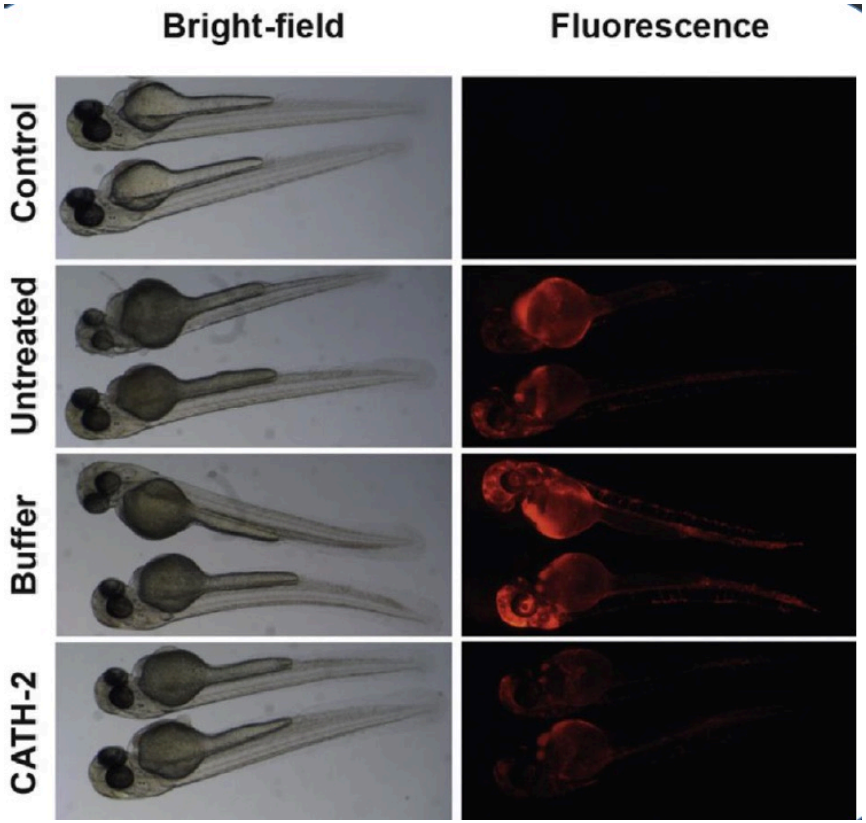






Cuperus et al. Sci. Rep., 2016

Efficacy of D-CATH-2 in a zebrafish infection model



Zebrafish embryos

Yolk injection
2.6 ng/kg D-CATH-2

Salmonella enteritidis
10-100
CFU/embryo

Schneider et al. Dev. Comp. Immunol., 2016

D-CATH-2 via embryonic route of administration

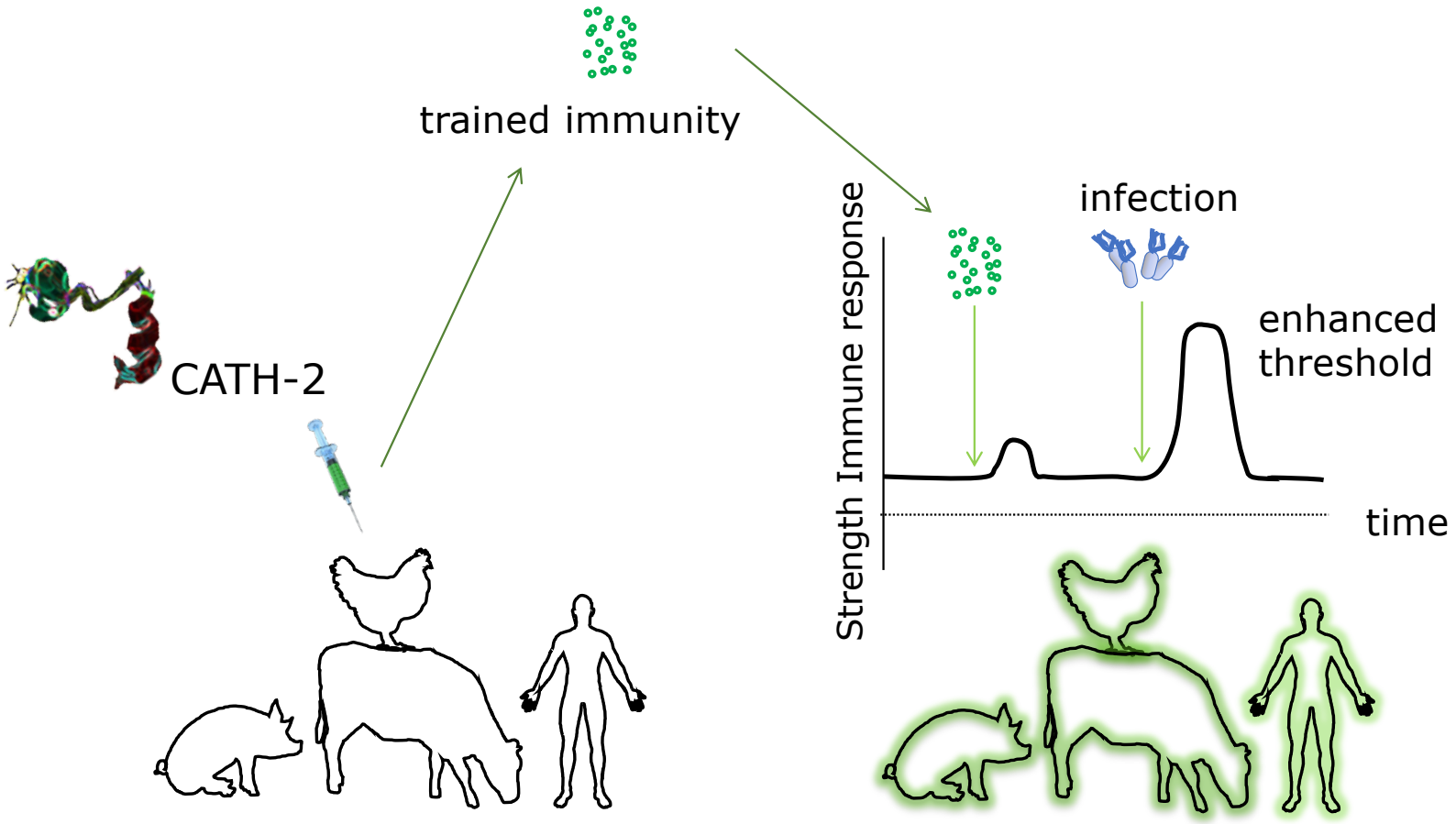
Target species	Challenge	Result	
Chicken 3 days pre-hatch	*E. coli* (i.t.) 7 days post hatch	Mortality Morbidity Bacterial load	30% 52% 93% ↓
Chicken 3 days pre-hatch	*S. enteriditis* (s.c.) 3 days post hatch	Mortality Morbidity	50% 67% ↓
Zebrafish 28 hpf	*S. enteriditis* 18-20 hpi	Mortality delayed	24 h

- **Peptide doses in models are too low to be directly antimicrobial**
- **Efficacy despite 6 to 10 day 'gap' between treatment and challenge!**

Amtimicrobial potency of HDPs in mammalian infection models

Presented by dr. Hancock in following presentation of this webinar

Peptide-induced innate immune memory?



Epidemiological observations of non-specific effects of vaccines

- Live vaccines induce cross-resistance
  - BCG
  - Measles-containing vaccines
  - Oral polio vaccines
  - Vaccinia against smallpox
- Inactivated vaccines induce cross-resistance
  - Diphteria-tetanus-pertussis containing vaccines
  - Hepatitis B vaccine
  - Inactivated polio vaccine

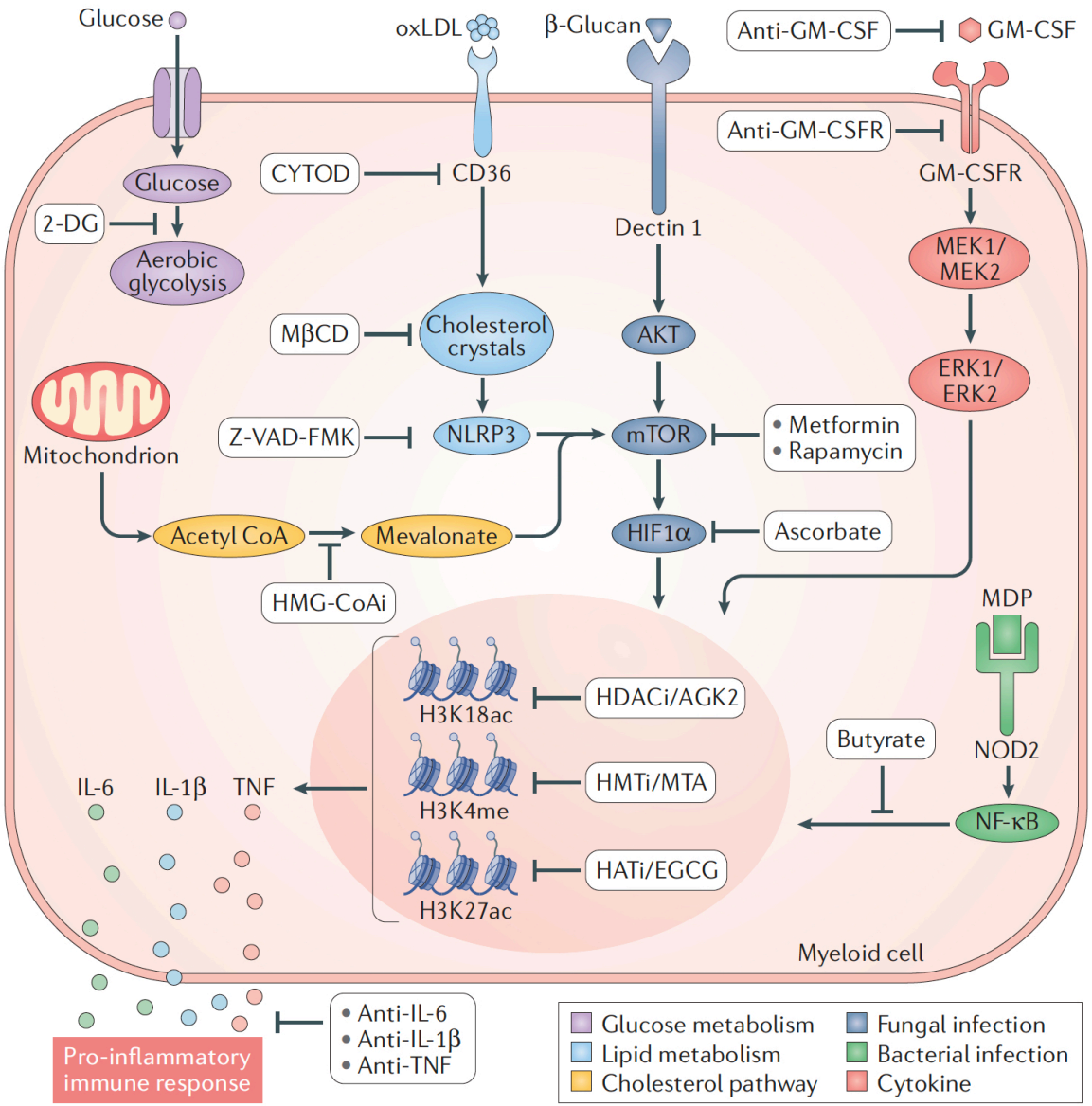
Adapted from Jensen et al. Semin. Immunol., 2016

Pathogen-associated molecules and cross-protection

Component	Source	Cross-protection	Reference
LPS (endotoxin)	G(-) bacteria	*Staphylococcus aureus*	Breyne (2017)
Peptidoglycan (muramyl dipeptide)	Bacteria	Toxoplasma	Krahenbuhl (1981)
Flagellin	G(-) bacteria	*Streptococcus pneumoniae* Rotavirus	Munoz (2010)
FimH (adhesin)	*Escherichia coli*	Influenza virus	Abdul-Careem (2011)
β-glucan	Fungi	*S. aureus* *S. pneumoniae*	Marakalala (2013)
Chitin	Fungi	*S. aureus* *E. coli*	Rizzetto (2016)
CpG oligonucleotide	Bacteria (synthetic)	*E. coli* Influenza virus	Ribes (2014) Jiang (2011); Norton (2010)

