Università
San Raffaele
Roma

The Airway Battlefield: Host-*Acinetobacter baumannii* Dynamics at the Airway Epithelial Interface

Cecilia Ambrosi PhD

May 13, 2025

Bacterial infections worldwide

Escherichia coli,
Staphylococcus aureus,
Klebsiella pneumoniae,
Streptococcus pneumoniae,
Acinetobacter baumannii,
Pseudomonas aeruginosa,
by order of number of deaths

1.27 million deaths per year are directly attributable to AMR!

Murray et al. *The Lancet* (https://doi.org/10.1016/S0140-6736(21)02724-0), 2021

Petrosillo et al., 2014. Chapter 20 - Acinetobacter Infections,
Editor(s): Önder Ergönül, Füsun Can, Lawrence Madoff, Murat Akova, Emerging Infectious Diseases, Academic Press, 2014

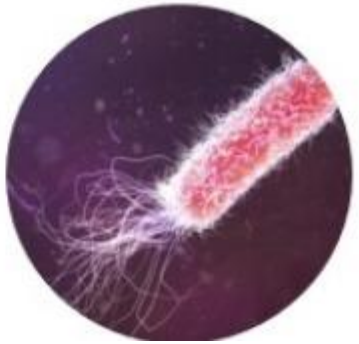
A. baumannii: one of the most dangerous ESKAPE pathogens

Enterococcus faecalis/faecium

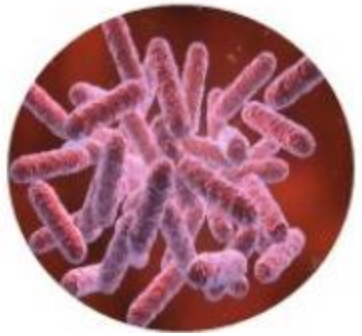
Staphylococcus aureus

Klebsiella pneumoniae

Acinetobacter baumannii



Pseudomonas aeruginosa



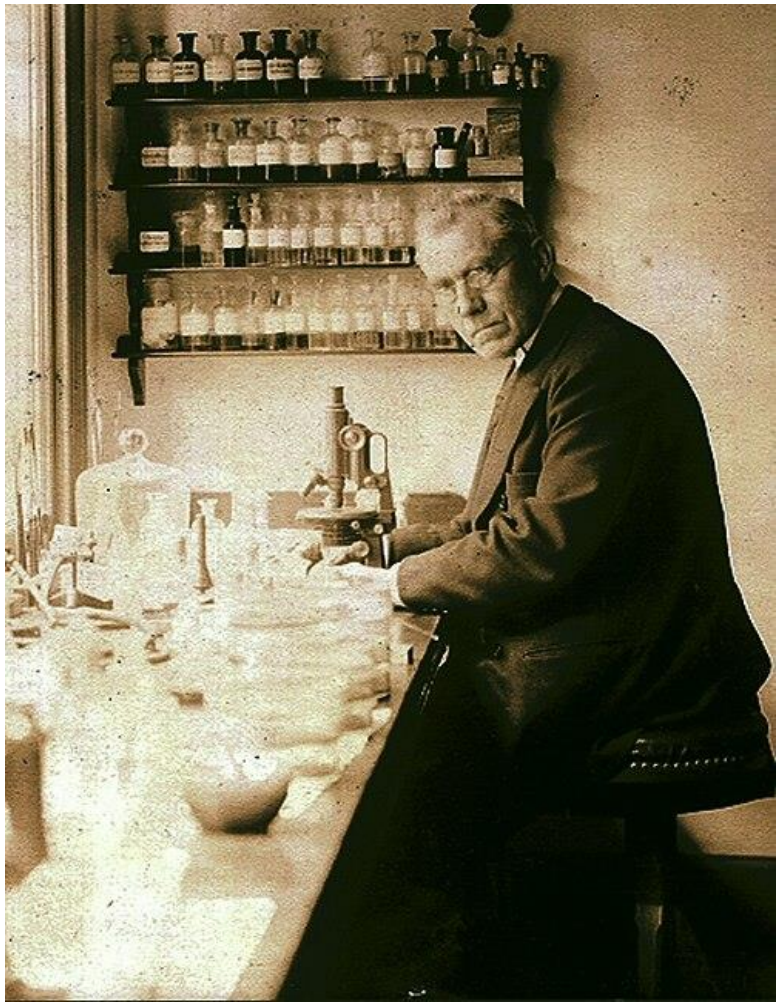
Enterobacter spp.

GRAM +VE

GRAM -VE

A step back: The discovery of *Acinetobacter* spp.

- 1911: Beijerinck discovered the *Acinetobacter* spp. genus using a medium containing calcium acetate → *Micrococcus calcoaceticus*
- 1954*:* Brisou and Prévot renamed the genus *Acinetobacter* spp. (2 species)
- 1968*:* Baumann et al. suggested its inclusion in the *Moraxellaceae* family
- 1986*:* Bouvet and Grimont proposed the definition of genospecies (genomic groups) with 12 genospecies
- Today*:* 108 species have been described, with 83 having validated nomenclature, including synonyms



Martinus Beijerinck

The *Acinetobacter calcoaceticus–Acinetobacter baumannii* complex (ACB complex)

A bunch of very closely related and display similar phenotypic and biochemical properties:

Acinetobacter baumannii
Acinetobacter pittii
Acinetobacter nosocomialis
Acinetobacter seifertii
Acinetobacter dijkshoorniae

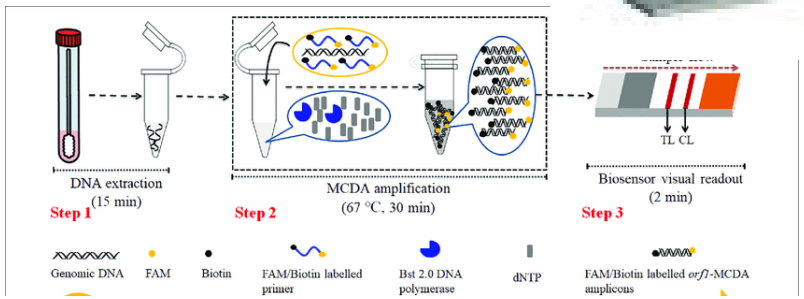
Nosocomial opportunistic pathogens

Acinetobacter calcoaceticus
(environmental species)

Species-Level Identification requires molecular testing

MALDI-TOF MS

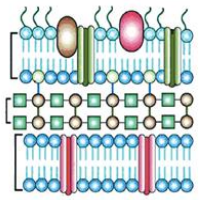
bla$_{OXA-51}$-like gene



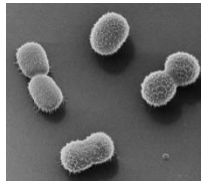
Multiple Cross Displacement Amplification (MCDA) coupled with Lateral Flow Biosensors (LFB) *pgaD* gene

Rangel, K., & De-Simone, S. G. (2024). Treatment and management of Acinetobacter pneumonia: lessons learned from recent world event.r*and drug resistance*, 507-529.

Acinetobacter baumannii: main features



Gram-negative



Coccobacillus in pairs

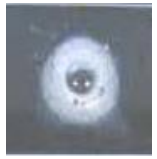
Strictly aerobic

Grows @ 44°C

Non-motile

Non-fermenter

Oxidase negative



Catalase positive

Considered a low-virulence pathogen until the 70's

Today, superbug status with strains resistant to all available antibiotics and a global incidence in healthcare facilities of more than 1,000,000 cases annually


pathogens

Review

Acinetobacter baumannii: An Ancient Commensal with Weapons of a Pathogen

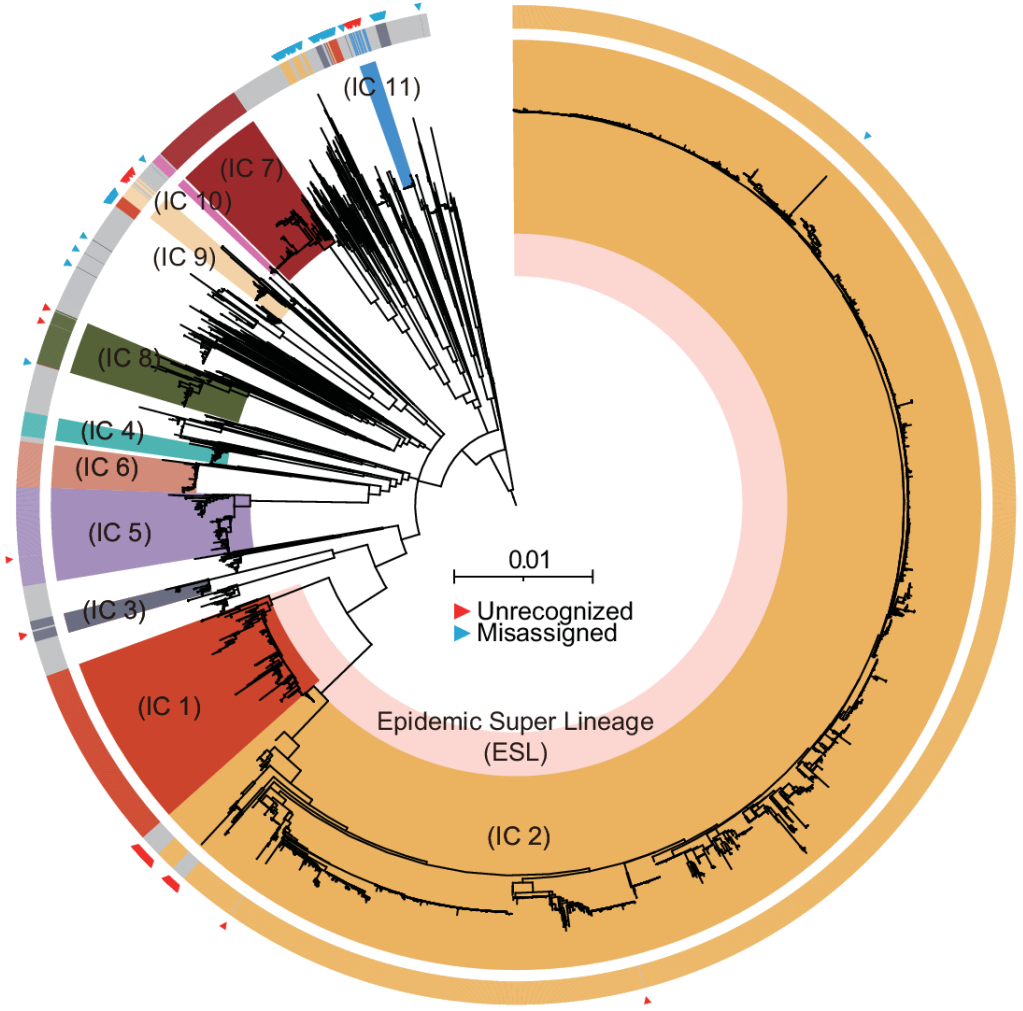

microorganisms

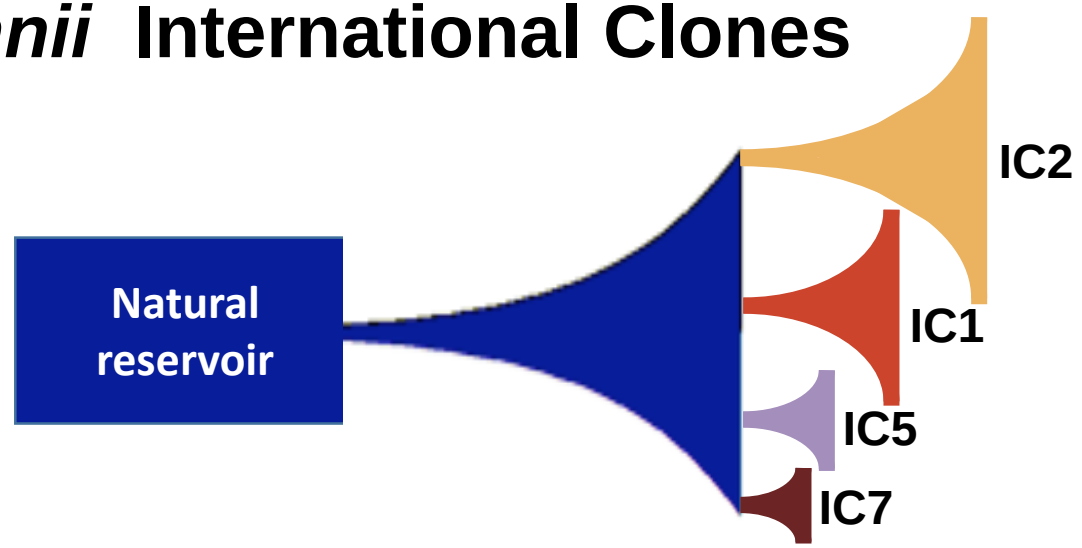
Review

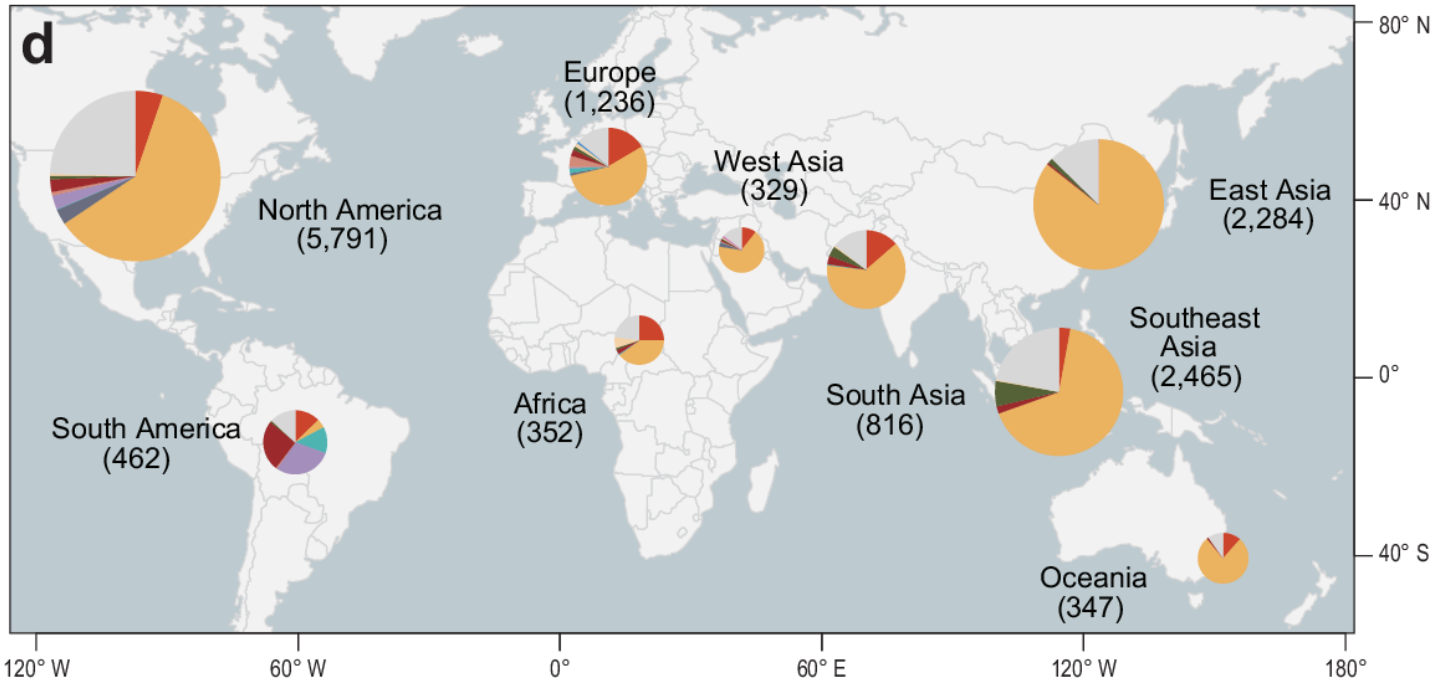
Gram-Negative Bacteria Holding Together in a Biofilm: The *Acinetobacter baumannii* Way

Distribution of *A. baumannii* International Clones

The majority of infections are caused by IC2







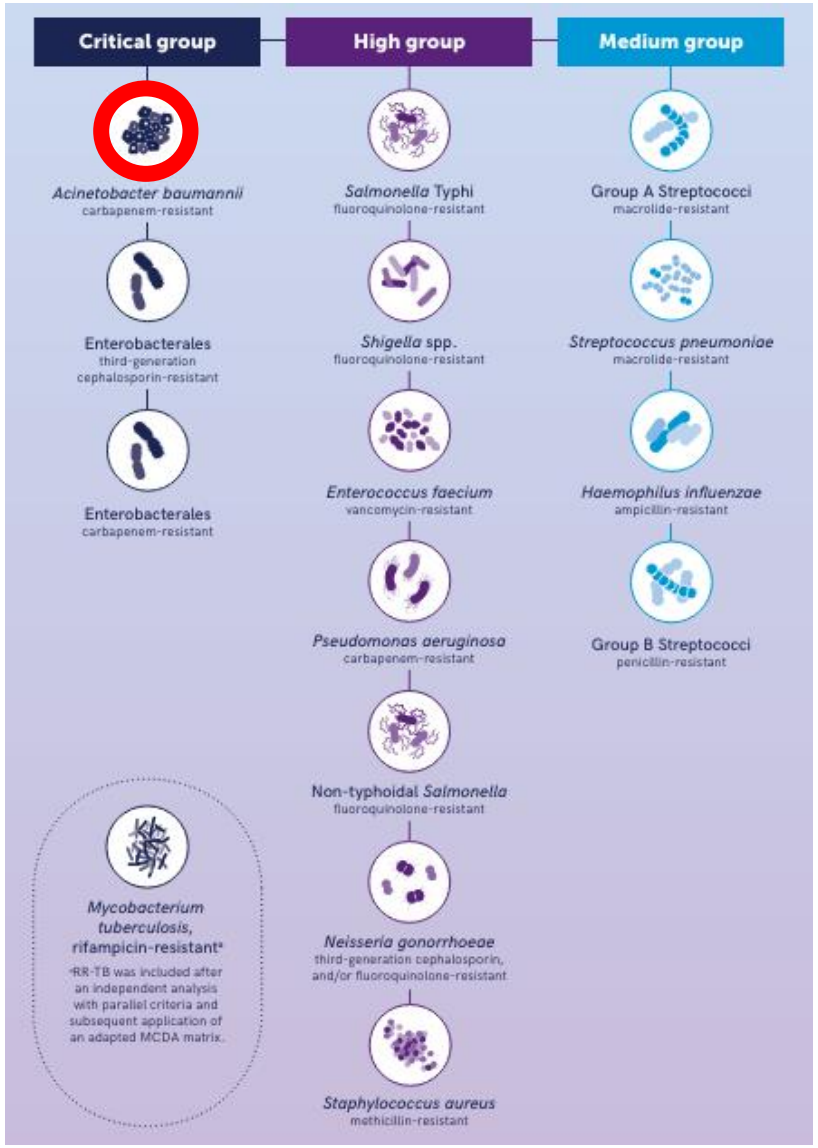
Li, S., Jiang, G., Wang, S. *et al.* Emergence and global spread of a dominant multidrug-resistant clade within *Acinetobacter baumannii*. *Nat Commun* **16**, 2787 (2025).