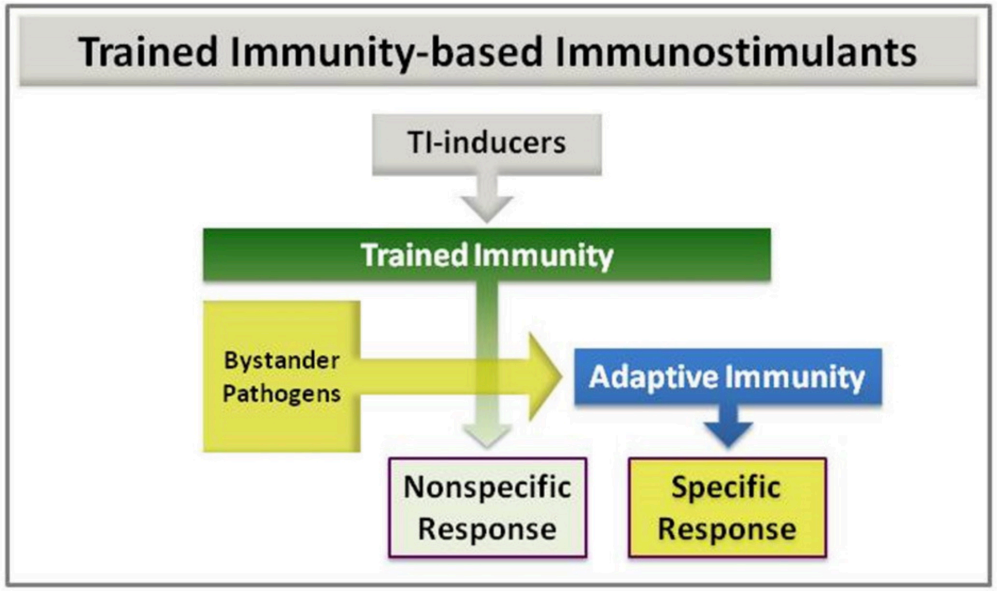
Adapted from Sánchez-Ramón et al. Front. Immunol., 2018

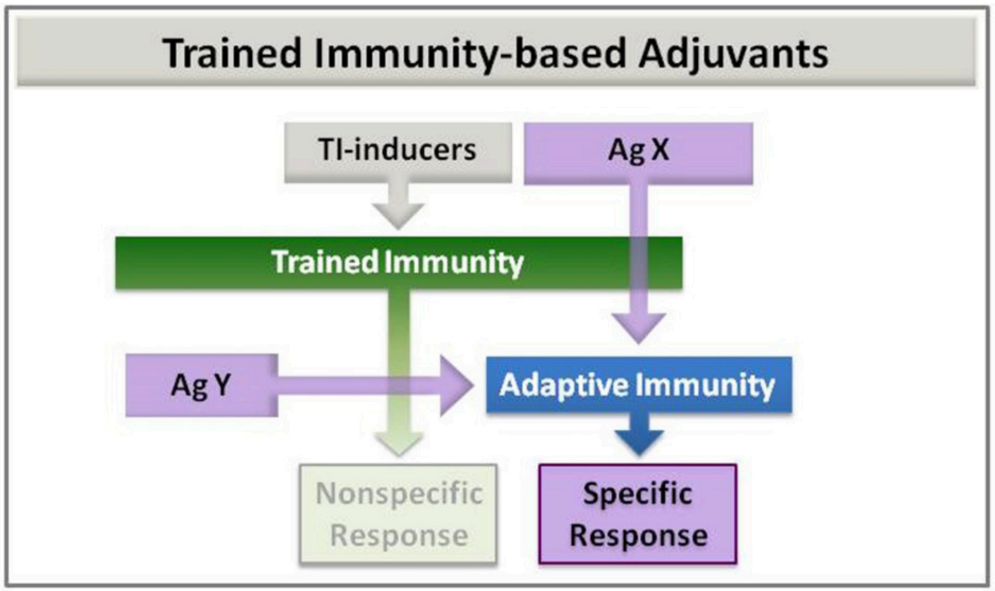
Trained immunity regulatory pathways



Mulder et al. Nat. Rev. Drug. Disc., 2019

Application of trained immunity inducers





Sánchez-Ramón et al. Front. Immunol., 2018

Applications of Host Defence Peptides

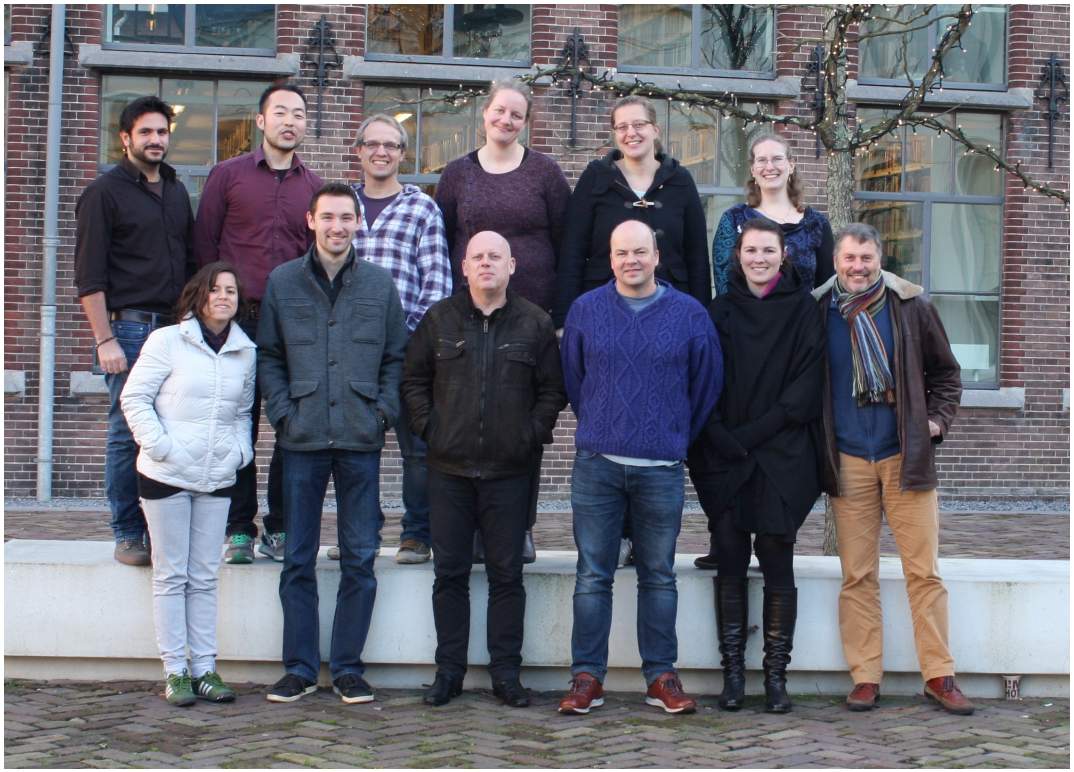
Therapeutic use:

- As immunomodulatory antimicrobials
- As adjunct to antibiotics

Prophylactic use:

- Regulation/reprogramming innate immune system in veterinary relevant species (generic protection)
- In vaccines as adjuvant

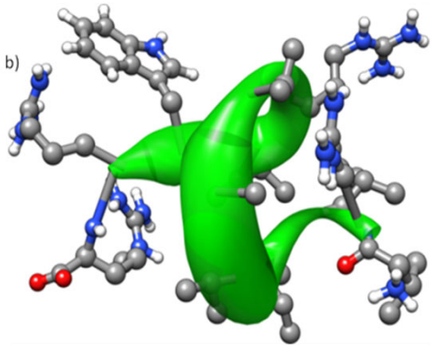
Section Molecular Host Defence

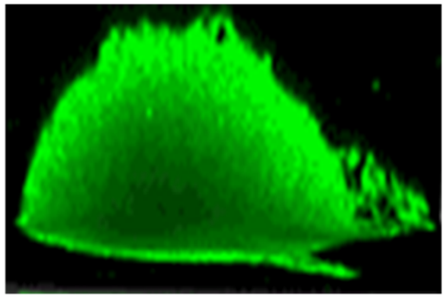


Andrea Bosso
Maarten Coorens
Tryntsje Cuperus
Soledad Ordonez
Viktoria Schneider
Weidong Zhang

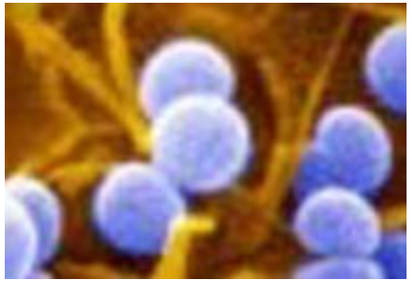
Albert van Dijk
Martin van Eijk
Marina Kraaij
Maaike Scheenstra

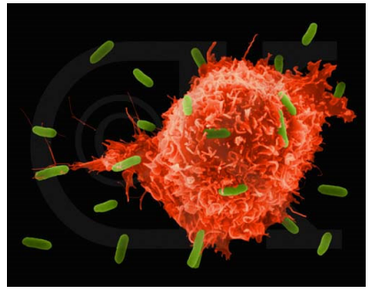
Hanne Tjeerdsma
Edwin Veldhuizen





Antibiotic Resistance. Immunomodulatory Strategies





R.E.W. (Bob) Hancock, PhD, OC
UBC, Vancouver, Canada

Conflict of Interest statement: Formed 2 virtual R&D companies to enable commercial development (through partnering) of peptides and sepsis diagnostics

Antibiotic Adjuvants/Adjuncts

Alternatives to antibiotics—a pipeline portfolio review

Lloyd Czaplewski, Richard Bax, Martha Clokie, Mike Dawson, Heather Fairhead, Vincent A Fischetti, Simon Foster, Brendan F Gilmore, Robert E W Hancock, David Harper, Ian R Henderson, Kai Hilpert, Brian V Jones, Aras Kadioglu, David Knowles, Sigríður Ólafsdóttir, David Payne, Steve Projan, Sunil Shaunak, Jared Silverman, Christopher M Thomas, Trevor J Trust, Peter Warn, John H Rex

Lancet Infect Dis 2016; 16: 239–51

Published Online January 12, 2016 http://dx.doi.org/10.1016/S1473-3099(15)00466-1

Antibiotics have saved countless lives and enabled the develop[illegible]ine over the past 70 years. However, it is clear that the success of antibiotics [illegible] we now expect a long-term and perhaps never-ending chall[illegible] resistant bacteria. A broader approach to address bac[illegible], we discuss alternatives to antibiotics, which we defined as non-compoun[illegible] other than classic antibacterial agents) that target bacteria or any

Host Directed therapies

Antibodies; Vaccines; Probiotics; Lysins; Wild Type and Engineered Bacteriophages; Immune Stimulation; Antimicrobial peptides; Innate Defence Regulators; Anti-biofilm Peptides

When do antibiotics fail?

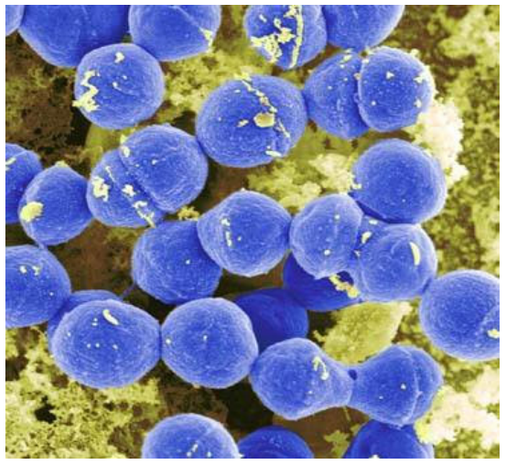
1. Resistance (mutations/plasmids etc.)
2. Sepsis (~20-35% death rate; 11 million deaths annually). Dysfunctional immune response.