Carbapenem-resistance: a global concern

World Health Organization

WHO Bacterial Priority Pathogens List, 2024

Bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance



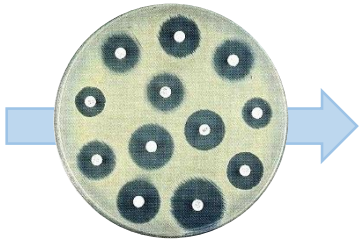
Materiale: Sangue Da CV
Isolati: 1 **Acinetobacter baumannii**

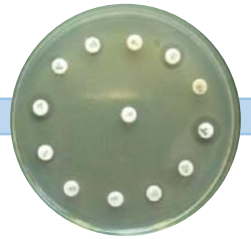
	Acinetobacter baumannii	
Amikacina	>=64	R
Ciprofloxacina	>=4	R
Colistina	<=0.5	S
Gentamicina	>=16	R
Imipenem	>=16	R
Meropenem (altro)	>=16	R
Meropenem Meningitidis	>=16	R
Piperacillina/tazobctam	>=128	
Tobramicina	>=16	R
Trimetoprim/Sulfam.	>=320	R

R:Resistente - I:Sensibile, aumentata esposizione - S:Sensibile
Antibiogramma interpretato secondo i criteri EUCAST

https://iris.who.int/bitstream/handle/10665/376776/9789240093461-eng.pdf?sequence=1

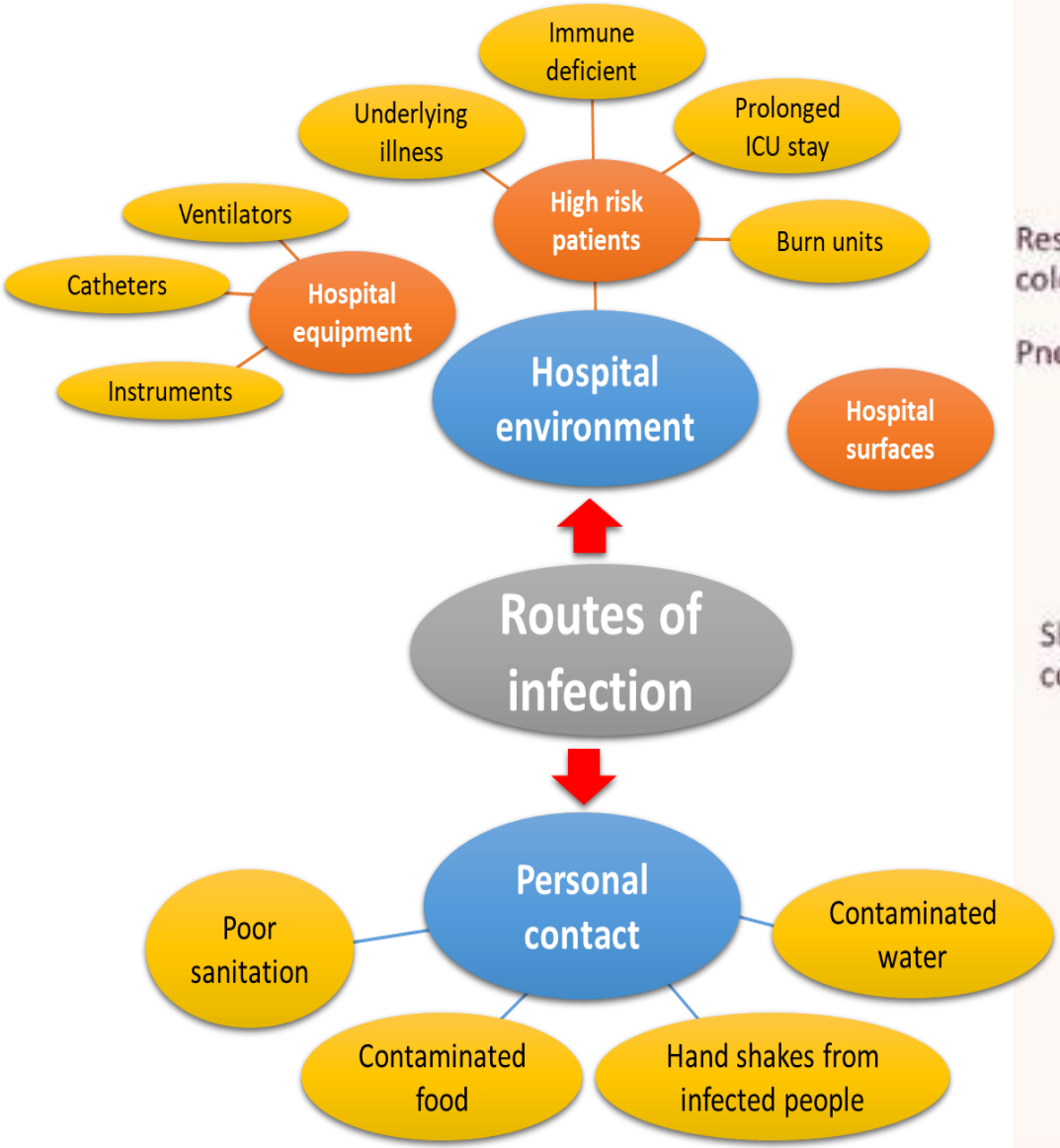
A. baumannii vicious cycle of MDR dissemination

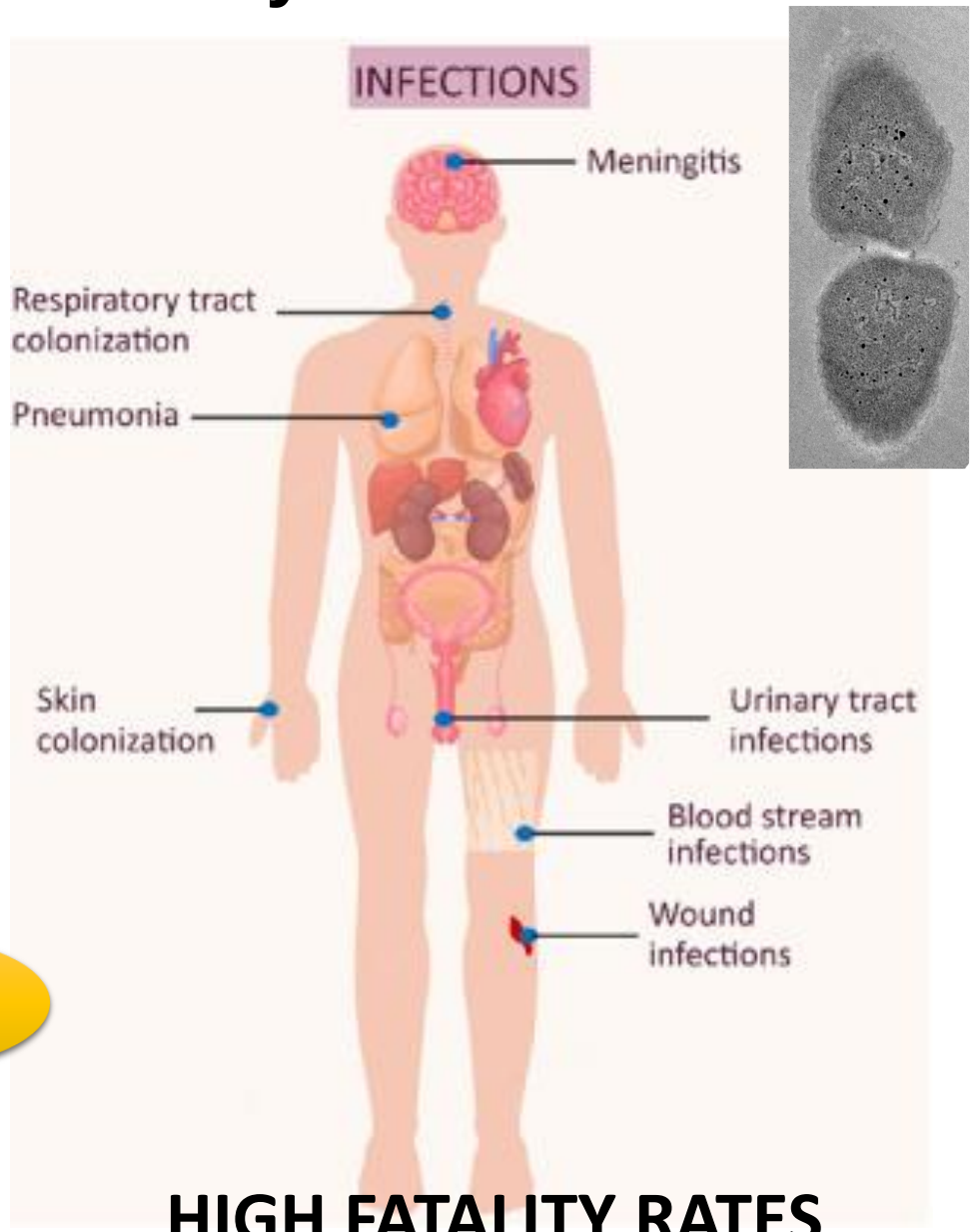




- Ventilators
- Ventilator circuits
- Patient monitors
- X-ray view boxes
- Equipment carts
- Bed rails
- Bedside tables
- Mattress
- Pillow
- Curtains
- Sink
- Floor mops
- Air humidifiers

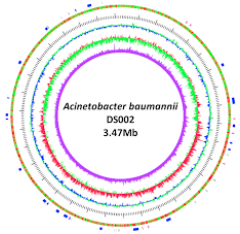
Routes of infections caused by *A. baumannii*



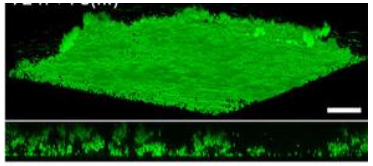


HIGH FATALITY RATES

A. baumannii: A number of virulence factors



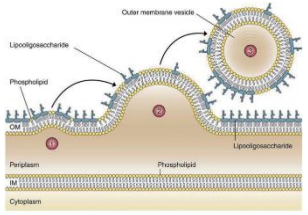
Anti-Microbial Resistance



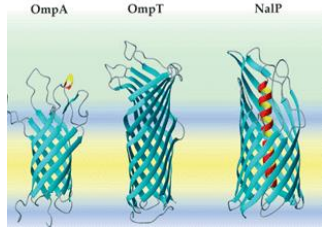
Biofilm

Desiccation Tolerance

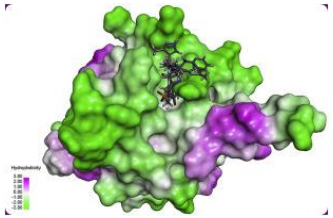
Twitching Motility



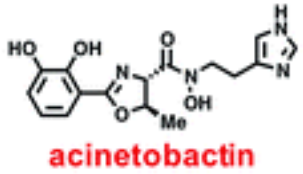
Outer Membrane Vesicles



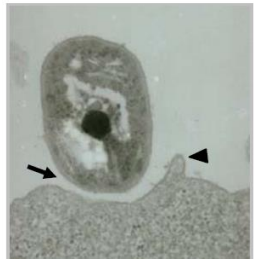
Outer Membrane Proteins



Hydrolytic Enzymes



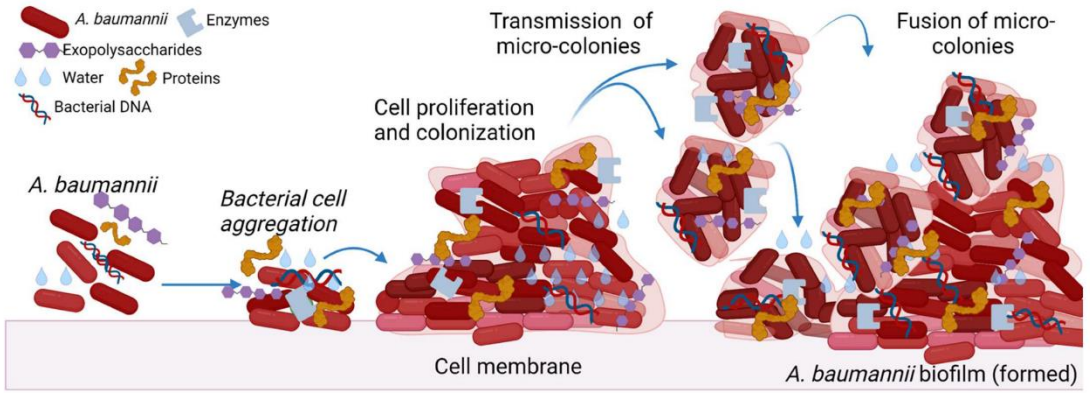
Iron Uptake Systems

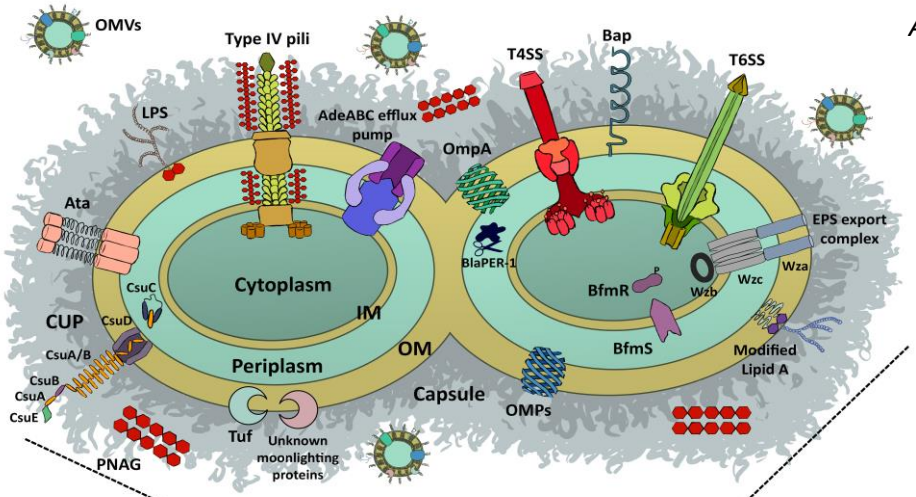


Host Cell Adhesion

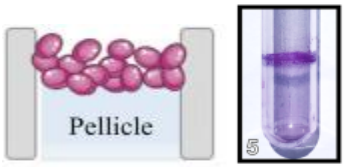
A. baumannii: A biofilm former

- Bap, biofilm-associated protein
- Ata, autotransporter
- Classical chaperone-usher pili
- Nonclassical chaperone-usher pili, type IV pili
- PNAG, poly-(1-6)-N-acetylglucosamine
- Tuf, moonlighting protein;
- OmpA
- AbaI (sensor & synthase) AbaR (regulator)





At the air–liquid interface

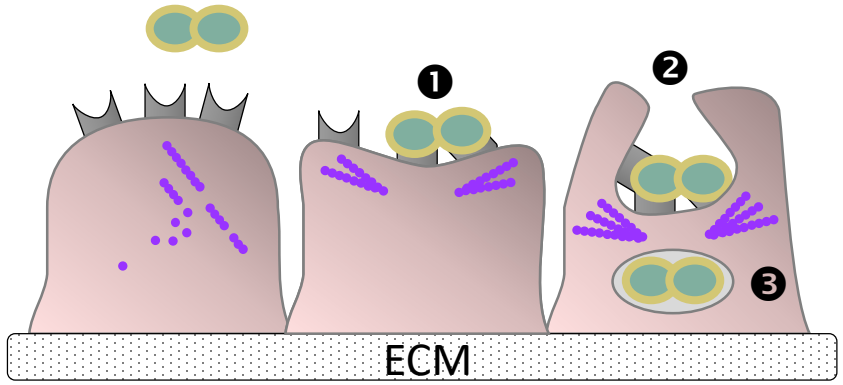


On solid surfaces

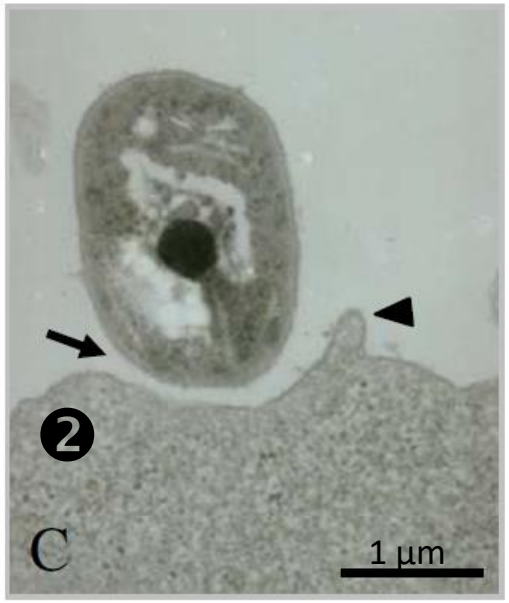
A. baumannii can adhere to & invade epithelial host cells