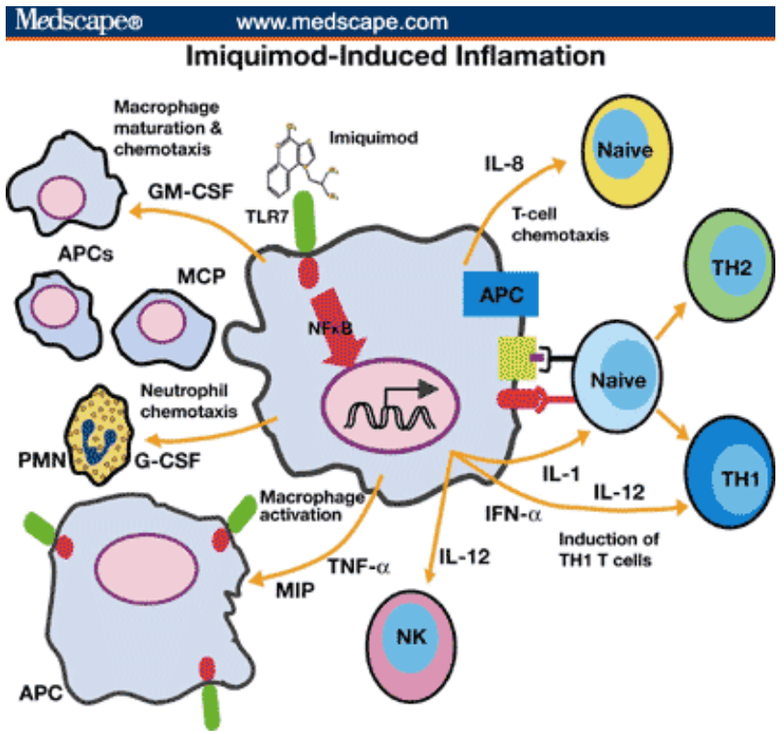
3. Chronic Infections especially biofilms (65% of all infections and very resistant). Associated with chronic inflammation.
4. Individuals with disturbed immune systems (chemotherapy, immuno-suppressive disease, genetic diseases, massive injuries or burns).

Indications 2 to 4 are rarely taken into account when developing new anti-infectives

Immune Modulation

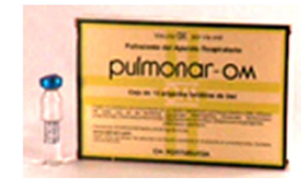
- **Successful therapy against infections requires help from the host: immune response**
- **Immune modulation is highly used in antiviral and anticancer therapy**
- **Less commonly used for bacterial infections**

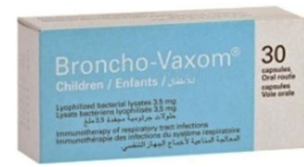




Hancock, R.E.W., A. Nijnik and D.J. Philpott. 2012. Modulating immunity as a therapy for bacterial infections. Nature Rev. Microbiol. 10:243-254.

Immune Modulation
Bacteria-based Products for Respiratory Diseases





- 35 placebo-controlled trials (4,060 participants) provided data in a form suitable for inclusion in the meta-analyses.
- Trial quality was generally poor and a high level of statistical heterogeneity was evident.
- By combining results, immunostimulants reduced incidence of Acute Respiratory Tract Infections by an average of 39% reduction cf. placebo.
- No difference in adverse events was evident between placebo and treated groups.
- Utilized in Latin America and Eastern Europe
- Many other immune modulators
- Need to understand mechanisms

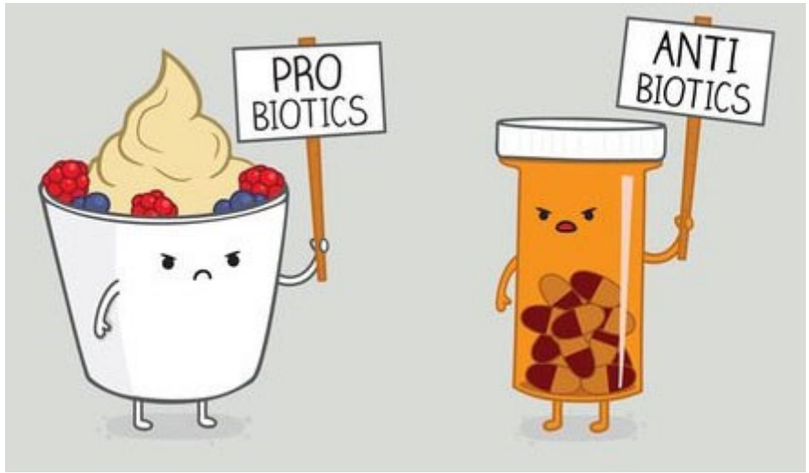
Any immunostimulant (IS) compared with placebo for preventing respiratory tract infection in children

Patient or population: children (age <18 years) susceptible to acute respiratory tract infections (ARTIs)
Settings: outpatient
Intervention: any IS
Comparison: placebo

Outcomes	Illustrative comparative risks* (95% CI)		No of participants (studies)	Quality of the evidence (GRADE)
	Assumed risk	Corresponding risk		
	Placebo	Any IS		
Number of ARTIs	The range of ARTIs in the control group was 0.92 to 6.2	The mean Number of ARTIs in the intervention groups was 1.24 lower (0.94 to 1.54 lower)	4060 (35 studies)	⊕⊕⊕ moderate[1]
Percent difference in ARTIs		The mean Percent difference in ARTIs in the intervention groups was 39 lower (31.31 to 46.37 lower)	4060 (35 studies)	⊕⊕⊕ moderate[1,2]
Incidence of gastrointestinal adverse events	21 per 1000	30 per 1000 (11 to 50 per 1000)	1457 (10 studies)	⊕⊕ low[1,3]
Incidence of skin adverse events	3 per 1000	7 per 1000 (-8 to 14 per 1000)	1469 (10 studies)	⊕⊕ low[1,3]

Del-Rio-Navarro BE, et al 2012. Immunostimulants for preventing respiratory tract infection in children. Cochrane Review 7:629–717.

Probiotics



- Use of bacteria to prevent infections
- Several mechanisms proposed, e.g. exclusion, immune modulation

Cosseau C, Hancock REW et al. 2008. Infection and Immunity 76:4163-4175.

- Already used successfully
  - As OTC medicines: *S. salivarius* clinically proven for Strep throat, Halitosis, Chronic sore throat
  - In fecal transplantation for *C. difficile*, and
  - Reduces sepsis (24% → 15%) & enterocolitis (4.6 → 2.5%) in 10,000 premature Very Low Birthweight Babies

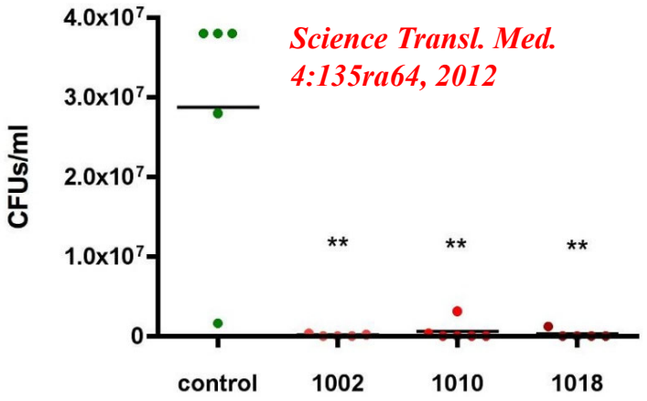
BLIS Technologies

- Consortia of defined bacteria can replace normal microbiota

Denkel et al. 2015 Antimicrobial Resistance & Infection Control 2015, 4(S1):O39

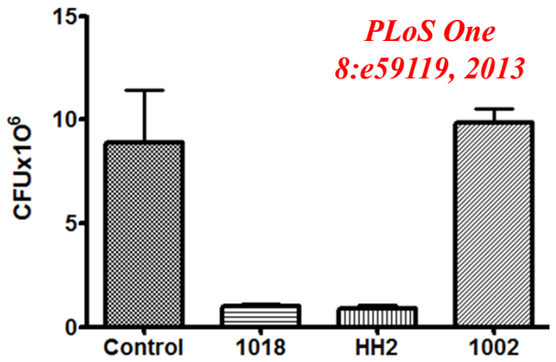
New immunomodulatory peptides show broad protection in Mouse Model Infections

Invasive *Staph. aureus* Mouse Model

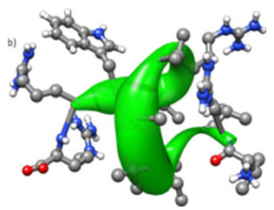


Science Transl. Med. 4:135ra64, 2012

MDR -TB Mouse Model

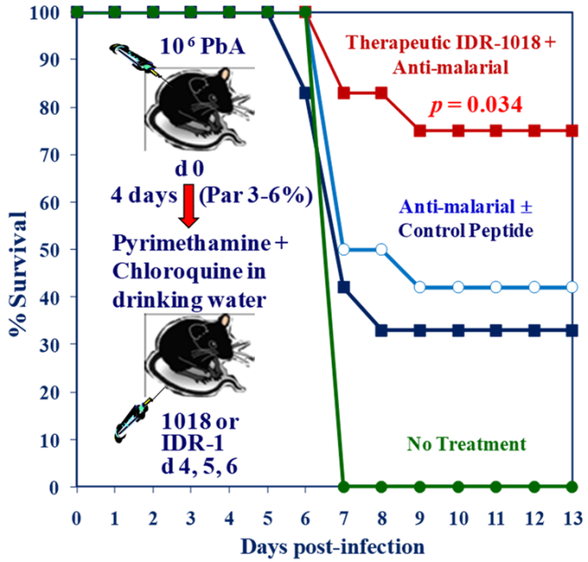


PLoS One 8:e59119, 2013



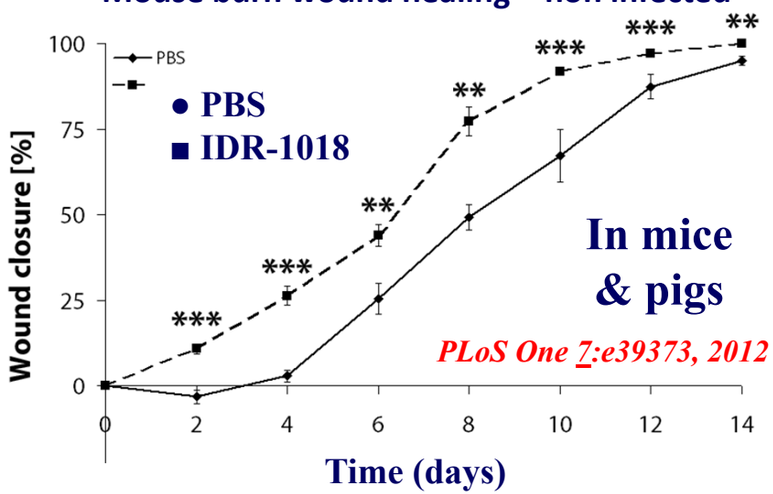
IDR 1018: VRLIVAVRIWRR-NH$_2$

Cerebral Malaria Mouse Model



Science Transl. Med. 4:135ra64, 2012

Mouse burn wound healing – non infected



In mice & pigs

PLoS One 7:e39373, 2012

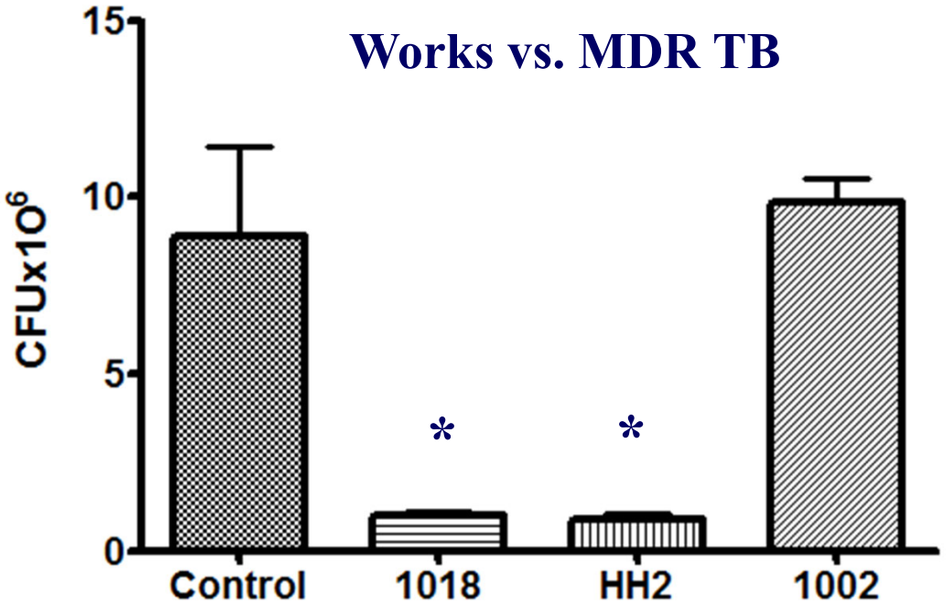
Also protects in animal models vs. *E. coli*, *Pseudomonas*, MRSA, *Salmonella*, *Klebsiella*, Tuberculosis; *Candida*; Pox & HSV viruses; Sterile inflammation; Malaria, Inflammatory Bowel Disease, Cystic Fibrosis (cells); LPS/hypoxia-ischemia; → Wound Healing

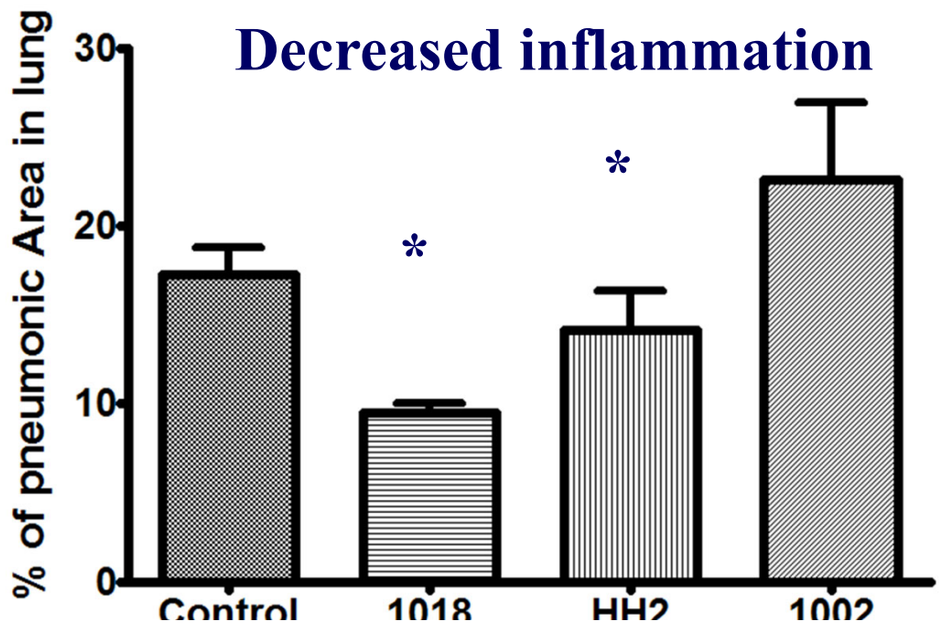
Grand Challenges in Global Health

Lars Steinstraesser, Louis Schofield, Ariel Achtman, Bruno Rivas, Rogelio Pando, Carina Mallard, Octavio Franco

Therapeutic IDR-1018 protects vs. MDR TB lung infections

- Balb/c mice infected intratracheally with high dose 10^6 virulent live *MTb* strain; Multi-drug Resistant (MDR) or H37Rv.
- After 2 months of infection, treatment via IT: 32 μg (~1 mg/kg) peptide every 2 days.
- Groups of 5 animals sacrificed after 4 wks of treatment and lung bacilli counted.

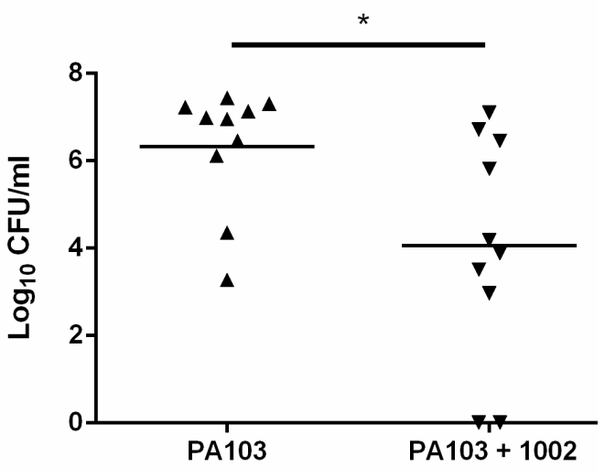


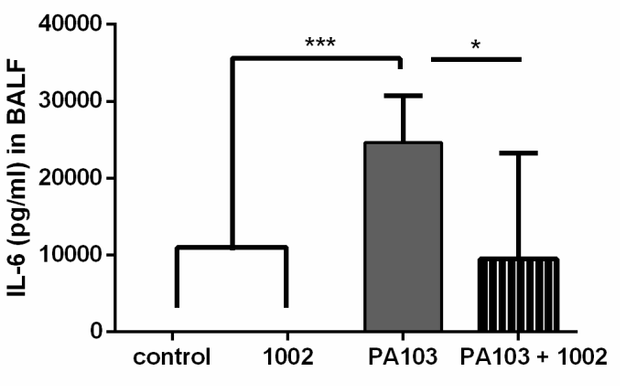


Bruno Rivas and Rogelio Hernandez Pando, UNA de México; PLoS One 8:e59119, 2013.

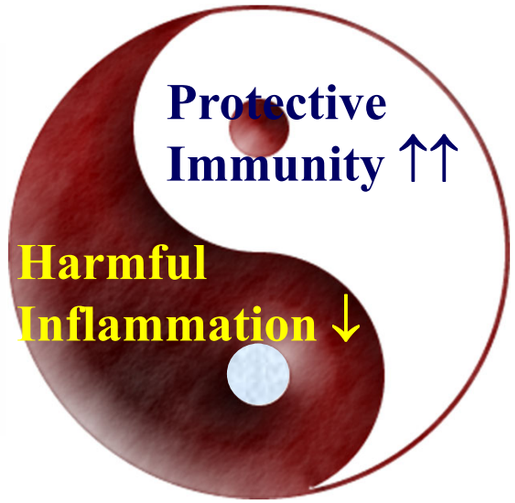
RNA-Seq and bioinformatics studies on mouse lungs are deciphering mechanisms in vivo

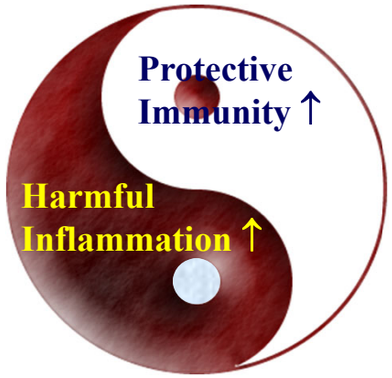
Intranasal delivery (single dose prophylactic)
***Pseudomonas aeruginosa* lung infections**



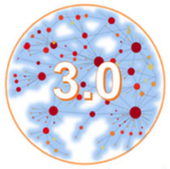


IDR-1002 given intranasally 24 hr prior to infection





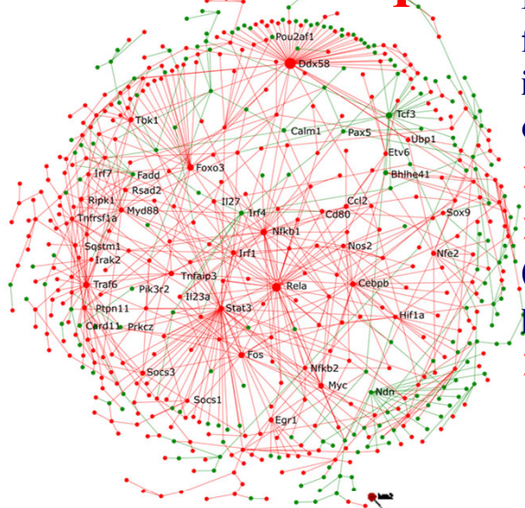
Effect of Microbes



NetworkAnalyst

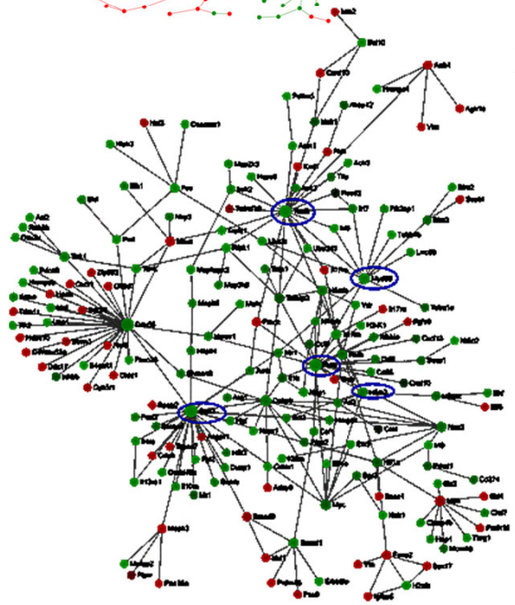
Effect of Peptides

RNA-Seq



Interaction network from whole lungs of mice infected with PA103 vs. control.

- **4,739 genes DE:**
- **Many immune (+ inflammatory) pathways ↑**
- **Extracellular matrix ↓**



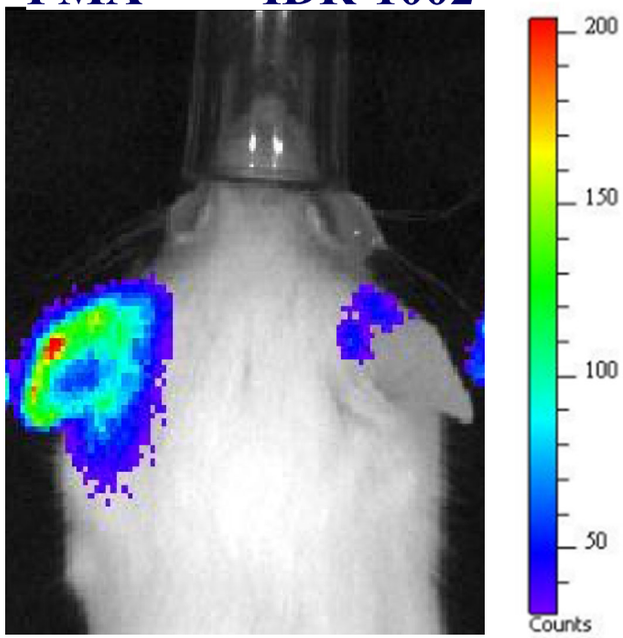
Network for PA103+IDR-1002 cf. PA103

- **2,111 genes DE**
- **Immune system pathways ↓**
- **Metabolism, T_h cell differentiation & VEGF regulation ↑**

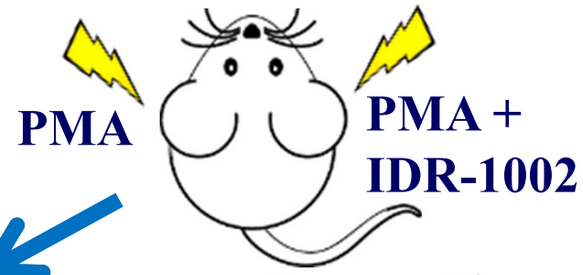
IDR peptides can treat sterile inflammation

- Mouse Ear sterile inflammation model
- Induced with phorbyl myristate (PMA)
- Treat with peptides topically @ -30 min