ZIPPER-LIKE MECHANISM

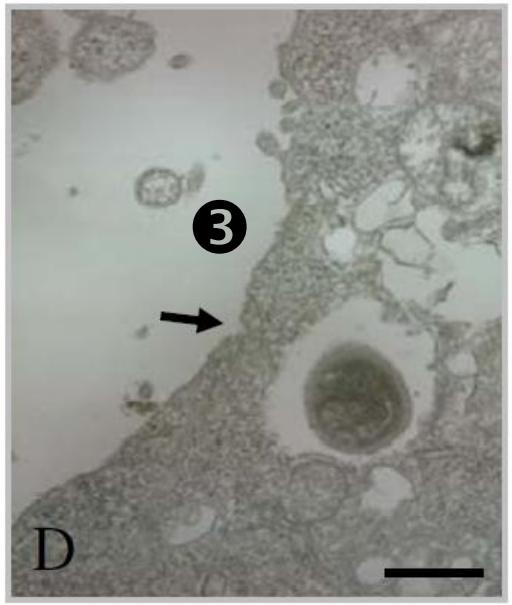
❶ Receptor-binding
❷ Membrane engulfment
❸ Endosomal trafficking

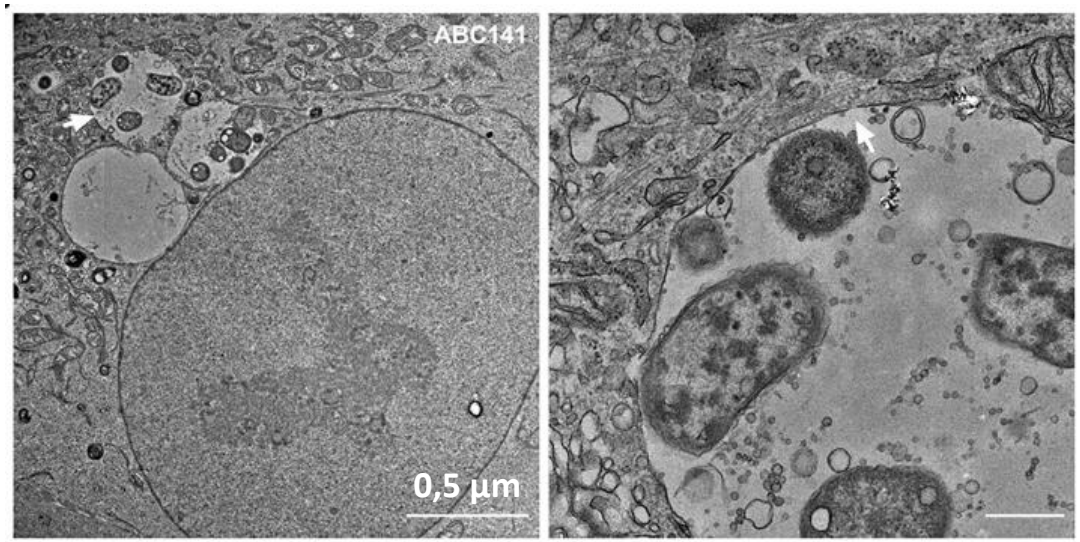


Receptor
A. baumannii
G- & F-actin

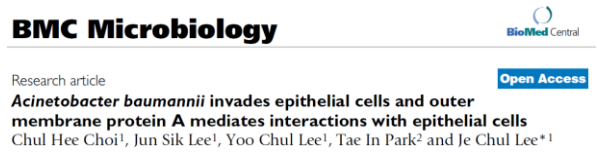


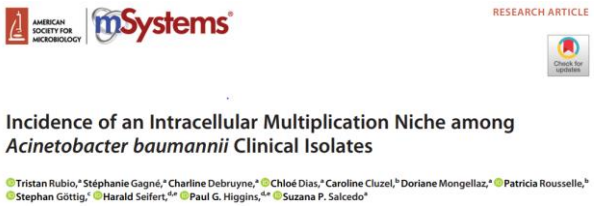
Human bronchial NCI-H292 cells





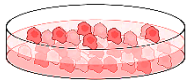
Human lung A549 cells

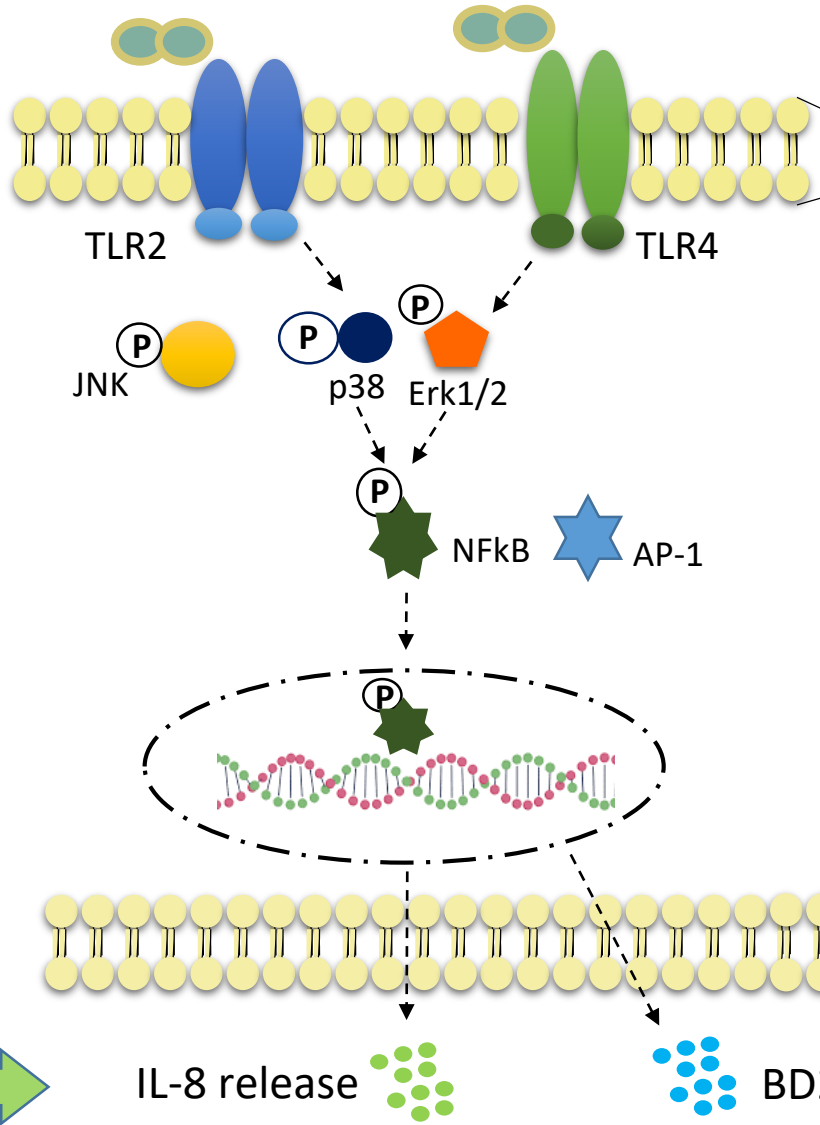

BMC Microbiology
BioMed Central
Research article
Open Access
Acinetobacter baumannii invades epithelial cells and outer membrane protein A mediates interactions with epithelial cells
Chul Hee Choi[1], Jun Sik Lee[1], Yoo Chul Lee[1], Tae In Park[2] and Je Chul Lee*[1]

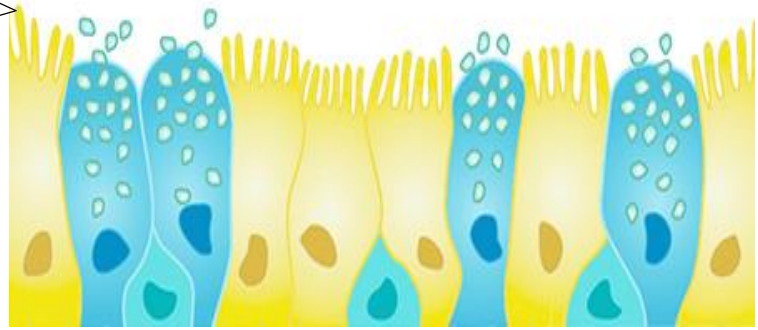

mSystems
RESEARCH ARTICLE
Incidence of an Intracellular Multiplication Niche among *Acinetobacter baumannii* Clinical Isolates
Tristan Rubio,[a] Stéphanie Gagné,[a] Charline Debruyne,[a] Chloé Dias,[a] Caroline Cluzel,[b] Doriane Mongellaz,[a] Patricia Rousselle,[b] Stephan Göttig,[c] Harald Seifert,[d,e] Paul G. Higgins,[d,e] Suzana P. Salcedo[a]

A. baumannii interacts with Toll-like receptors 2 & 4

Human lung epithelial cells
(A549 cell line)







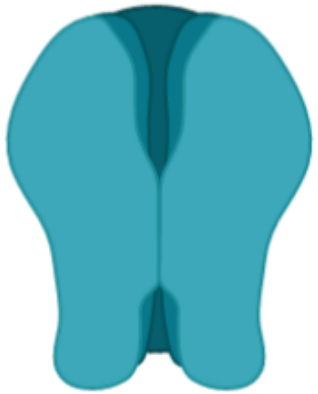
OPEN ACCESS Freely available online PLoS one

Dissection of Host Cell Signal Transduction during *Acinetobacter baumannii* – Triggered Inflammatory Response

Catalina March[1,2], Verónica Regueiro[1,2], Enrique Llobet[1,2], David Moranta[1,2], Pau Morey[1,2], Junkal Garmendia[1,2,3], José A Bengoechea[1,2,4,5]*

Host receptors for bacterial adhesion

CFTR
cystic fibrosis transmembrane conductance regulator



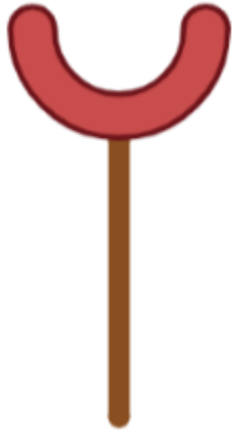
Fibronectin

Collagen type IV

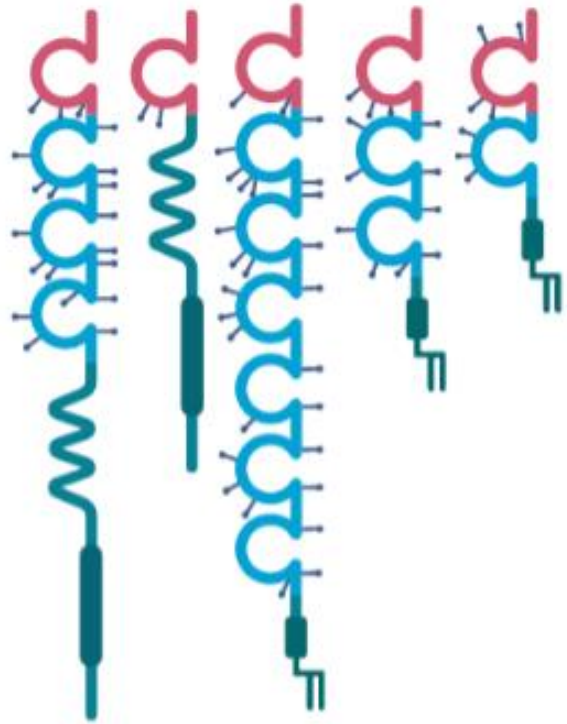
DAF
decay accelerating factor

PAFR
platelet-activating factor receptor

Gp96
heat shock protein gp96



CEACAMs
carcinoembryonic antigen related cell adhesion molecules

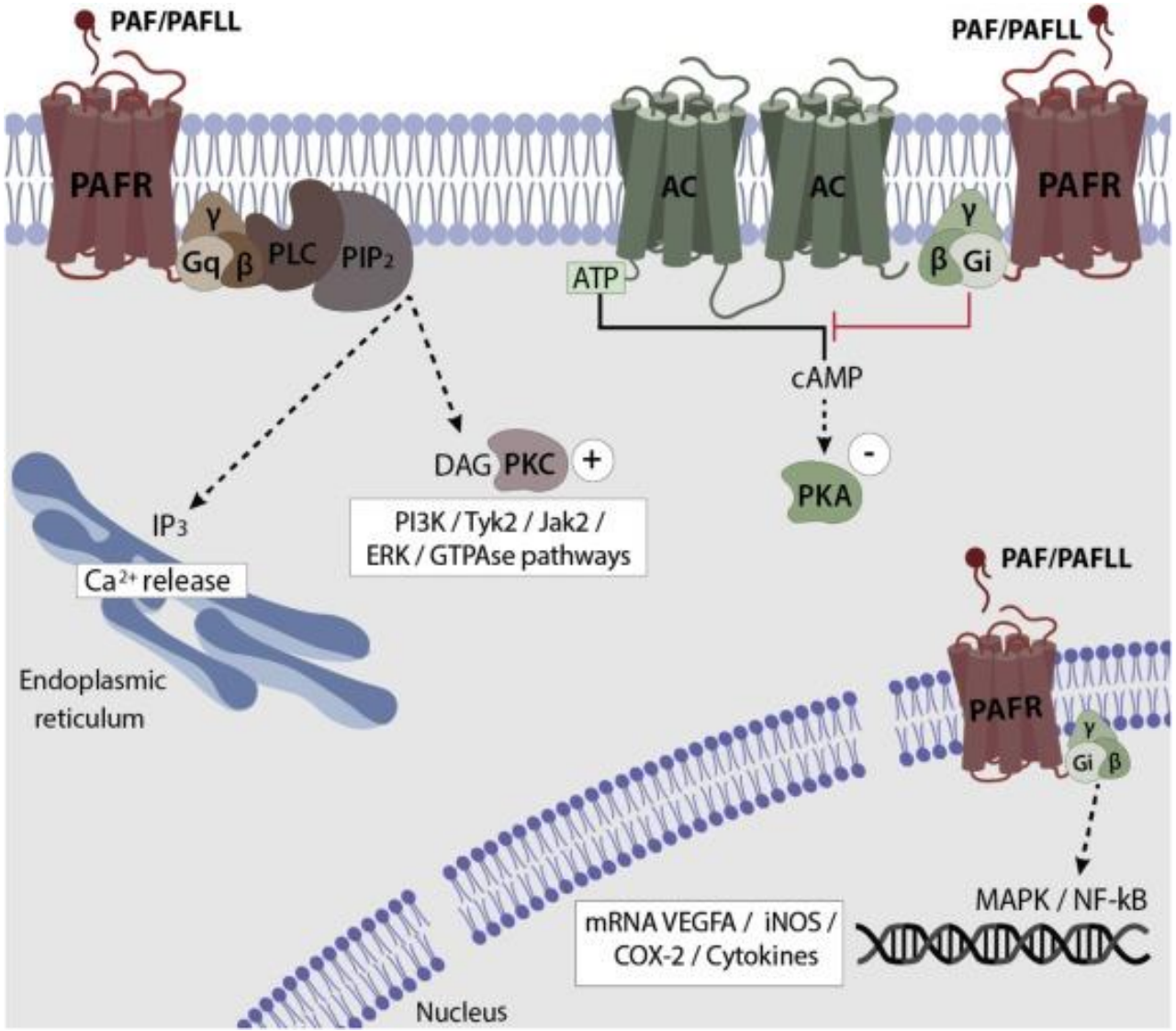


MICROBIOLOGY REVIEW MICROBIOLOGY SOCIETY

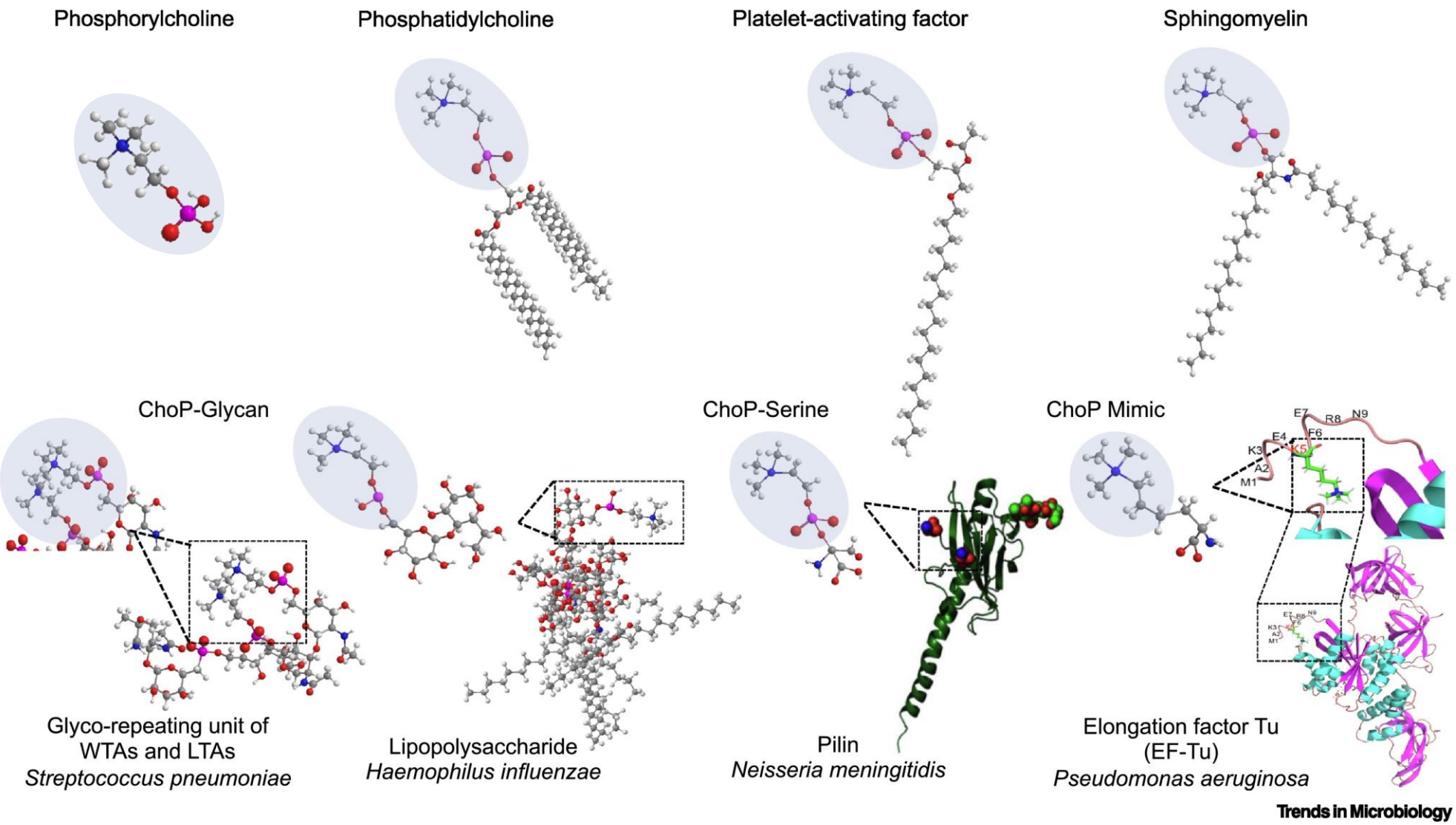
Temporal upregulation of host surface receptors provides a window of opportunity for bacterial adhesion and disease

Rajendra Kc,[1] Shakti D. Shukla,[2,3] Eugene H. Walters[1] and Ronan F. O'Toole[1,*]

Platelet-activating factor (PAF): A phospholipid mediator of inflammation



Phosphorylcholine (ChoP): A phosphate bonded to a choline group

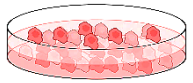


Zhang, Y., Jen, F. E. C., Fox, K. L., Edwards, J. L., & Jennings, M. P. (2023). The biosynthesis and role of phosphorylcholine in pathogenic and nonpathogenic bacteria. *Trends in microbiology*, *31*(7), 692-706.

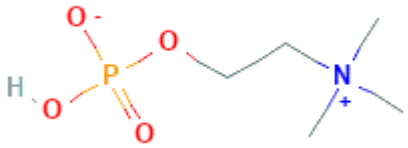
A. baumannii interacts with PAFR

PAFR
platelet-activating factor receptor

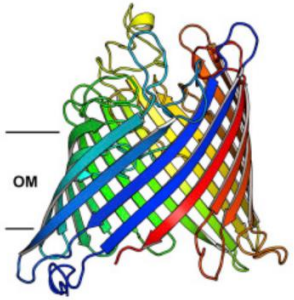
Human lung epithelial cells (A549 cell line)



Phosphorylcholine (ChoP)



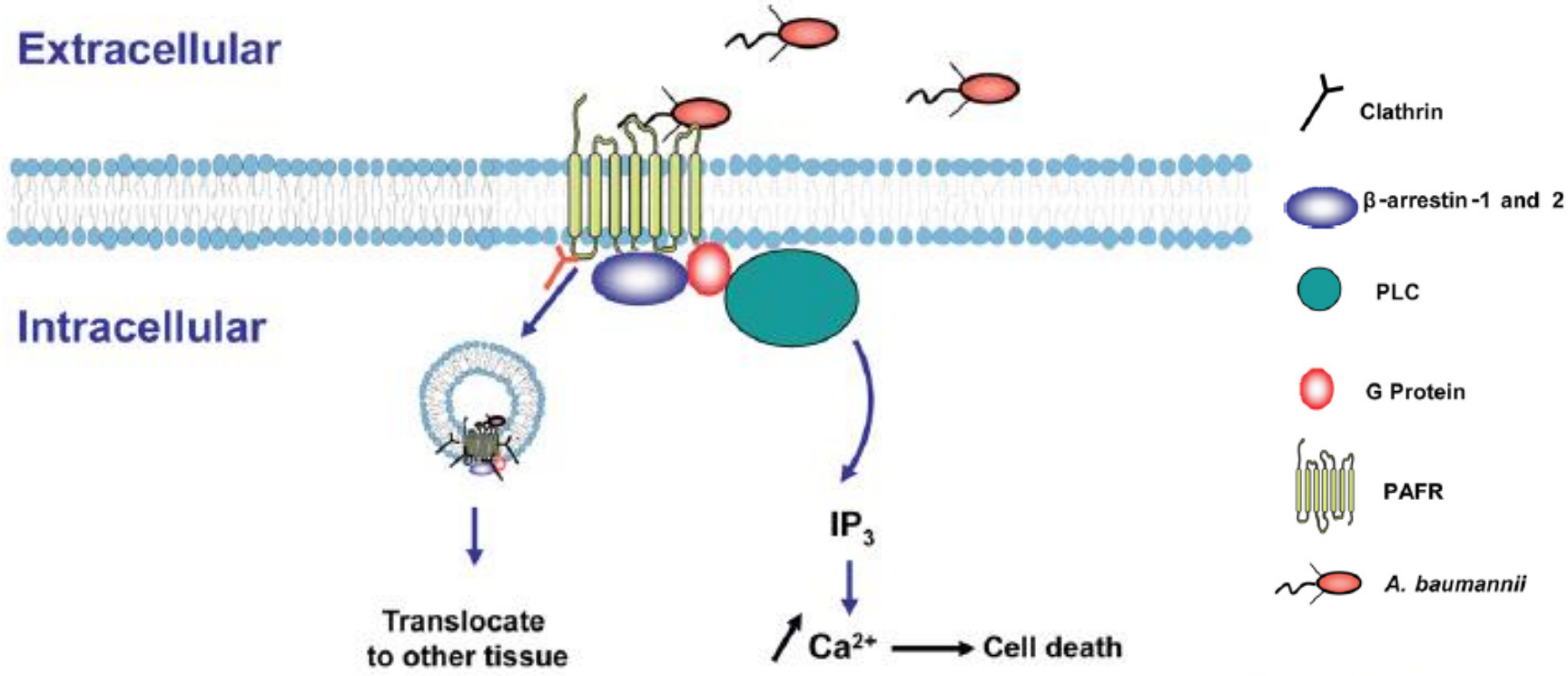
OccAB1/OprD



Structure

Structural Insights into Outer Membrane Permeability of *Acinetobacter baumannii*

Michael Zahn,
Satya Prathyusha Bhamidimarri,
Arnaud Baslé, Mathias Winterhalter,
Bert van den Berg



THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 287, NO. 32, pp. 26901–26910, August 3, 2012

Platelet-activating Factor Receptor Initiates Contact of *Acinetobacter baumannii* Expressing Phosphorylcholine with Host Cells

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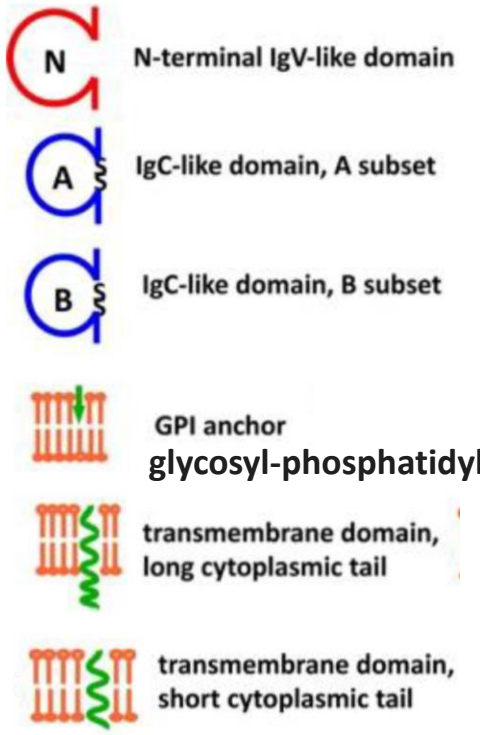
Younes Smani, Fernando Docobo-Pérez, Rafael López-Rojas, Juan Domínguez-Herrera, José Ibáñez-Martínez, and Jerónimo Pachón

Carcinoembryonic antigen-related cell adhesion molecules (CEACAMs)

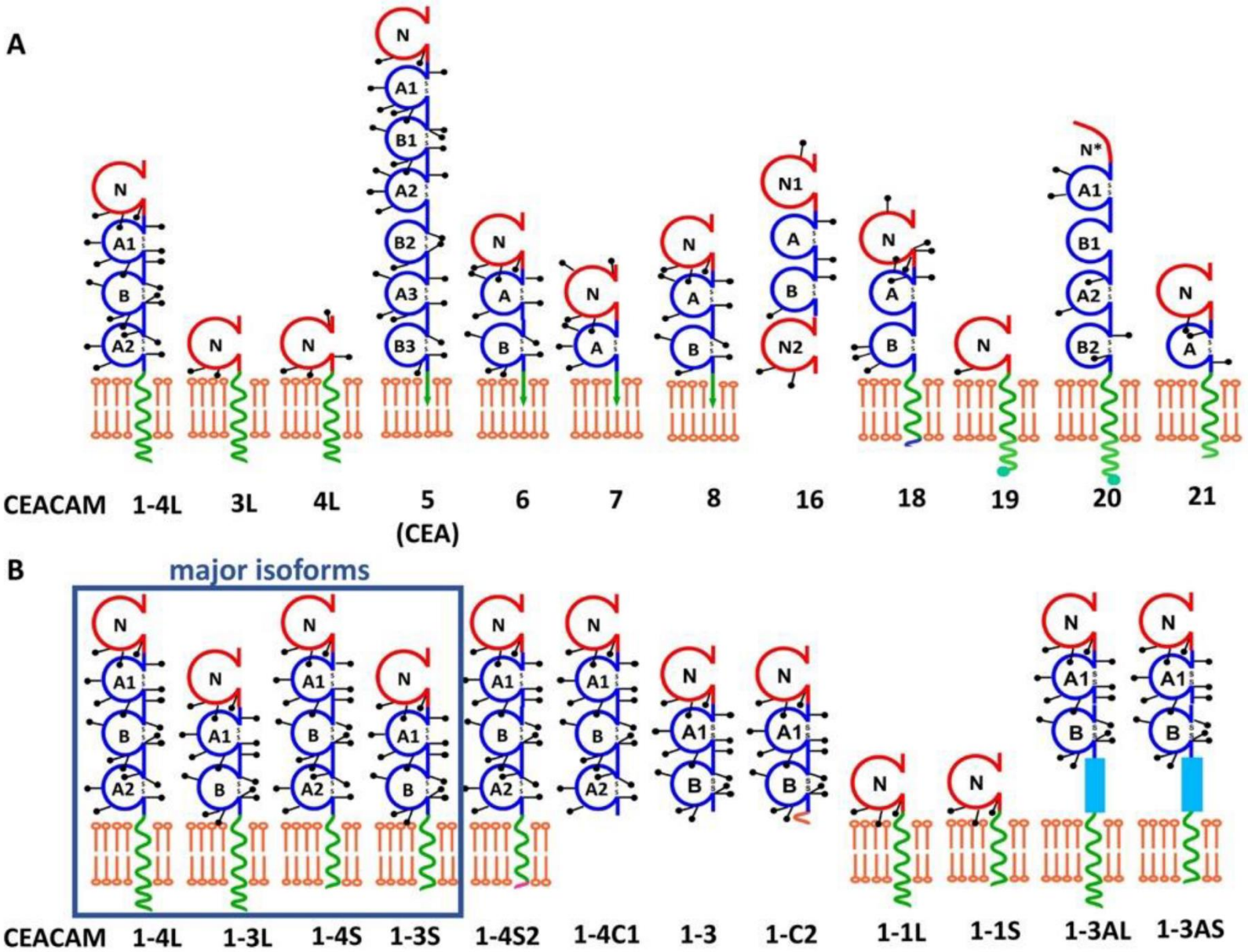
A subgroup of the CEA family of immunoglobulin-related proteins, encoded in the human genome by 12 genes that affect various normal and pathogenic processes associated with cellular growth and differentiation.

Name & Alternative name	CEACAM1 (CD66a, BGP, C-CAM)	CEACAM3 (CD66D, CEA, CGM1, W264, W282)	CEACAM4 (CGM7)	CEACAM5 (CD66e, CEA)	CEACAM6 (CD66c, NCA)	CEACAM7 (CGM2)	CEACAM8 (CD66b, CGM6)	CEACAM16 (CEAL2, DFNA4B, DFNB113)	CEACAM18	CEACAM19 (CEACM19 CEAL1)	CEACAM20 (UNQ9366)	CEACAM21 (CEACAM3R 29124_1)
Tissues/cells	**Epithelial cells, endothelial cells, monocytes, granulocytes, act.T/B cells, myeloid cells, NK**	Granulocytes	Primary human granulocytes	**Epithelial cells**	Granulocytes, **epithelial cells,** vascular endothelial cells, monocytes	**Epithelial cells**	Neutrophils	A non-collagenous protein of the tectorial membrane	Variety of tissues	Variety of tissues	Microvilli of the brush boarder in the epithelial cells	Variety of tissues
Function(s)	Tumor suppression, immune regulation, angiogenesis, insulin clear	Neutrophil-specific receptor	Involving immune and proliferation-related pathways	Intercellular contact(with CEA CAM6), cell adhesion migration	Intercellular contact (with CEACAM 5, 8) neutrophil adhesion	Involving inflammatory response, biomarker of tumors	Intercellular contact (with CEACAM6), immune regulation	Structure of the cochlea essential for normal hearing	Tumor suppression			

The complex structure of CEACAM proteins



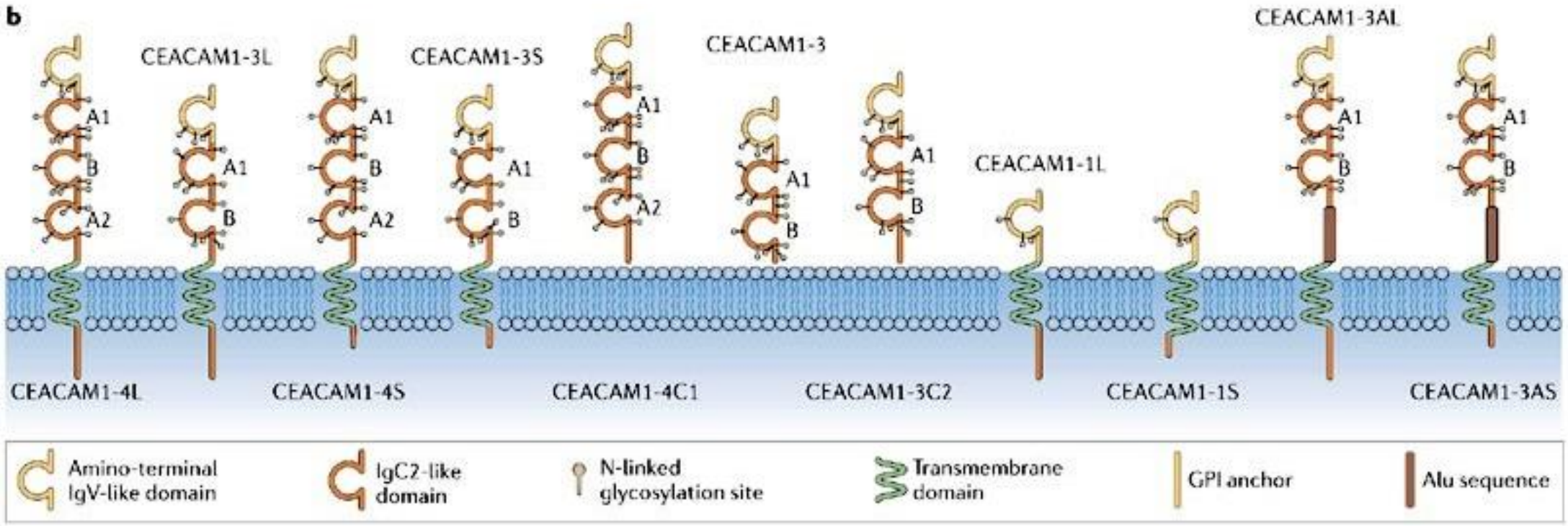
Spikes on the extracellular domain represent glycosylation sites



International Journal of Molecular Sciences
MDPI
Review
CEACAM1 in Liver Injury, Metabolic and Immune Regulation
Andrea Kristina Horst [1,*,†], Sonia M. Najjar [2,3,†], Christoph Wagener [4] and Gisa Tiegs [1]