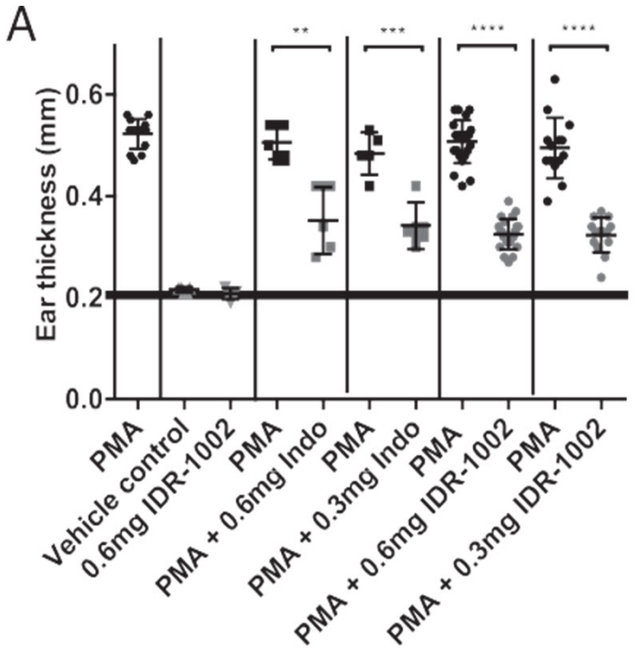
PMA | PMA + IDR-1002

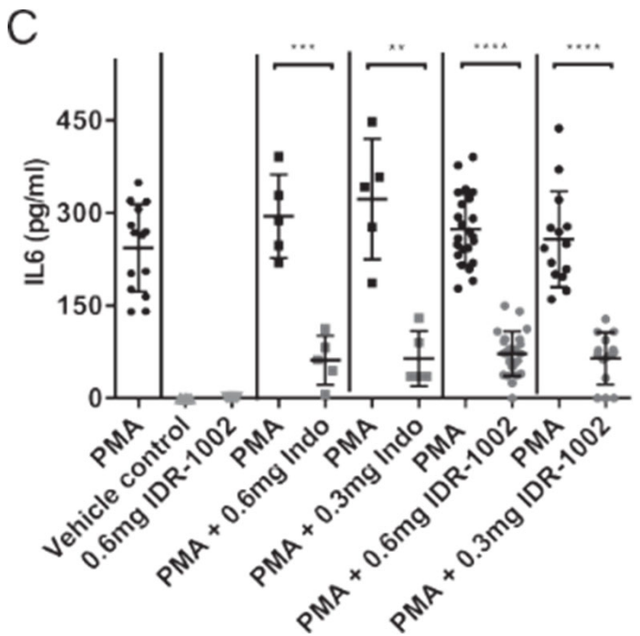


Assessing production of reactive oxygen and nitrogen species with probe LO-12



Ear swelling @ 6 hours

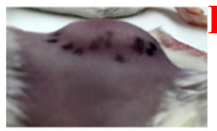




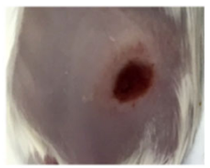
- Suppressive effects on IL-6, MCP-1, KC
- Occurs at level of transcription (IRF8)
- ≡ Indomethacin

Catherine Wu et al. 2017. J. Immunol. PMID:28993516

Modelling High Density Infections by *ESKAPE* pathogens in mice - Tracking infection and inflammation in a chronic model



Day 1



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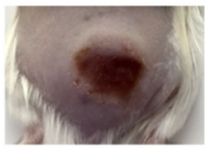

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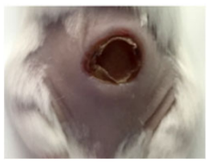

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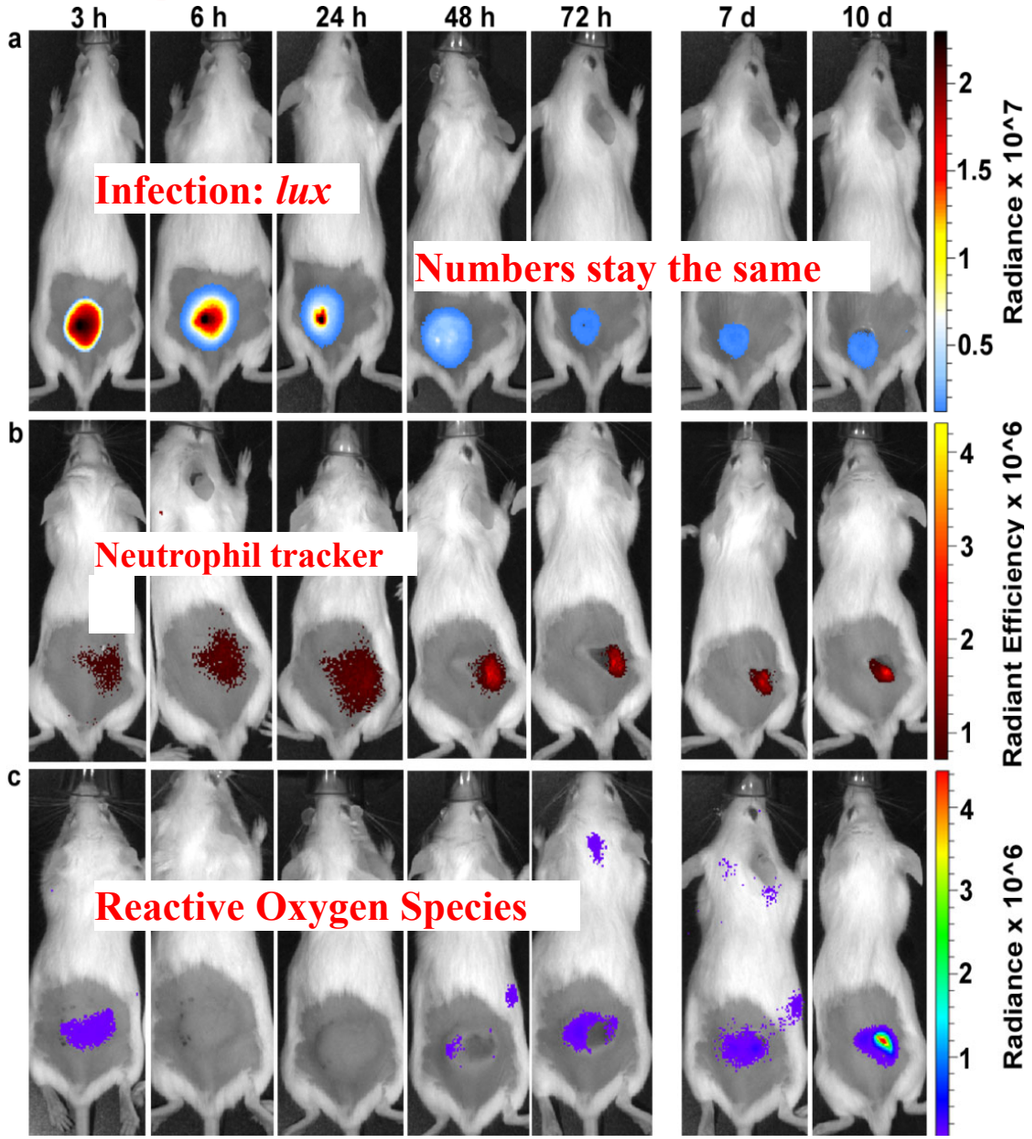
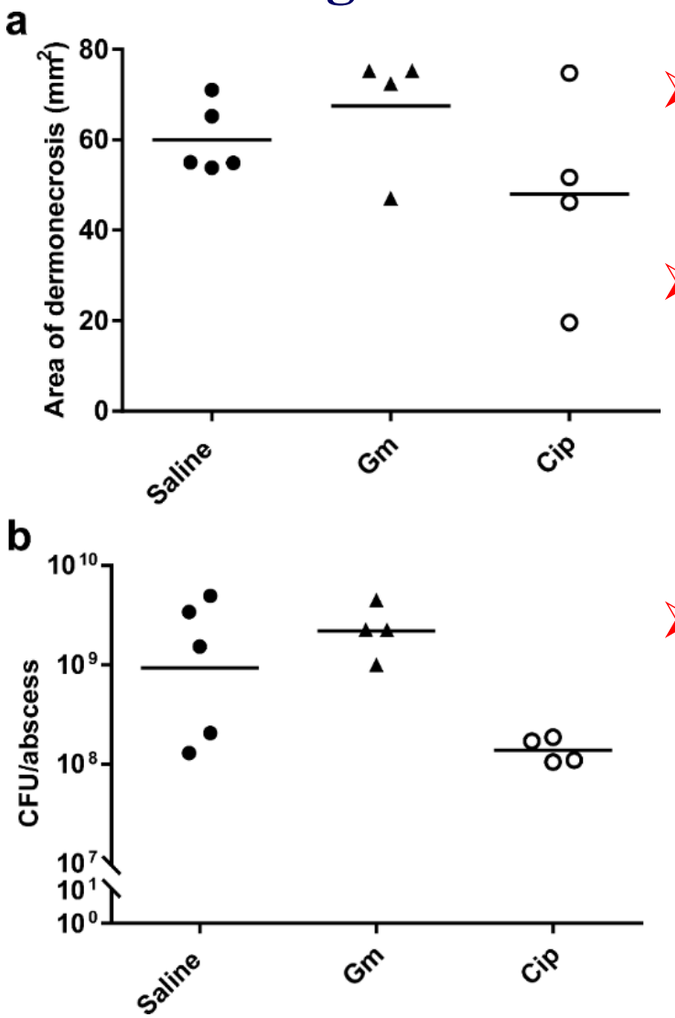
Pletzer et al, 2017. mBio 8:e00140-17

P. aeruginosa LESB5



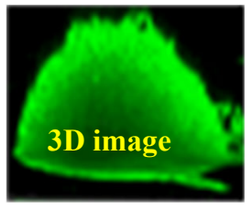
Intravenous antibiotics at 6X the human dosage did not work



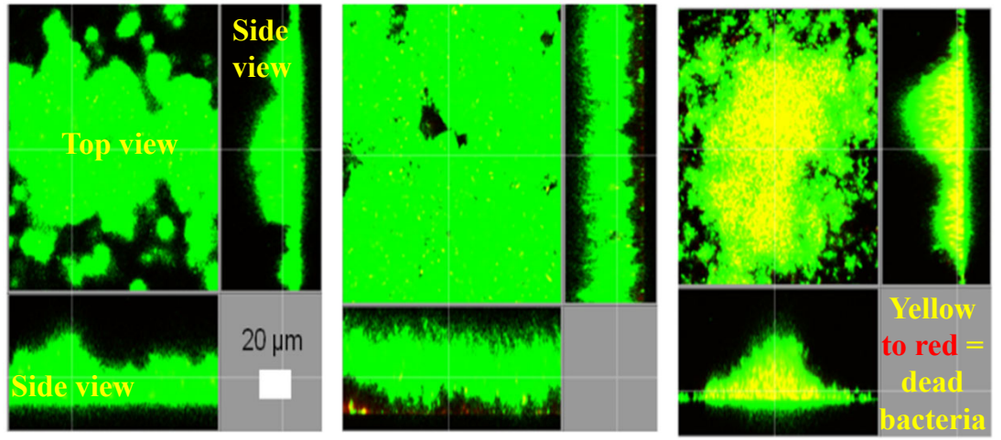
- Intra abscess worked poorly
- For some antibiotics 1,000X the human dose didn't work
- Challenging model for recalcitrant chronic infections (cf. biofilms)

Biofilms: an alternative growth state & highly resistant to most conventional antibiotics

- **Biofilms are found everywhere; form on surfaces and are often an adaptation to stress**
- **Cause >65% of all bacterial infections (clinically relevant) e.g. devices, lung, bladder, wound, oral, ears, nose & throat, skin, etc.**
- **Chronic infections that lead to chronic and harmful inflammation**
- **Highly adaptively resistant**
- **No specific treatments!!**

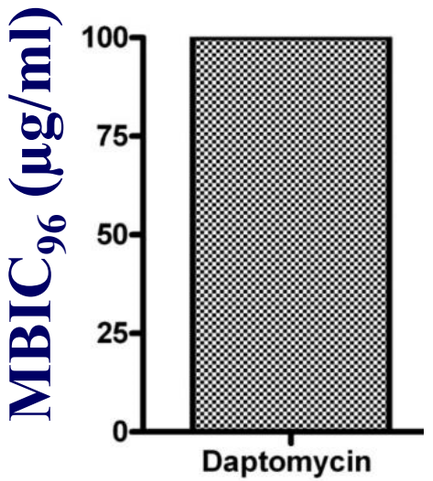


Pseudomonas aeruginosa



Untreated | + ciprofloxacin | + ciprofloxacin (100X MIC)

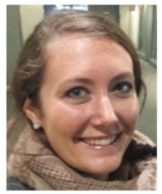
MIC_{Cipro} = 0.25 µg/ml (MIC)