The 11th different isoforms of CEACAM1

CEACAM1: 9 exons that can be alternatively spliced



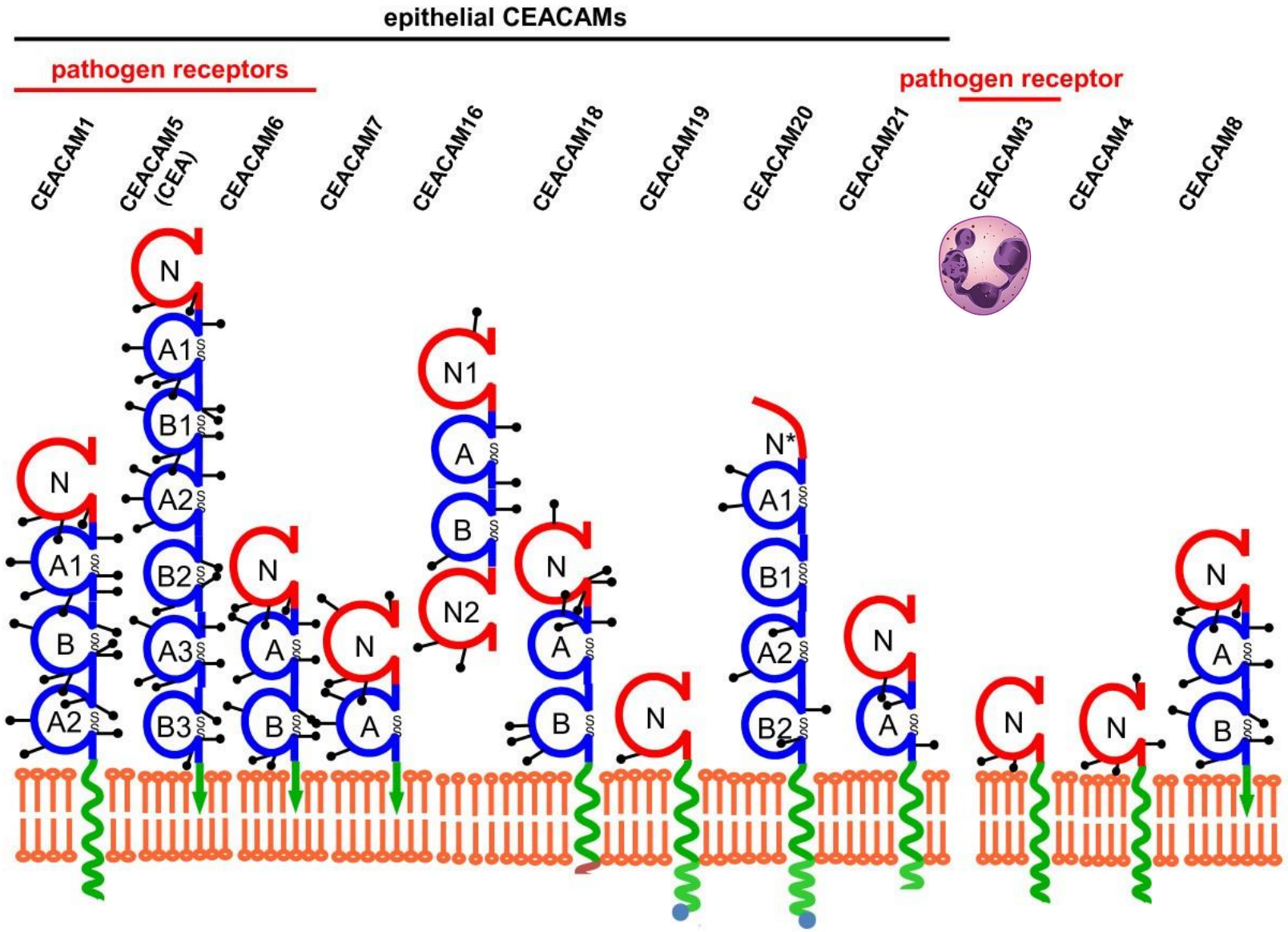
ITIM, immune-receptor tyrosine-based inhibitory motif that provides intracellular inhibitory signaling

REVIEWS

CEACAM1: contact-dependent control of immunity

Scott D. Gray-Owen* and Richard S. Blumberg‡

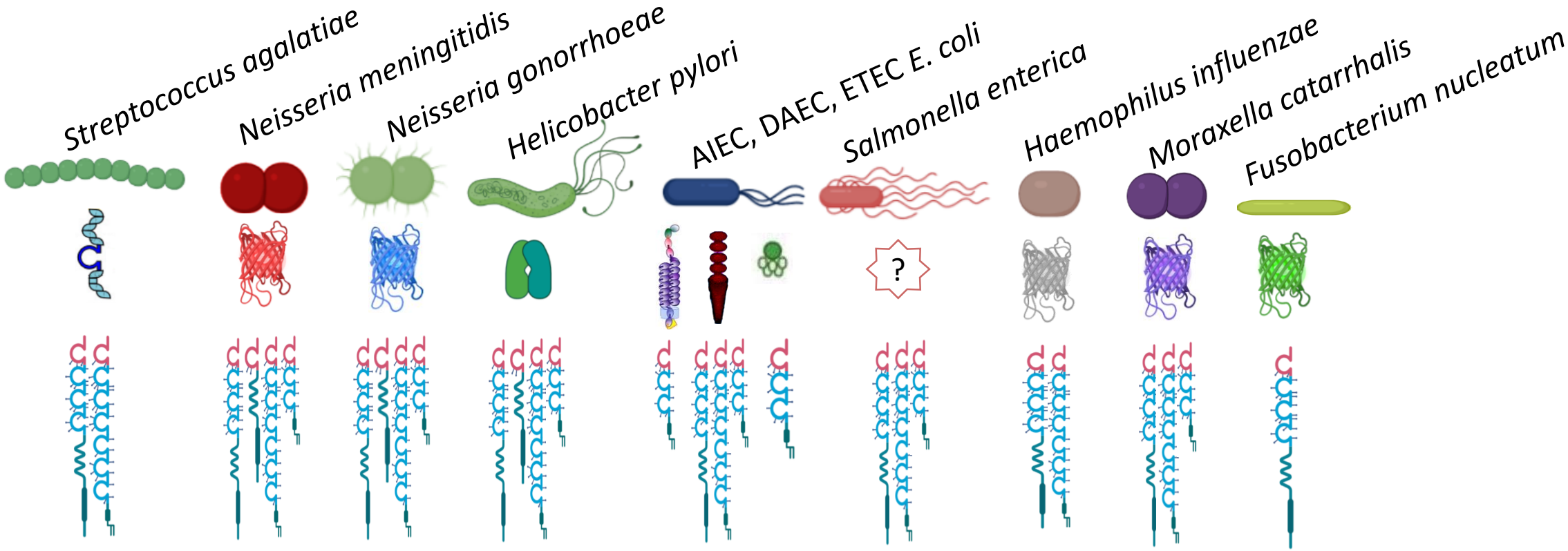
Pathogenic & non-pathogenic bacteria exploit CEACAMs during mucosal colonization



REVIEW Open Access

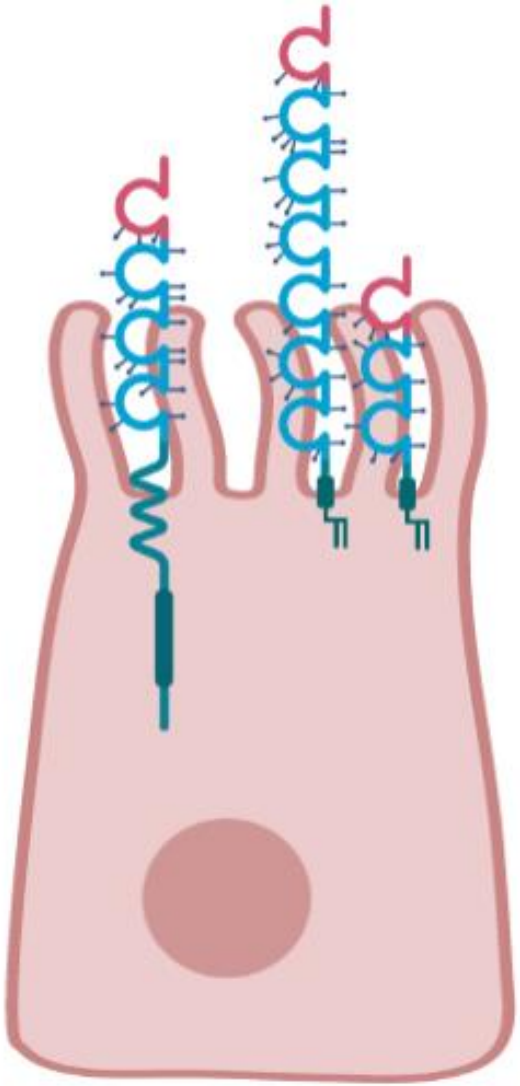
Signaling by epithelial members of the CEACAM family – mucosal docking sites for pathogenic bacteria

CEACAM family members are microbial targets



Many, if not most, bacteria probably use one or more adhesins to colonize host cells

Why CEACAM family members are microbial targets?



- Prominent surface exposure on the host cell
- Wide range of hosts due to their structural conservation
- Bacterial internalization
- Manipulation of host immune responses

Genes & Immunity www.nature.com/gene

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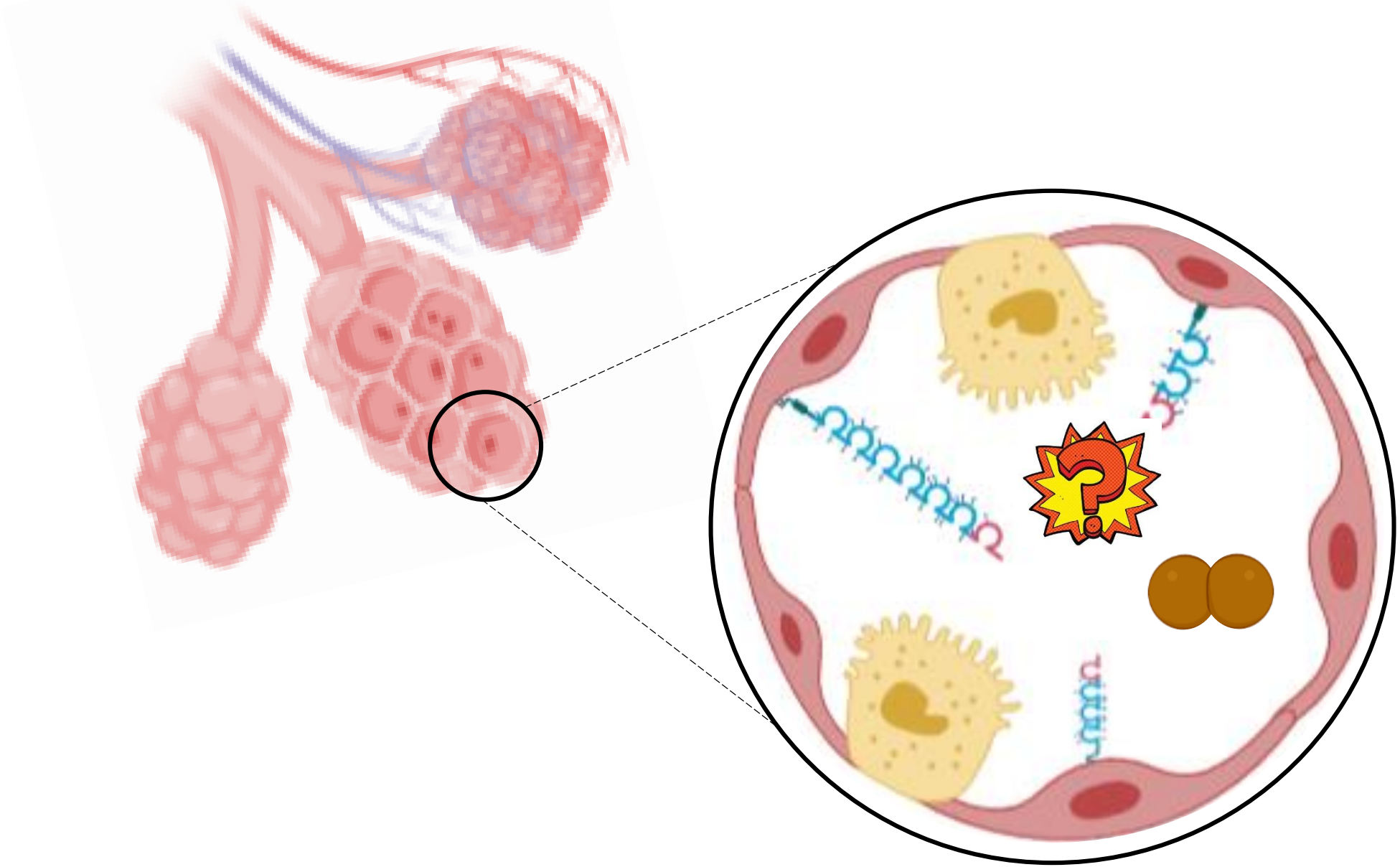
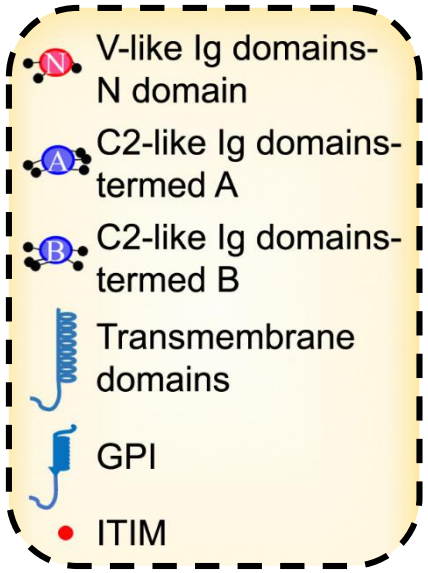
Microscale communication between bacterial pathogens and the host epithelium

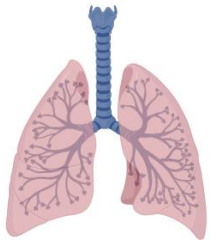
Ann-Kathrin Mix[1], Griseldis Goob[1], Erik Sontowski[1] and Christof R. Hauck[1,2]✉

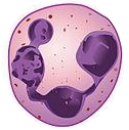
Does also *A. baumannii* bind to CEACAMs?

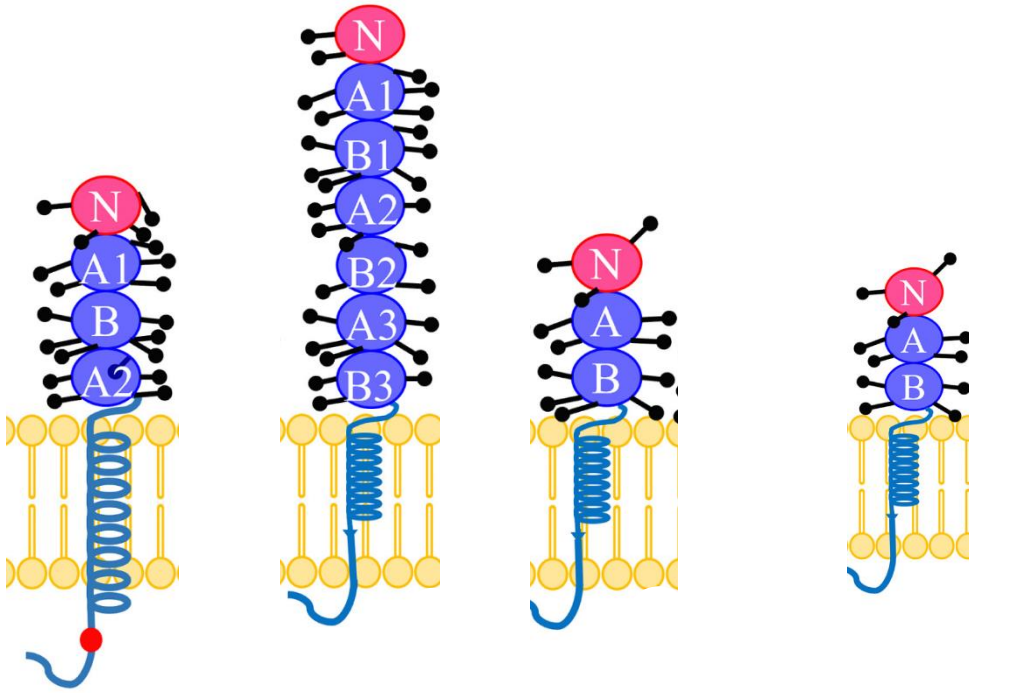


Schematic summary of our experimental plan









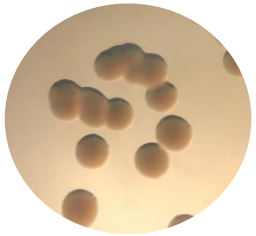
CEACAM1 CEACAM5 CEACAM6 CEACAM8

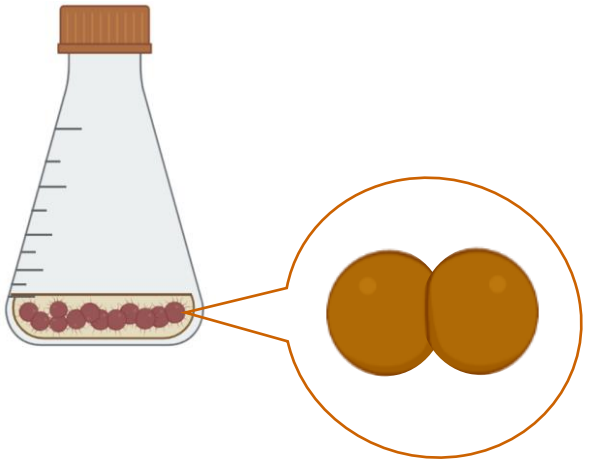
A. baumannii AB5075

RESEARCH ARTICLE

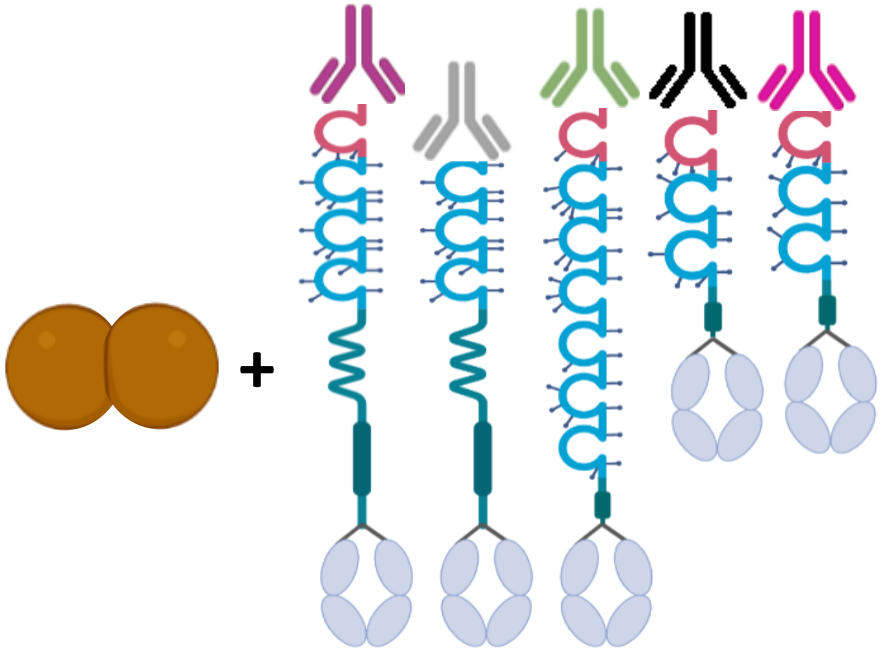
AB5075, a Highly Virulent Isolate of *Acinetobacter baumannii*, as a Model Strain for the Evaluation of Pathogenesis and Antimicrobial Treatments