***S. aureus* MRSA**

← MIC = 0.2 µg/ml

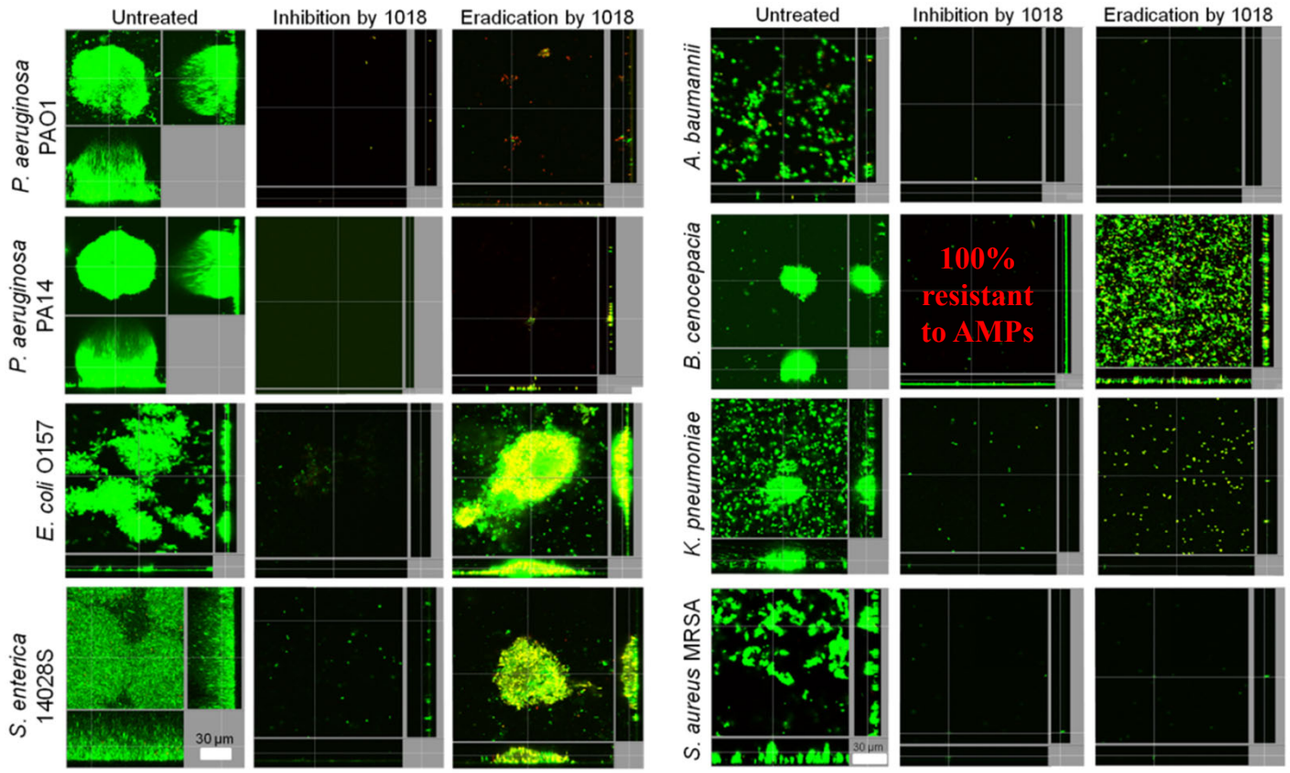
Cesar de la Fuente Nunez



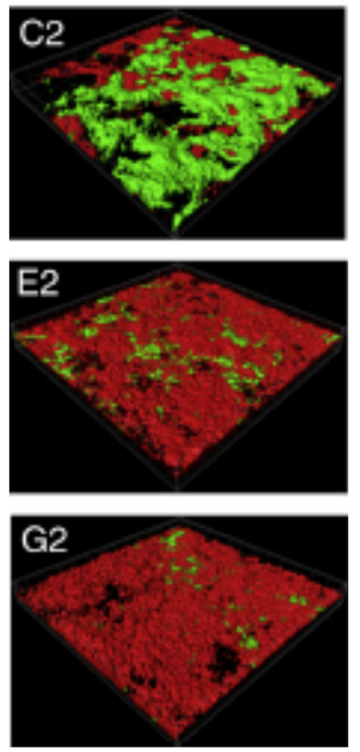
Fany Reffeuveille

Broad Spectrum Anti-Biofilm Activity when added at 0 or 2 days of biofilm formation

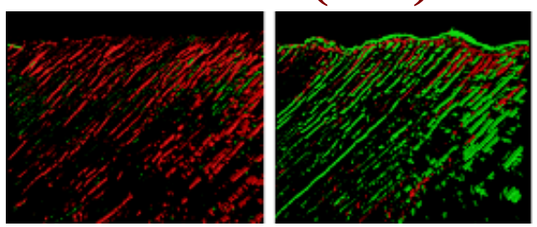
Kills all ESKAPE pathogens



Peptides work rapidly (3 min) vs multispecies oral biofilms & synergize with EDTA & Chlorhexidine →



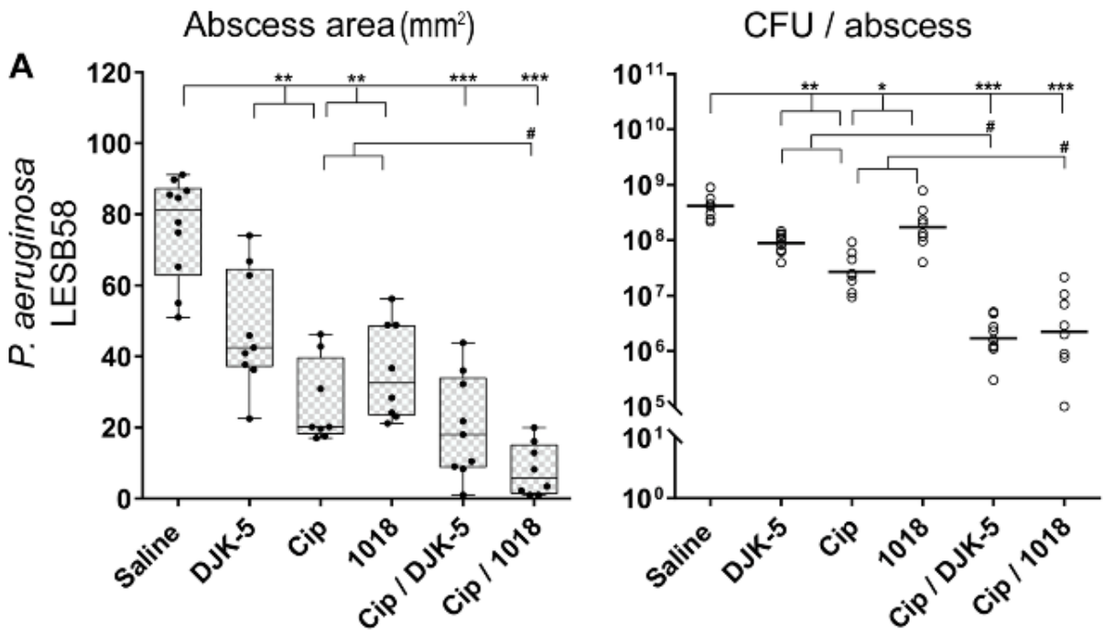
And work on dentin biofilms (left) ↓

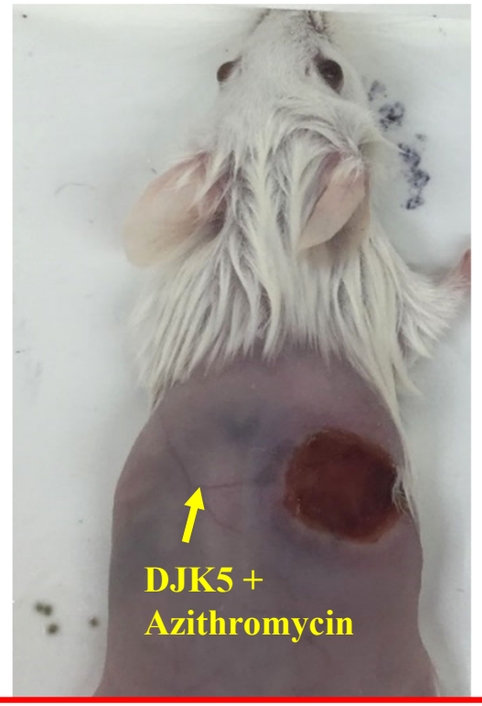


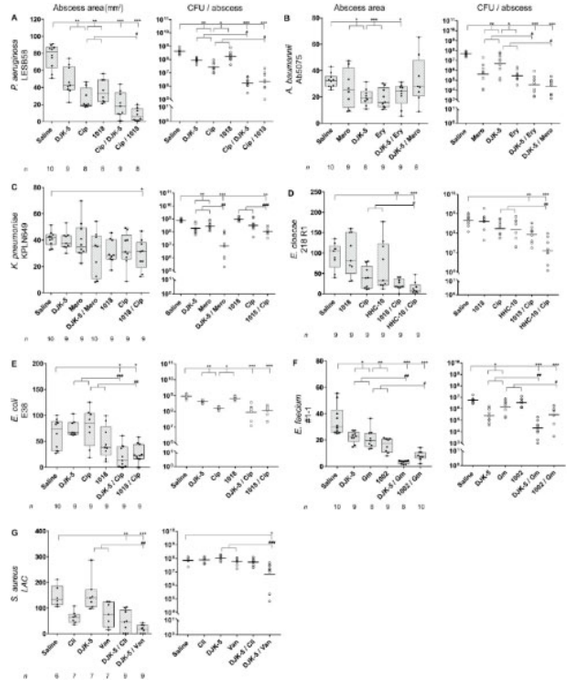
Broad Spectrum Anti-Biofilm Activity when added at 0 or 2 days of biofilm formation

Cesar De la Fuente Nunez, Fany Refeuveille et al. 2014. PLoS Pathogens 10(5):e1004152; De la Fuente Nunez et al. 2015. Chemistry & Biology 22:196–205; Wang, Z, et al. 2015. PLoS One 10, e0132512.; Zhang et al. 2016. PLoS One 11: e0166997; Wang et al. 2018. J. Endodontics 44:1709-13

Anti-biofilm peptides are effective against high density infections and synergize with conventional antibiotics in vivo





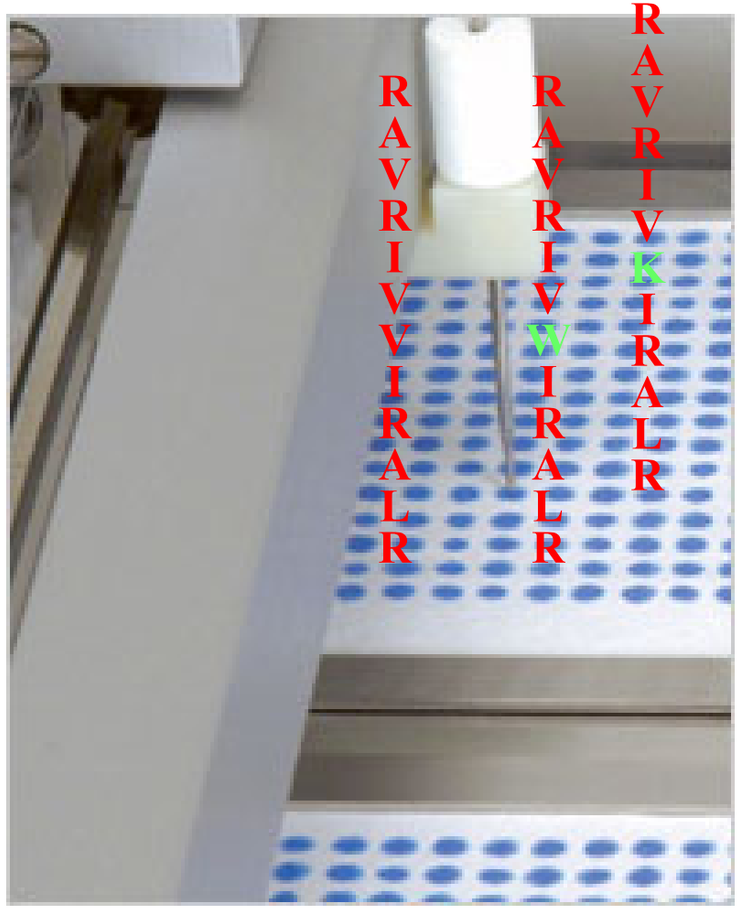


- Peptides … work bet… DJK5 with …ycin (given …ss on the *left* …scess visible), cf. untreated (*right side*).
- Observe… *E. coli* (several antibiotics)

NB: Larger effect on abscess formation (pathology) than bacterial numbers. Can we also impact on biofilm-mediated inflammation?