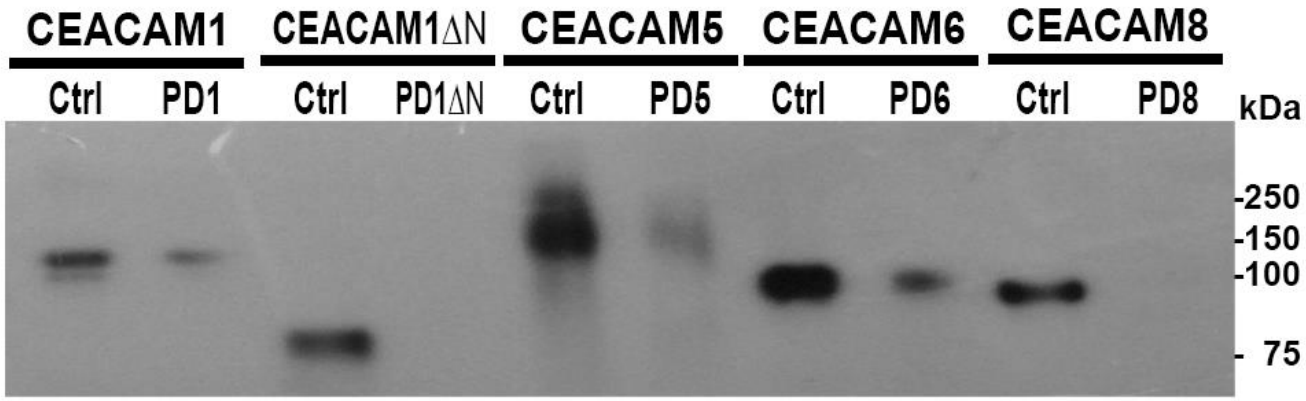
Anna C. Jacobs,[a] Mitchell G. Thompson,[a] Chad C. Black,[a] Jennifer L. Kessler,[a] Lily P. Clark,[a] Christin N. McQueary,[a] Hanan Y. Gancz,[a] Brendan W. Corey,[a] Jay K. Moon,[a] Yuanzheng Si,[a] Matthew T. Owen,[b] Justin D. Hallock,[b] Yoon I. Kwak,[c] Amy Summers,[a] Charles Z. Li,[a] David A. Rasko,[d] William F. Penwell,[e] Cary L. Honnold,[f] Matthew C. Wise,[f] Paige E. Waterman,[c] Emil P. Lesho,[c] Rena L. Stewart,[b] Luis A. Actis,[e] Thomas J. Palys,[a] David W. Craft,[a] Daniel V. Zurawski[a]

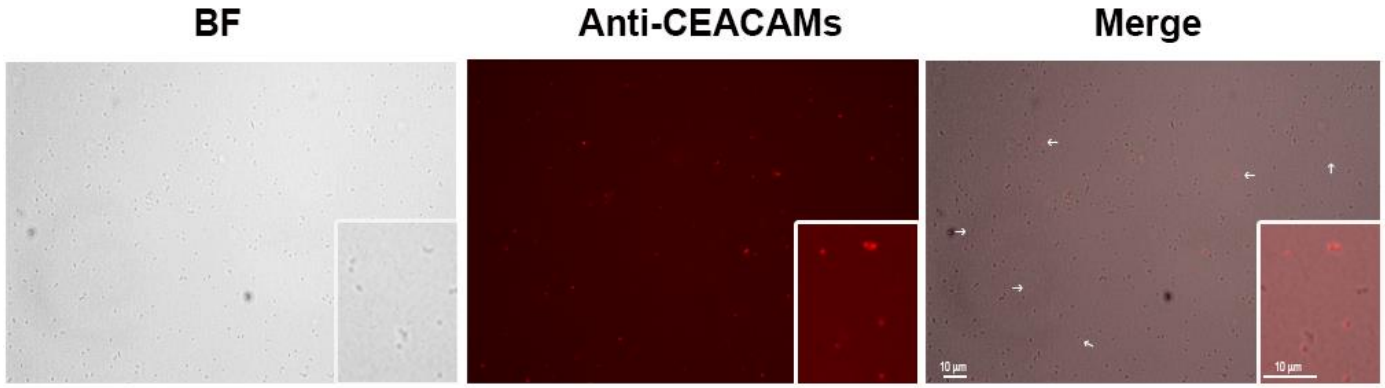




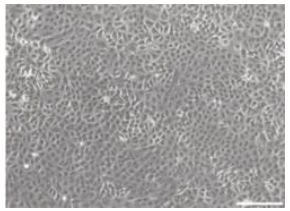
A. baumannii binds to CEACAM receptors



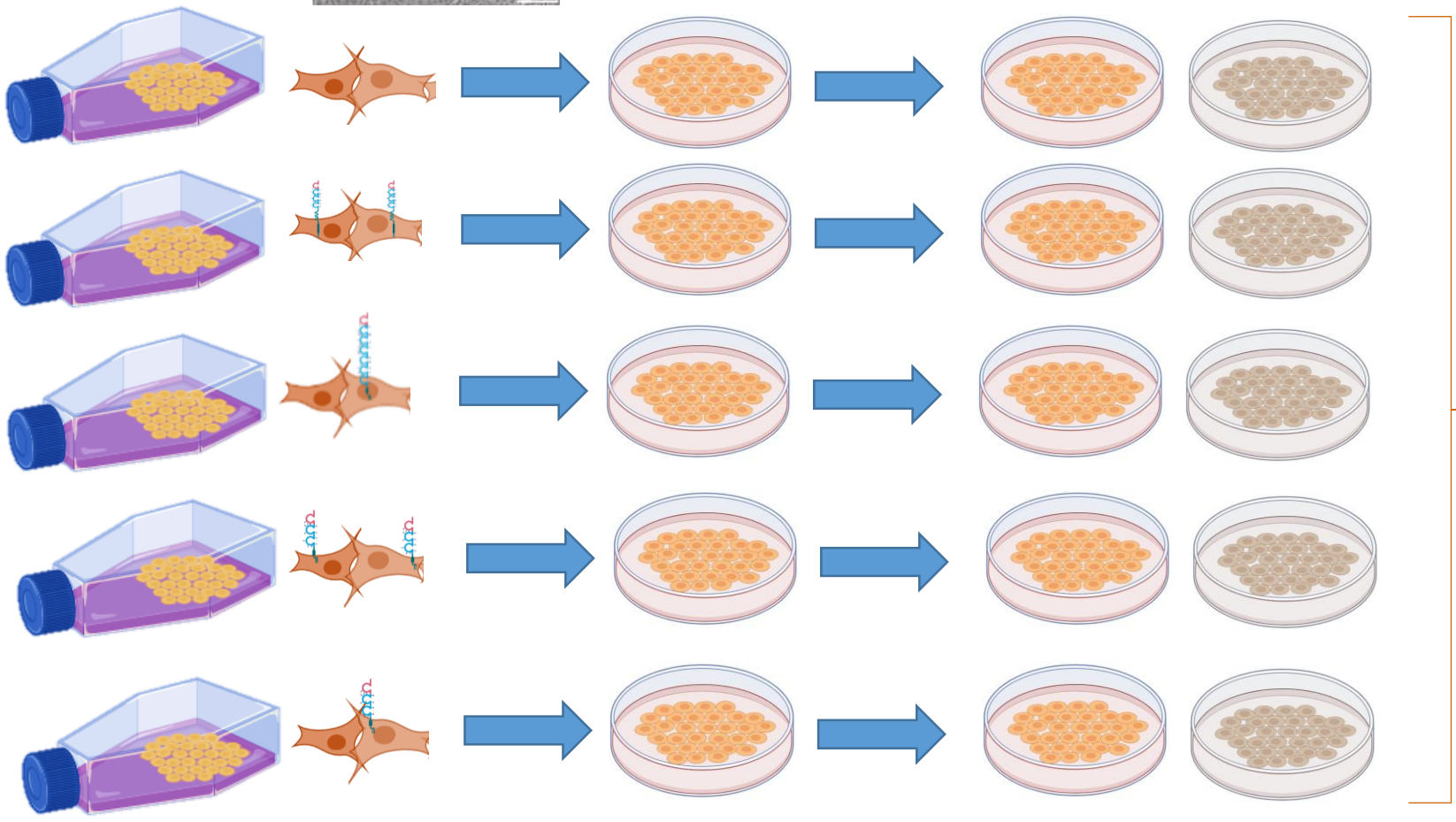


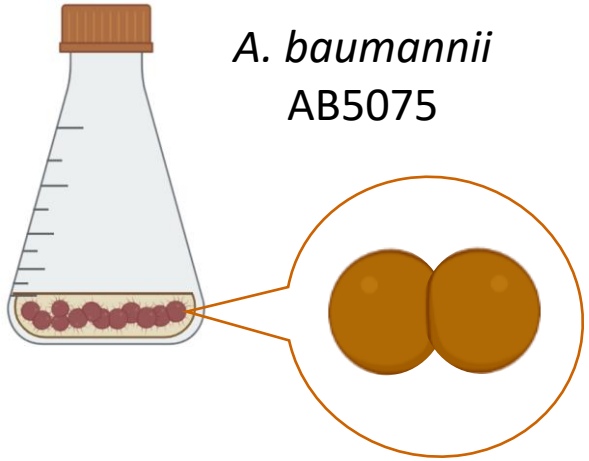


Host-*A. baumannii* interaction



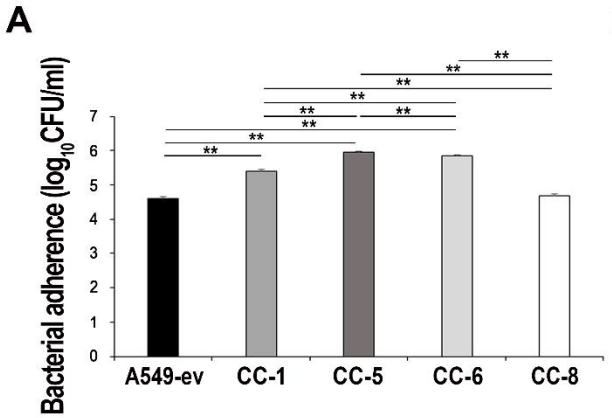
A549 [ATCC CCL-185]:
epithelial cells isolated from lung tissue derived from a 58-year-old, white, male with lung cancer

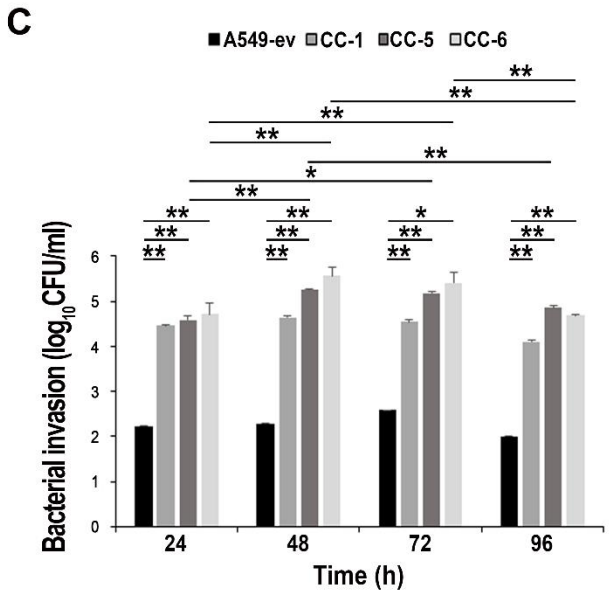


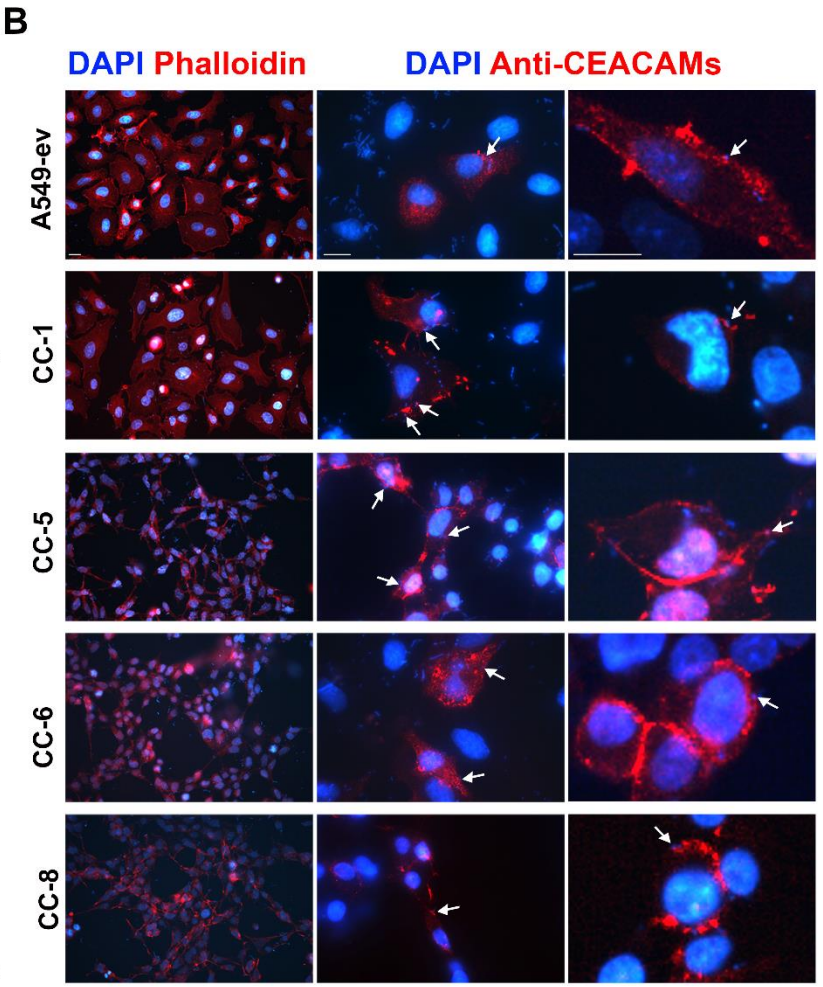


A. baumannii
AB5075

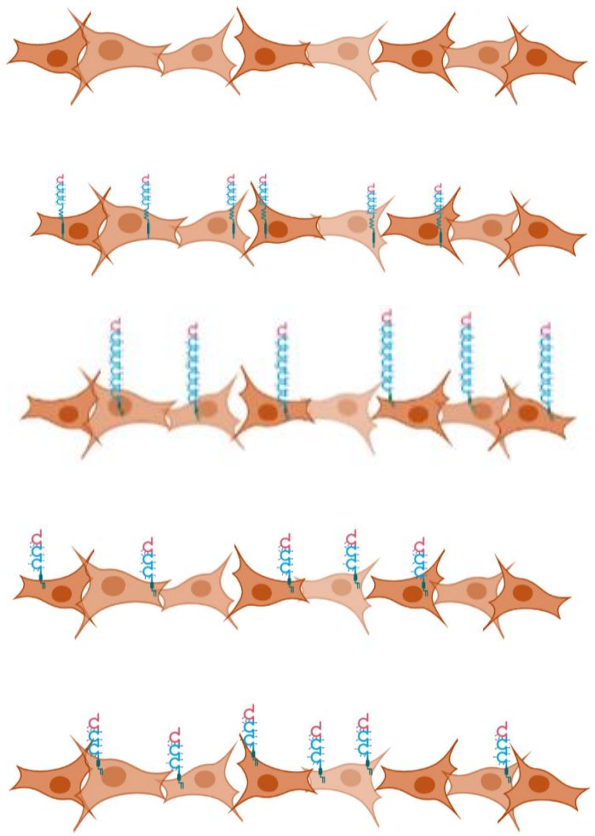
Increased *A. baumannii* adherence to and invasion of CEACAM-expressing lung epithelial cells







A549 cell lines



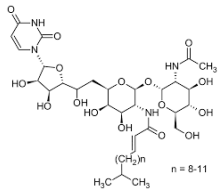
mSystems

RESEARCH ARTICLE
Host Microbe Biology

Acinetobacter baumannii Targets Human Carcinoembryonic Antigen-Related Cell Adhesion Molecules (CEACAMs) for Invasion of Pneumocytes

A. baumannii-CEACAM1 interaction does not involve N-glycan moieties of the receptor

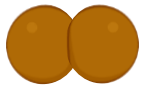
Tunicamycin



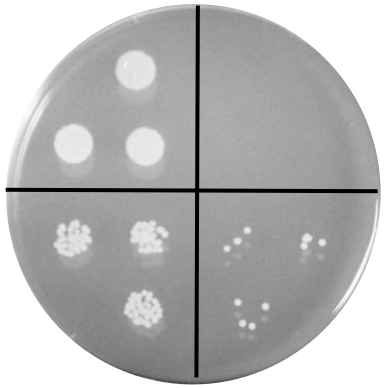
+

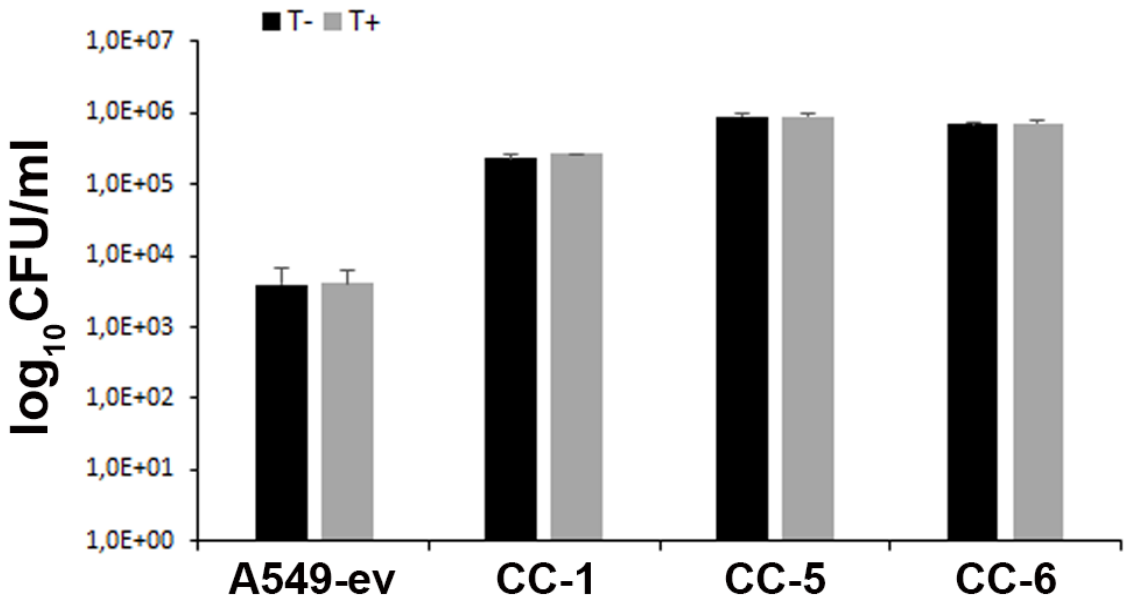
A549 cell lines

AB5075

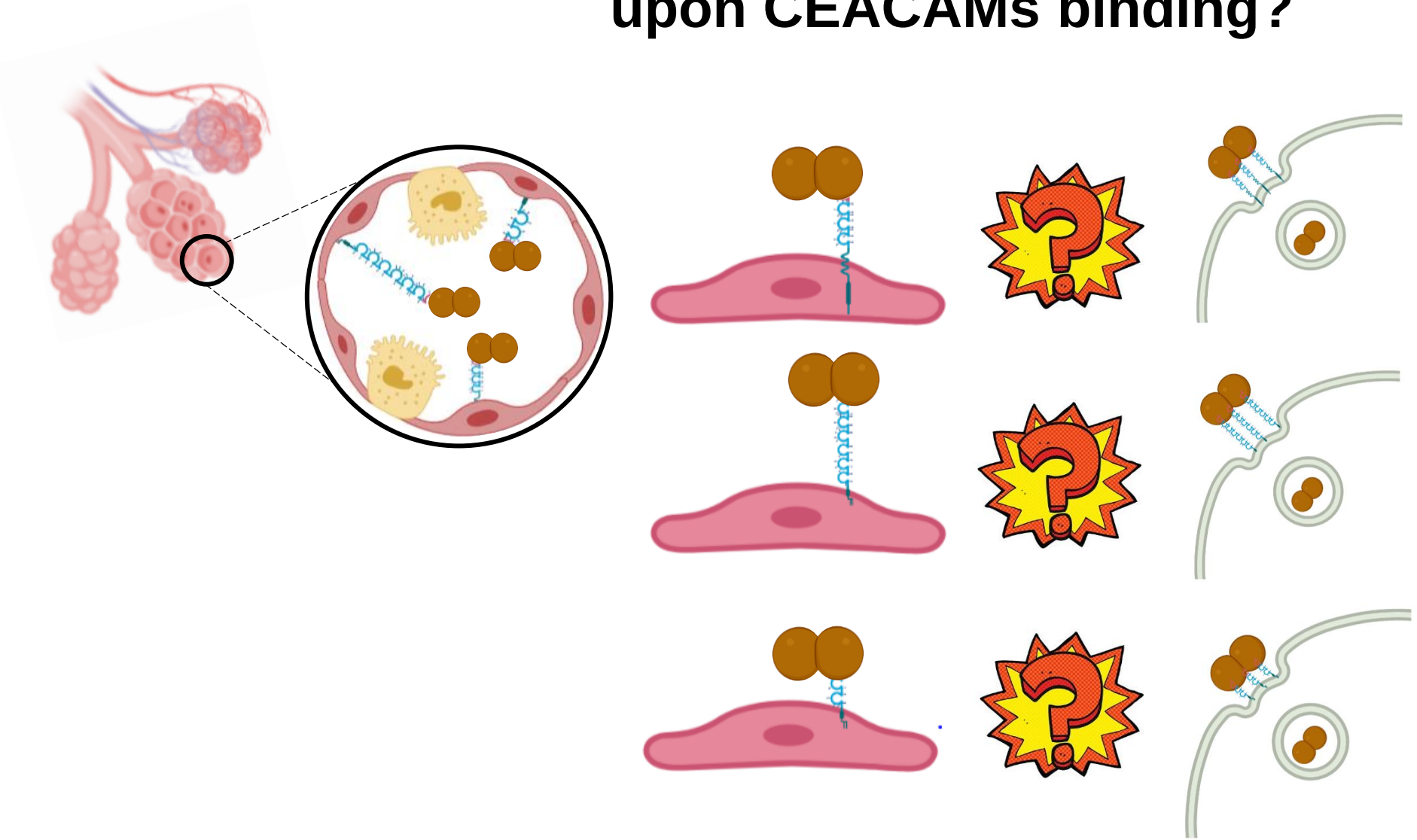


CFU/ml @ 2.5 h.p.i.

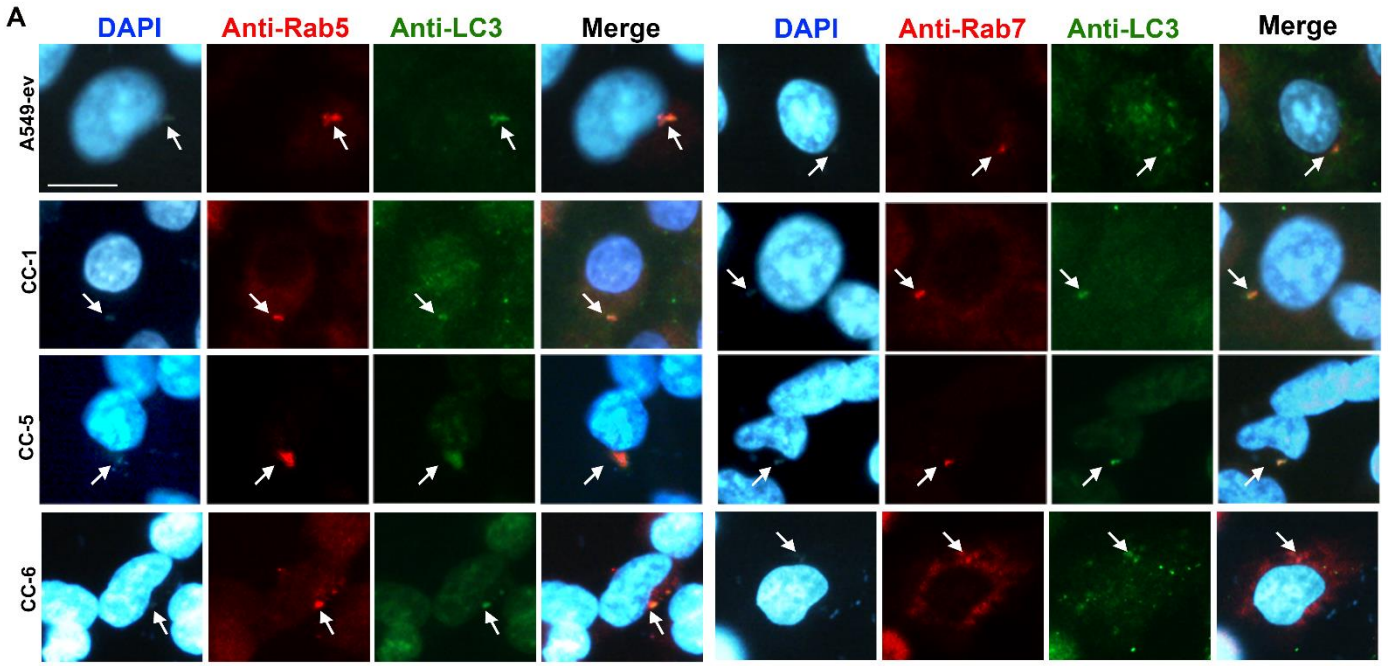


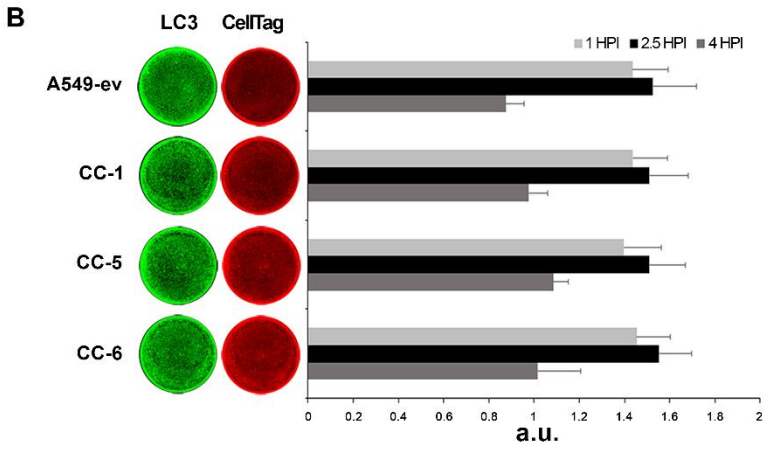


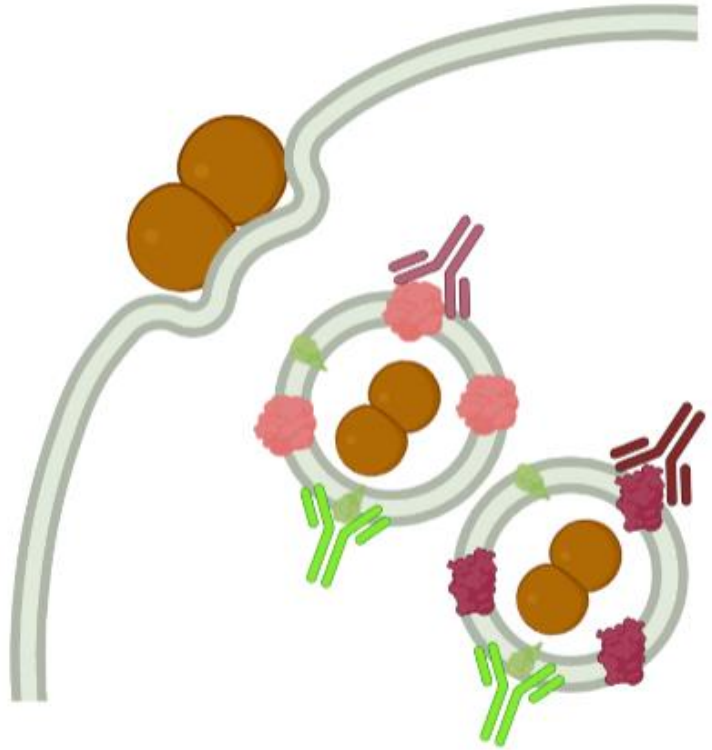
What is the *A. baumannii* intracellular lifestyle upon CEACAMs binding?



A. baumannii colocalizes with endocytic markers and LC3





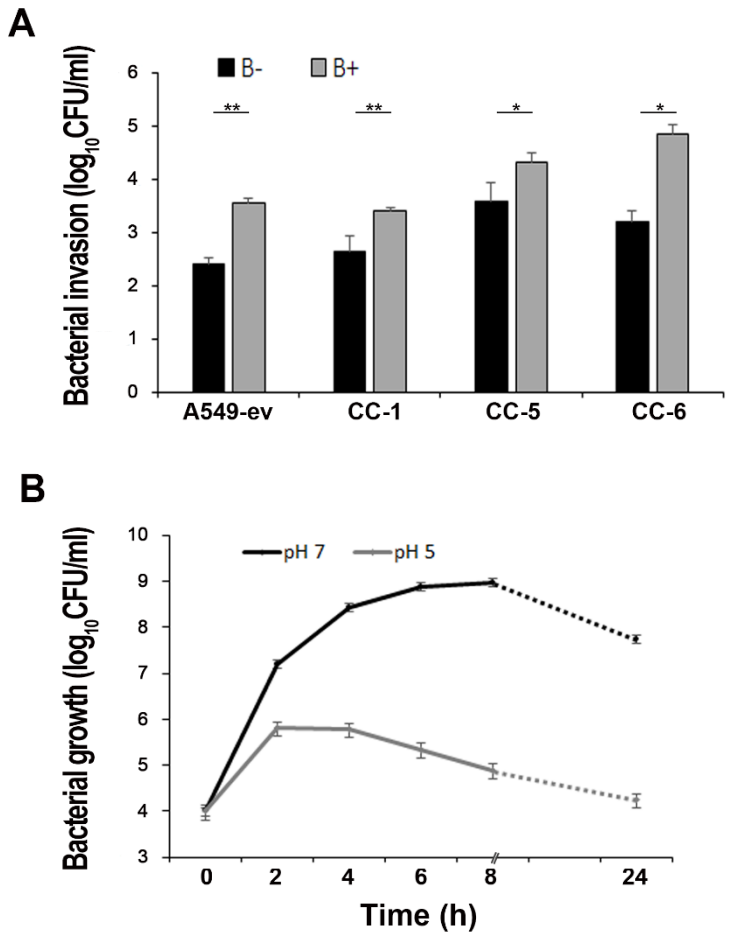


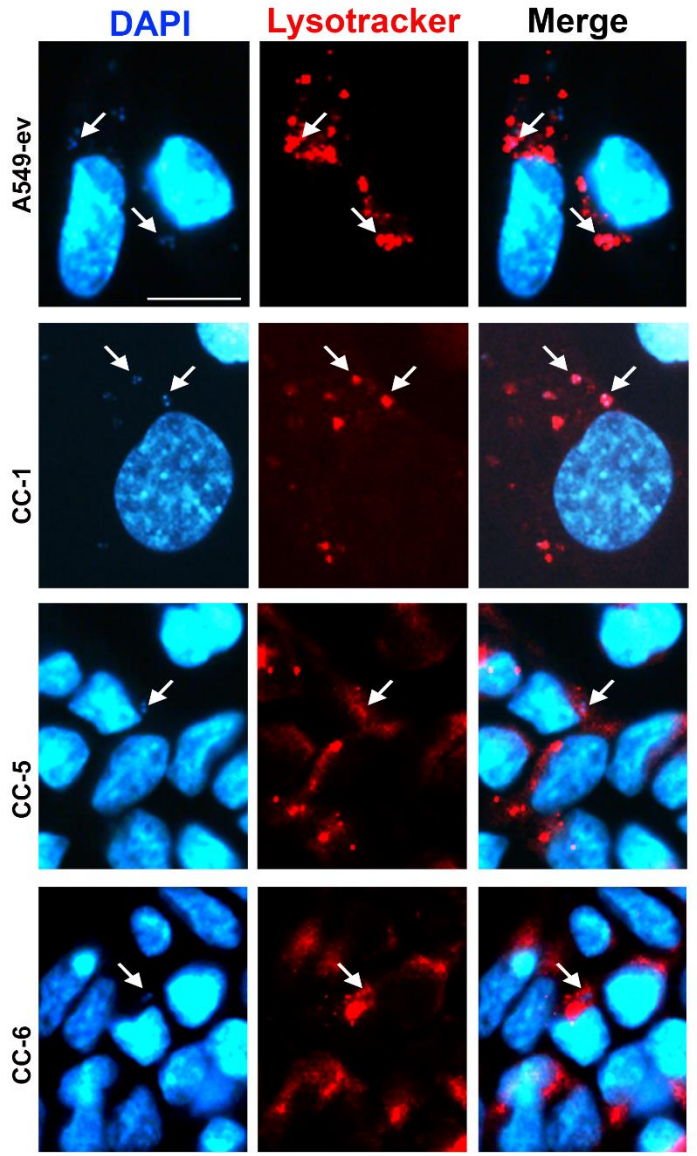
Rab5

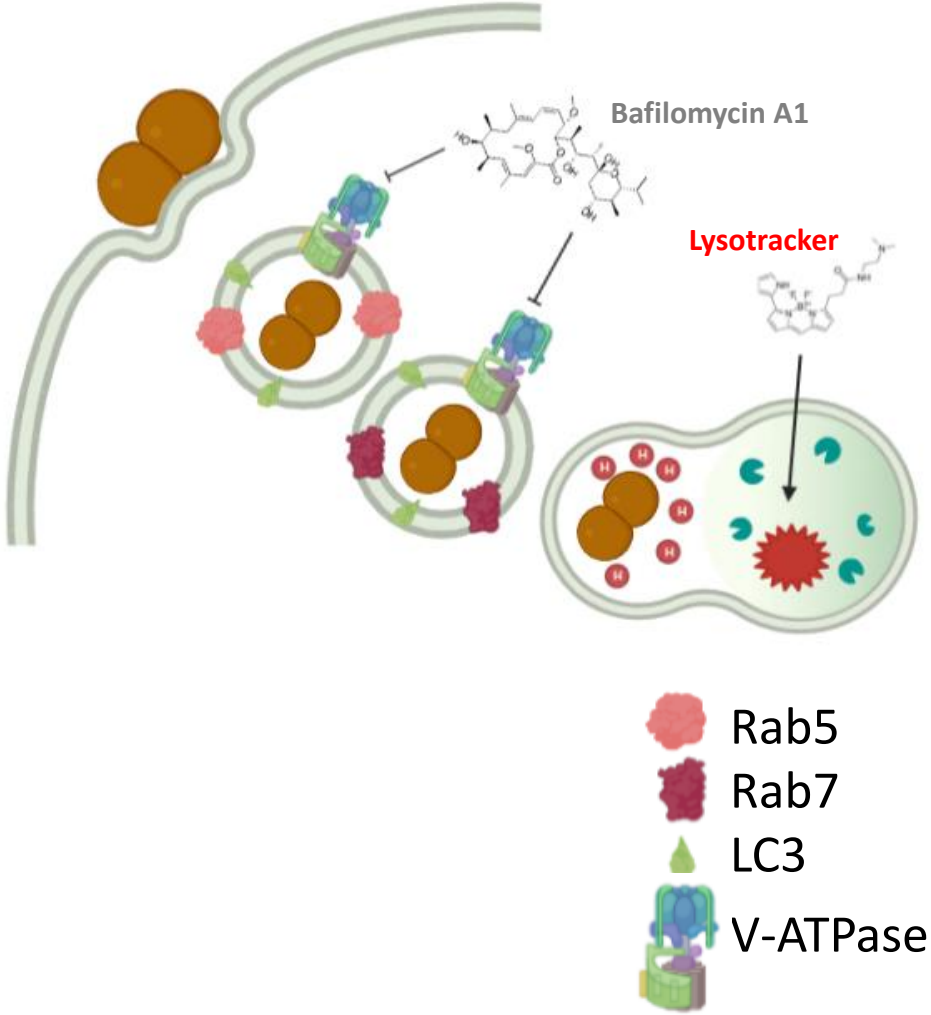
Rab7

LC3

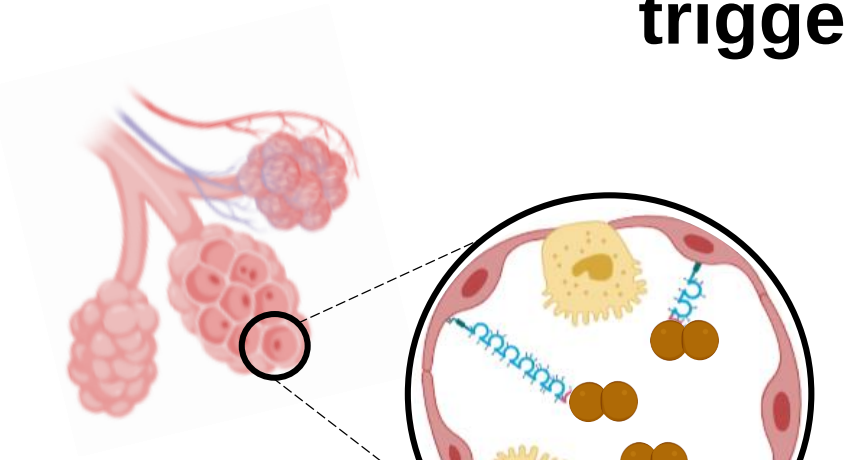
Acidification has detrimental effects on *A. baumannii* viability