Pletzer et al. 2018. PLoS Pathogens 14(6):e1007084

Random Screening for New Peptides:
Spot Synthesis of Peptides using a Pipetting Robot



Solid phase synthesis (peptide array) method
synthesizes peptides from C-terminus on cellulose sheets.

Structure Activity Relationships of Immune Modulation & Anti-Biofilm activities overlap

Based on lead peptides. Substituting every position with 9 most common amino acids → Identification of many peptides with better or similar activities (dark red cf. grey).

Evan Haney *et al. Peptides 71:276-85, 2015; Sci. Reports 8:1871, 2018*

Anti-biofilm

Antibiofilm	Substituted Amino Acid	1002 Sequence											
		V	Q	R	W	L	I	V	W	R	I	R	K
Cationic	R	32.9	40.5	26.8	64.1	37.8	86.7	41.9	76.8	26.8	84.7	26.8	37.6
	K	24.8	31.3	38.8	113.0	21.0	98.5	71.9	130.8	50.0	129.2	25.7	26.8
Polar, uncharged	Q	23.2	26.8	29.7	64.4	51.8	78.0	55.5	80.5	59.1	61.3	33.0	35.0
Small, Hydrophobic	G	31.7	26.0	44.0	57.8	40.9	74.1	45.9	58.3	56.3	65.0	40.3	49.7
	A	45.1	32.2	35.2	47.8	29.7	72.0	42.2	47.5	42.4	55.4	48.1	62.6
Large, Hydrophobic	W	23.7	38.8	32.4	26.8	23.6	47.4	37.5	26.8	27.1	29.1	62.6	28.7
	V	26.8	28.4	50.7	31.4	25.1	29.5	26.8	43.8	53.1	37.0	32.1	33.0
	L	30.5	28.2	40.9	66.9	26.8	47.7	43.0	43.4	43.2	34.8	38.7	36.2
	I	36.1	29.7	37.8	37.6	16.0	26.8	26.8	31.5	47.2	26.8	33.1	34.3

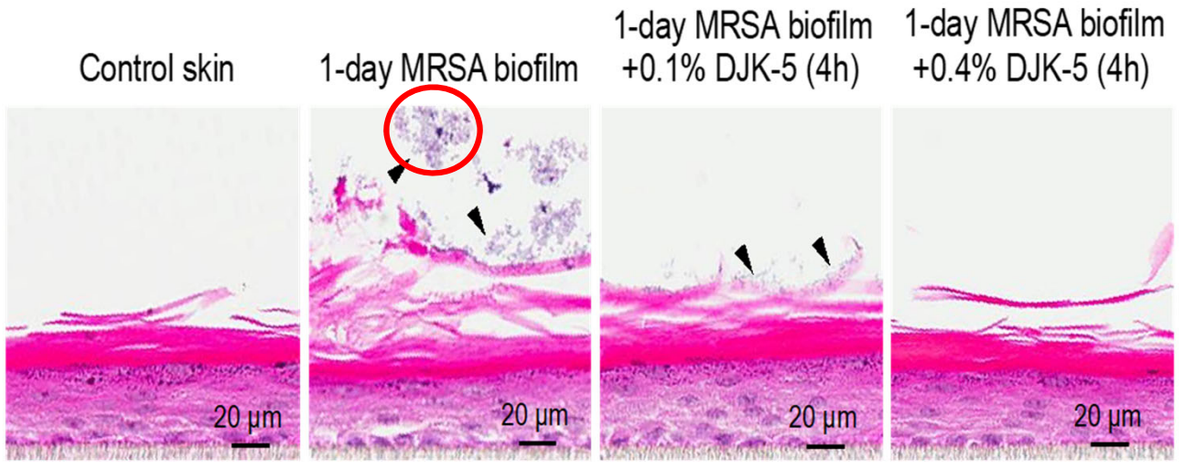
Chemokine induction

MCP1	Substituted Amino Acid	1002 Sequence											
		V	Q	R	W	L	I	V	W	R	I	R	K
Cationic	R	929	2069	807	252	835	328	1317	366	807	519	807	484
	K	2793	681	522	398	962	223	271	826	410	335	800	807
Polar, uncharged	Q	845	807	403	3443	7412	3928	2360	350	2217	444	3855	681
Small, Hydrophobic	G	2319	593	1706	6915	427	237	229	473	1616	604	513	229
	A	2076	1635	566	260	291	450	252	386	1960	884	592	428
Large, Hydrophobic	W	745	1655	688	807	293	309	356	807	366	1263	276	524
	V	807	2152	446	1730	4651	714	807	1313	386	404	466	295
	L	1438	1770	374	966	807	553	532	1524	333	527	1074	644
	I	5640	944	1612	568	2926	807	504	1067	512	807	503	257

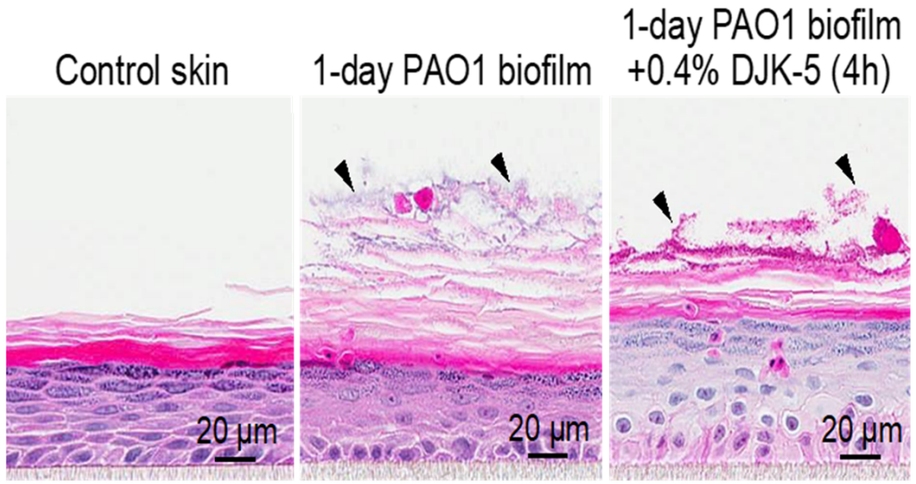
Anti-inflammatory

IL-1β	Substituted Amino Acid	1002 Sequence											
		V	Q	R	W	L	I	V	W	R	I	R	K
Cationic	R	0.47	0.71	0.68	1.12	1.18	1.20	1.21	1.01	0.68	0.87	0.68	0.86
	K	0.40	0.67	0.67	1.18	1.46	1.13	1.25	1.04	0.92	0.93	0.79	0.68
Polar, uncharged	Q	0.65	0.68	0.44	1.16	1.25	1.25	1.24	1.25	1.14	0.98	0.90	0.95
Small, Hydrophobic	G	0.65	0.72	0.63	0.90	1.23	1.06	0.92	0.87	0.99	0.78	0.88	1.04
	A	0.79	0.84	0.62	0.89	0.97	0.99	0.96	1.05	0.95	0.91	1.02	0.98
Large, Hydrophobic	W	0.87	0.71	0.69	0.68	0.97	0.83	0.83	0.68	0.62	0.68	0.28	1.01
	V	0.68	0.82	0.85	1.08	1.01	1.04	0.68	0.71	0.88	0.60	0.24	1.00
	L	1.06	1.01	0.41	0.89	0.68	0.90	0.90	0.69	0.89	0.82	0.21	0.79
	I	0.96	0.80	1.00	1.09	0.79	0.68	0.58	0.92	1.07	0.68	0.32	1.08

Peptides work in a Human Skin Model reducing biofilms and suppressing inflammation

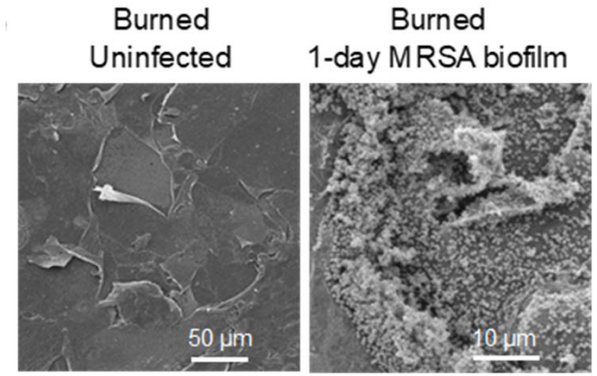


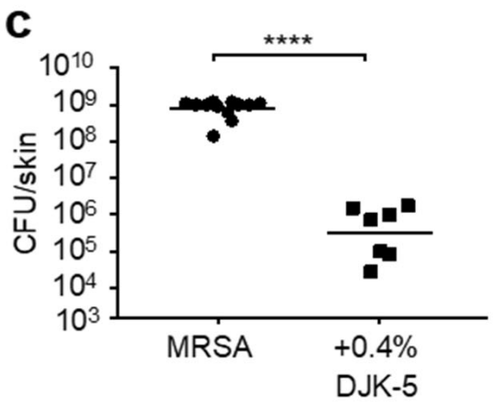
↑ *Staphylococcus aureus*
↓ *Pseudomonas aeruginosa*

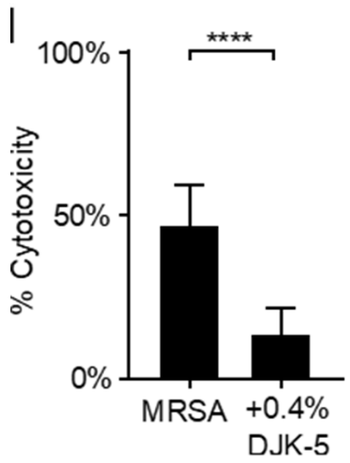


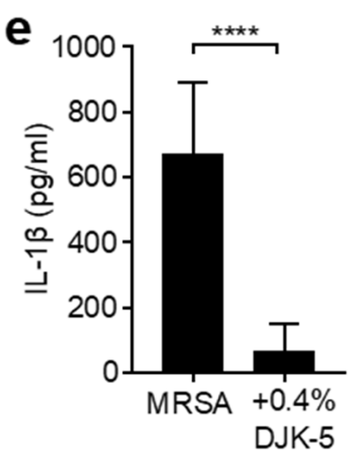
4 sec thermal injury at 100°C

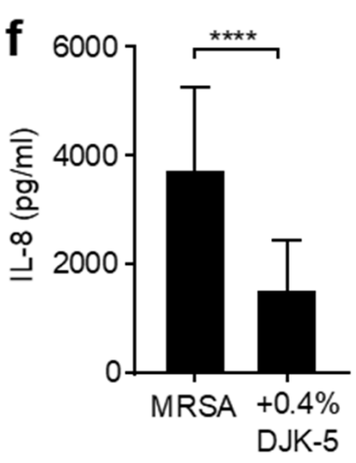
Catherine Wu.
NPJ Biofilms and Microbes in press





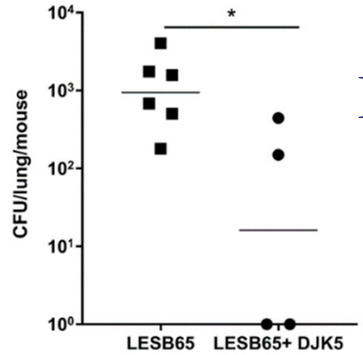






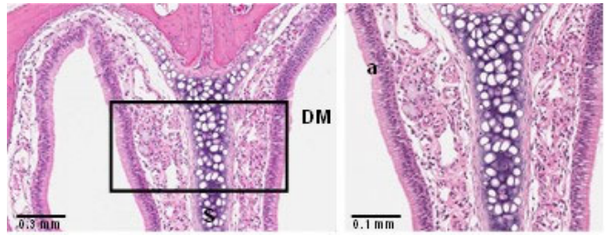
Treating infection plus inflammation: In-vivo chronic lung/sinus infections using LESB65 in alginate

- Sinusitis very common; >29.2 million in USA; est. aggregated cost $8B.
- CRS is caused by a bacterial biofilm infection of the sinus cavity that causes chronic inflammation.
- Antibiotics are used a lot but are <u>not</u> effective for CRS. No approved drugs for management of CRS.



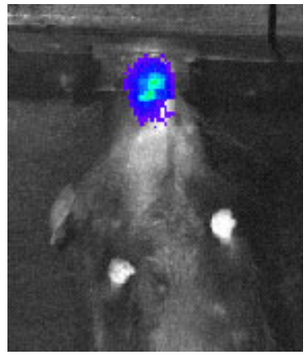
Lung

5 days post-infection

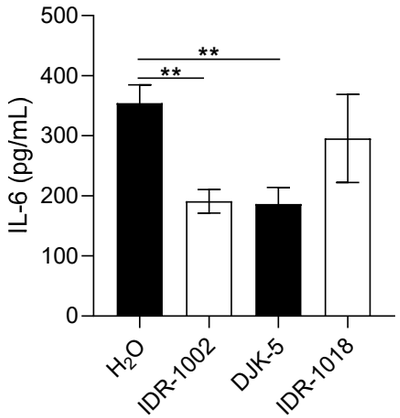


Potent inflammation

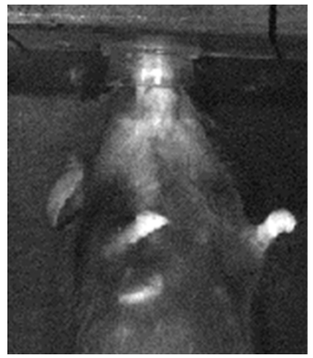
P. aeruginosa (*lux*) LESB65 in alginate



Biofilm infection



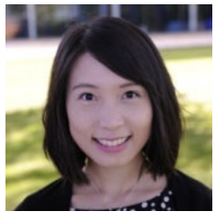
DJK5 treatment (Respimat)



Grace Choi

Morgan Alford

Crispr mutant library methods reveal 185 mutant genes that are required for *Salmonella* infection of macrophages

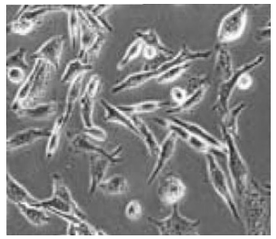


Amy Yeung, Gordon Dougan, Allan Bradley, Chris Hale, Sanger Team. 2019. mBio 10(5):e02169-19,

GeCKOv2 gRNA human library

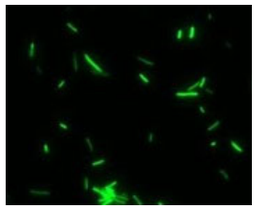
Mutant cells of Cas9-THP-1 monocytes

PMA



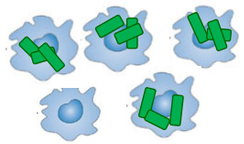
Hi-Seq

\+ **Macrophages**

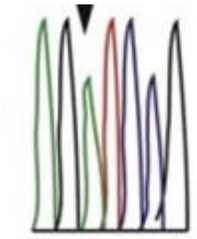


30min infection

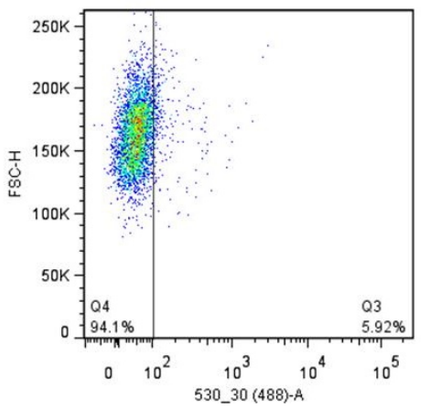
***S.* Typhimurium MOI = 400**



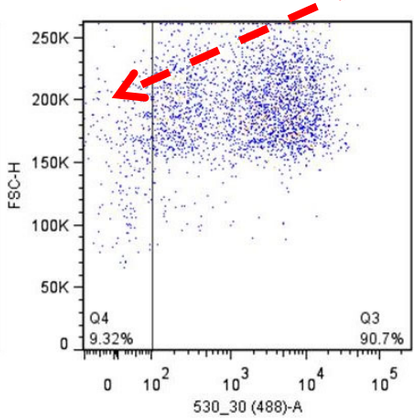
FACS sort resistant macrophages



Hi-Seq to identify gRNAs → Resistance

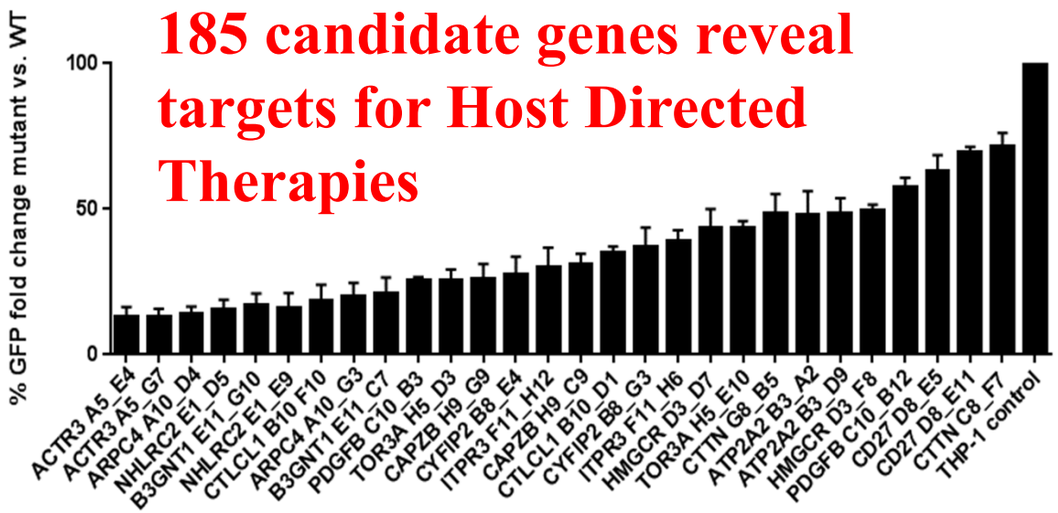


Mutant macrophages - No GFP *Salmonella* (control)

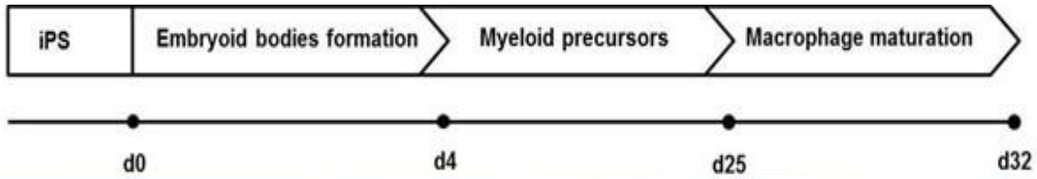


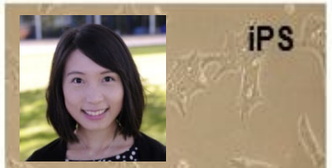
Mutant macrophages + GFP *Salmonella*

185 candidate genes reveal targets for Host Directed Therapies

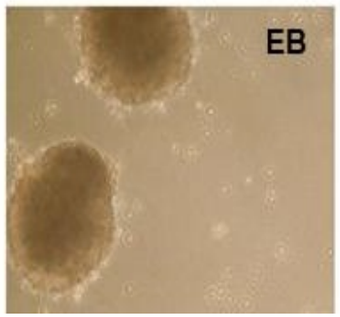


New Model for finding Host-directed therapies: Human stem Cells

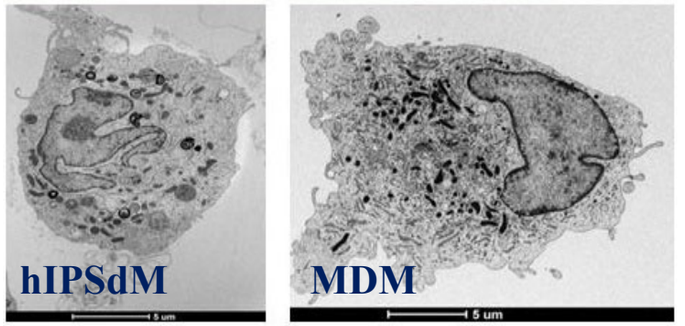


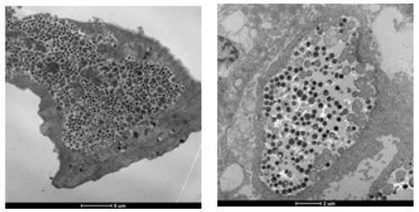


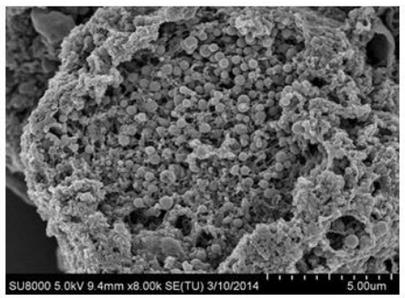
Amy Yeung,
Gordon Dougan,
Sanger Team,
Amy Lee, Erin Gill



Human induced pluripotent Stem cell derived Macrophages (hIPSdM)



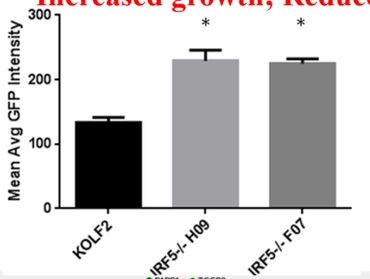


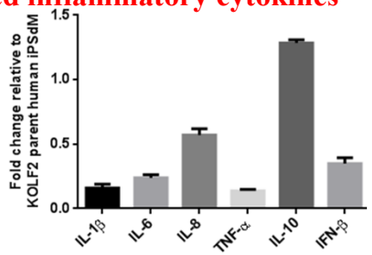


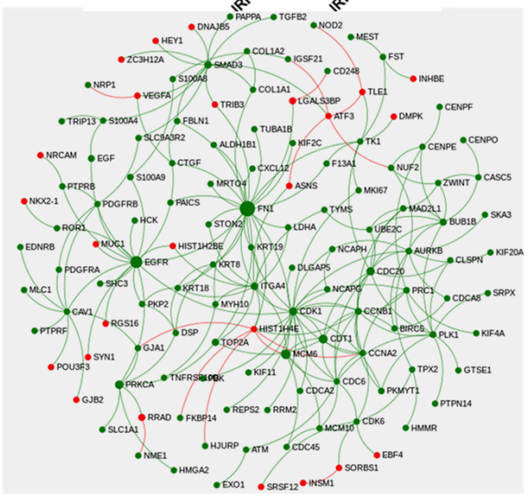
***Chlamydia* infection at 24 hr**

Yeung et al. 2017. Nature Communications 8:15013

Increased growth; Reduced inflammatory cytokines



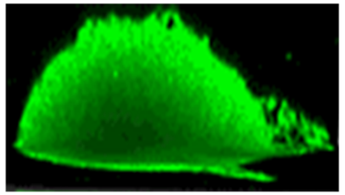




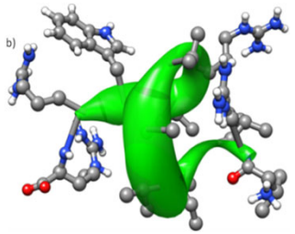
***Chlamydia* → 2,029 genes with altered expression in hIPSdM & human macrophages**

←Effect of IRF5$^{-/-}$ on Gene expression after *Chlamydia* infection; *Amy Lee*

- ***Chlamydia* is the world's leading cause of STD & preventable blindness.**
- **Human *Chlamydia* only grows in human cells; Macrophages involved in pathology**
- **hIPSdM provide a tractable model**
- **Enables study of infection genetics for essential genes/targets (Cas9/CRISPR)**



Conclusions



- We need new anti-infectives. Resistance is very complex. And we are not properly addressing the reasons for antibiotic failure.
- Given our dismal recent success, what if we do not discover new antibiotics?
- Immune modulation as an adjunctive treatment provides significant opportunities. Not discussed - Vaccines, Therapeutic Monoclonal Antibodies

Acknowledgements: Cast of dozens of trainees, Much funding from taxpayers and companies, Many excellent collaborators

Thank you for joining us

Visit now revive.gardp.org/webinars to find more webinars about antimicrobial drug R&D