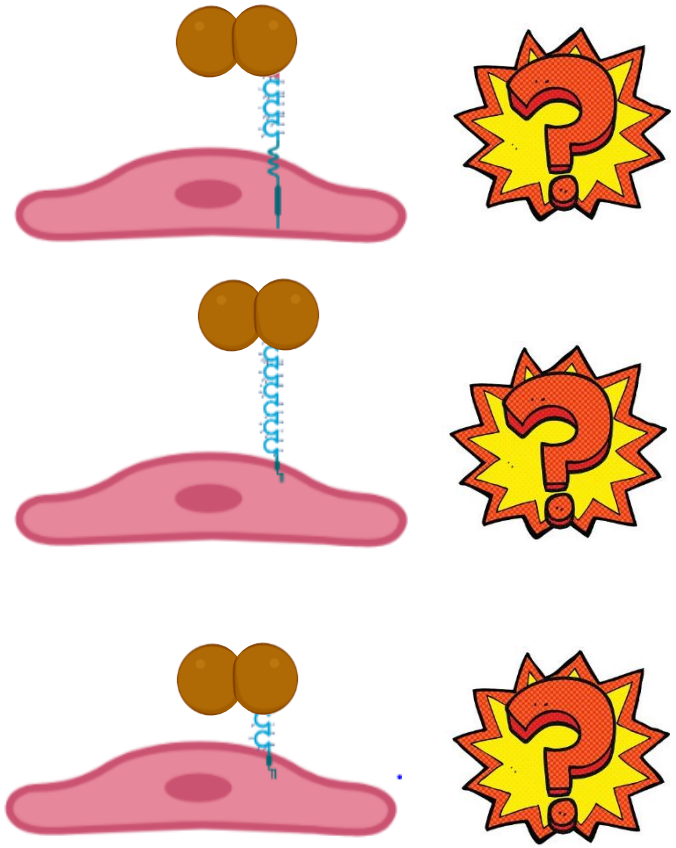


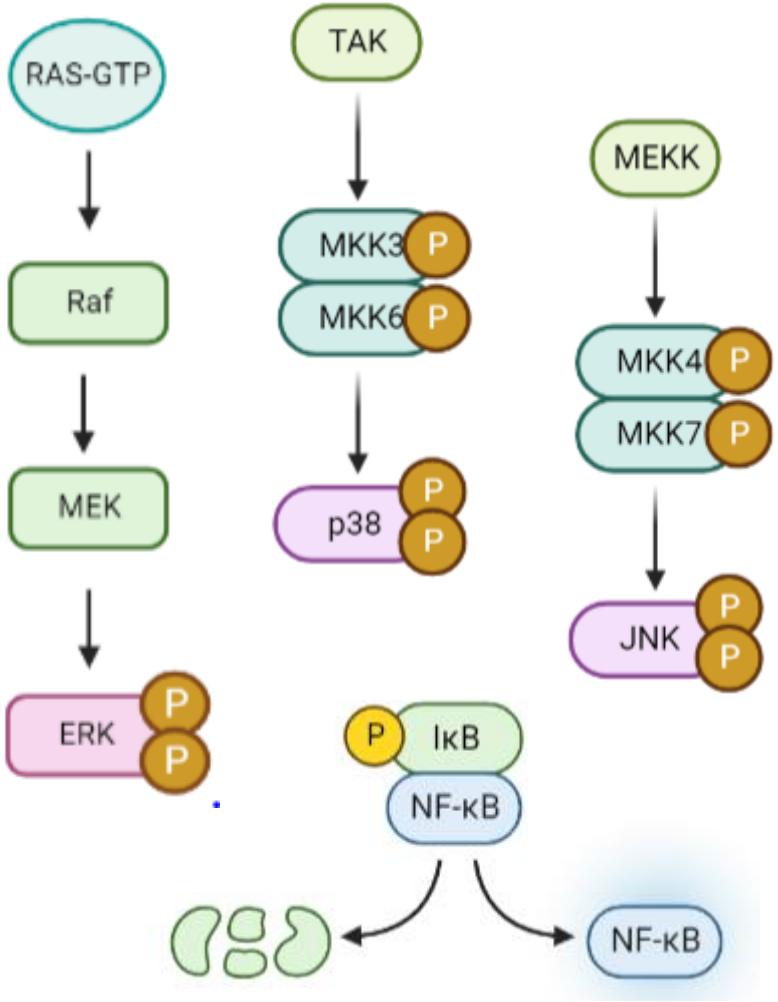




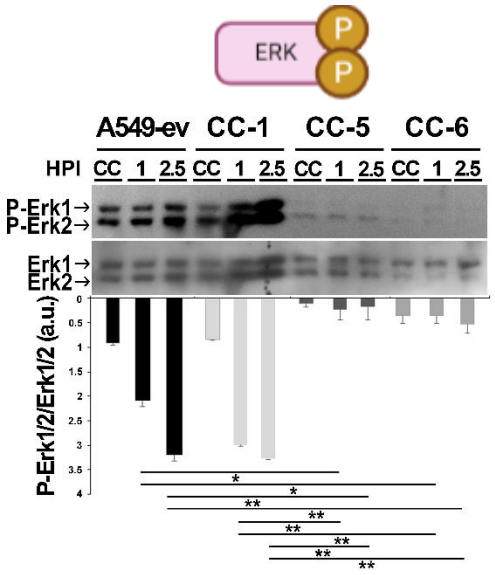
What microscale communication *A. baumannii* triggers upon CEACAMs binding?

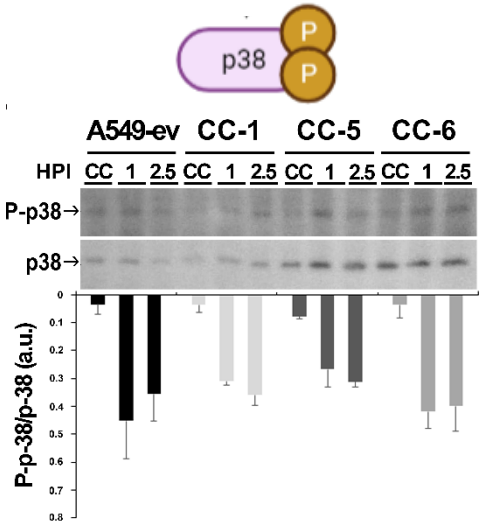


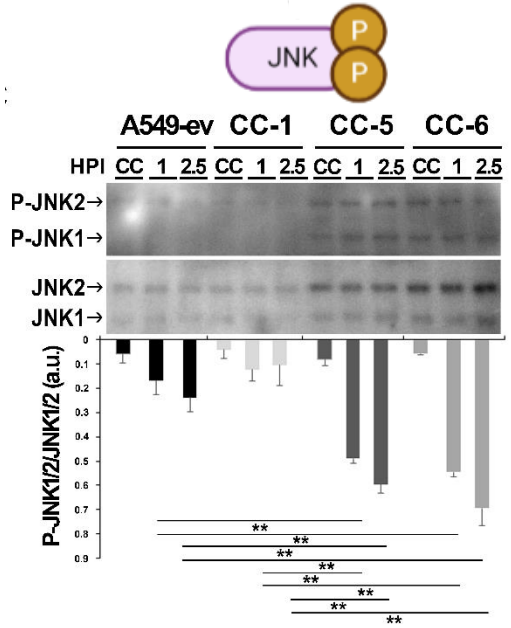


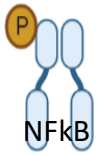


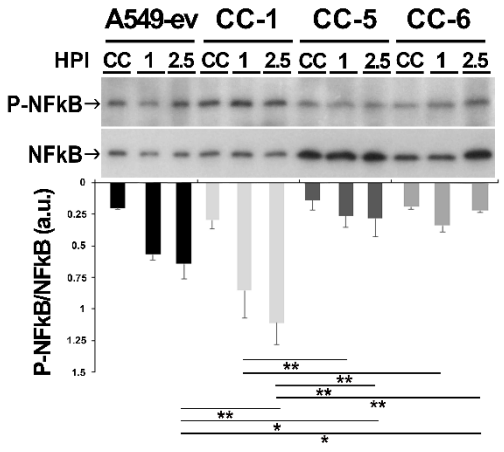
Engagement of CEACAMs by *A. baumannii* transduces different signaling pathways

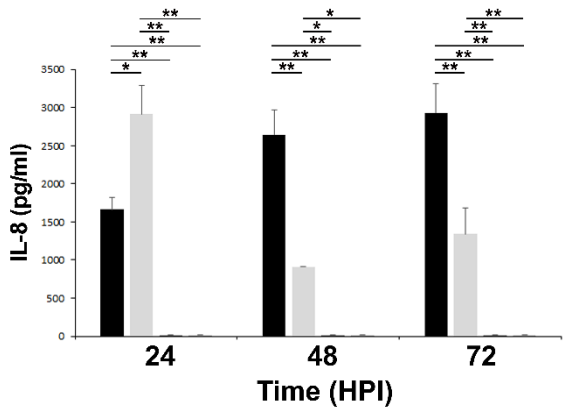


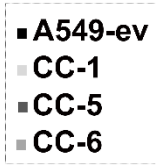


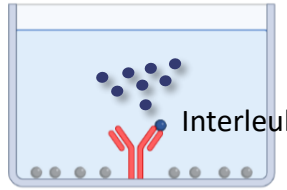






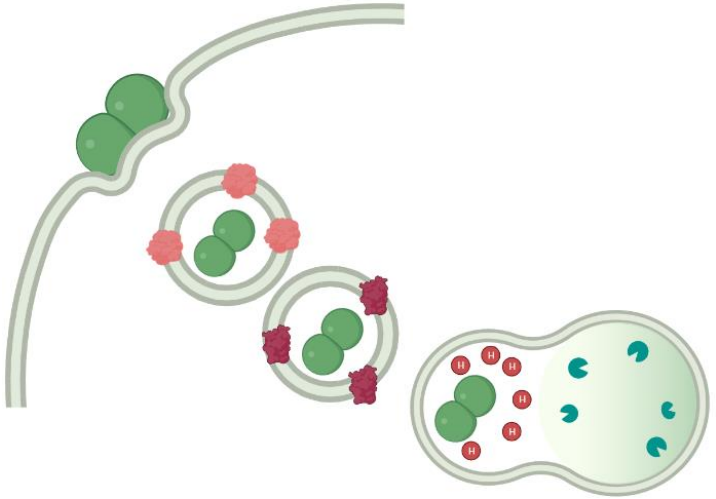




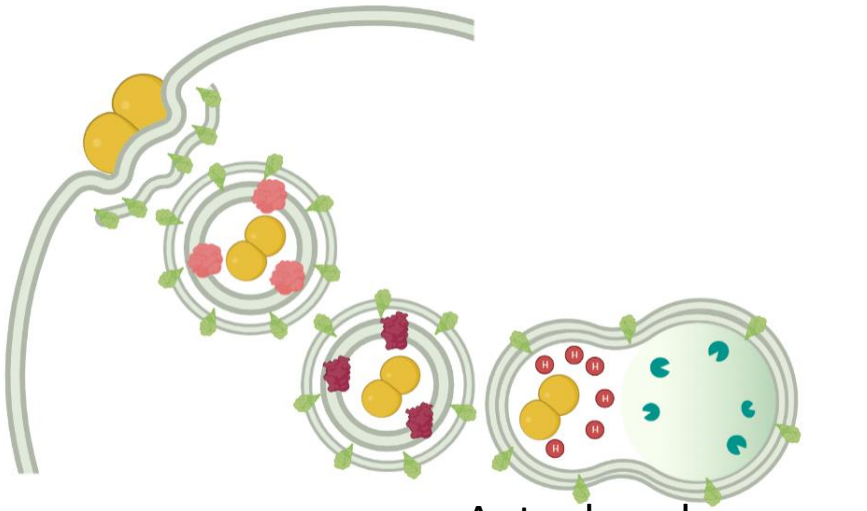


The noncanonical autophagy process: LC3-associated phagocytosis (LAP)

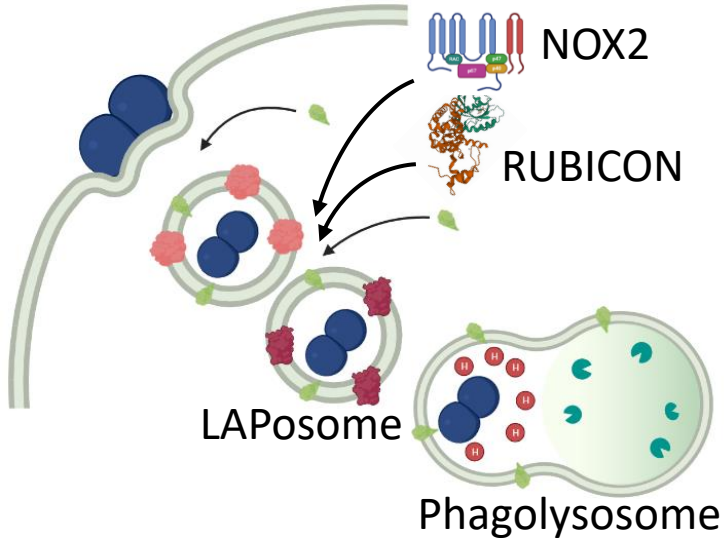
Classical phagocytosis



Macroautophagy



LAP



Rab5 Rab7 LC3 Lysosome

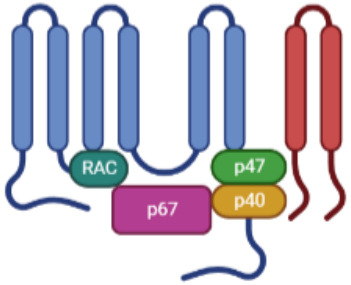
ARTICLES

nature cell biology

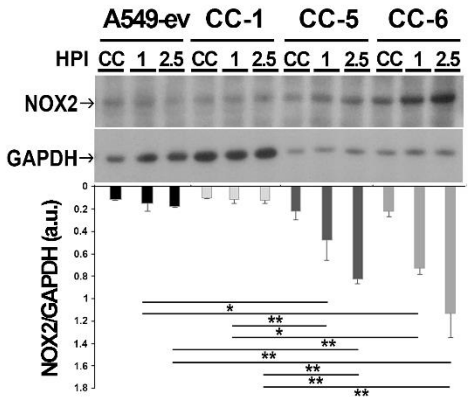
Molecular characterization of LC3-associated phagocytosis reveals distinct roles for Rubicon, NOX2 and autophagy proteins

Jennifer Martinez[1,7], R. K. Subbarao Malireddi[1], Qun Lu[2], Larissa Dias Cunha[1], Stephane Pelletier[1,3], Sebastien Gingras[1,3], Robert Orchard[2], Jun-Lin Guan[4], Haiyan Tan[5], Junmin Peng[5,6], Thirumala-Devi Kanneganti[1], Herbert W. Virgin[2] and Douglas R. Green[1,8]

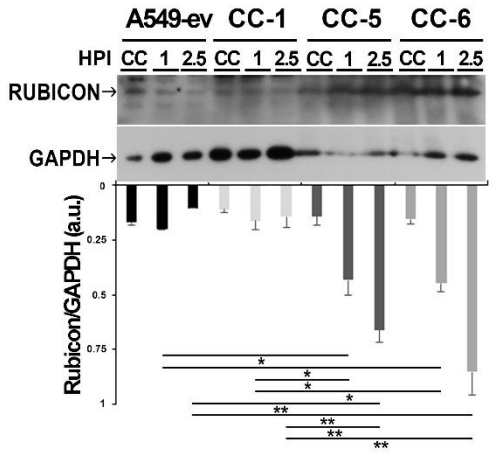
Engagement of CEACAM5 and CEACAM6 by *A. baumannii* increases NOX2 and Rubicon expression

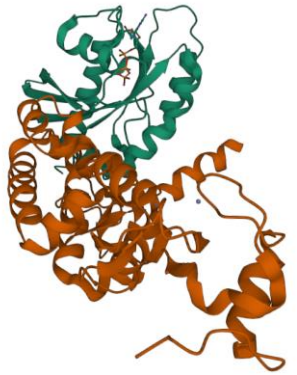


A

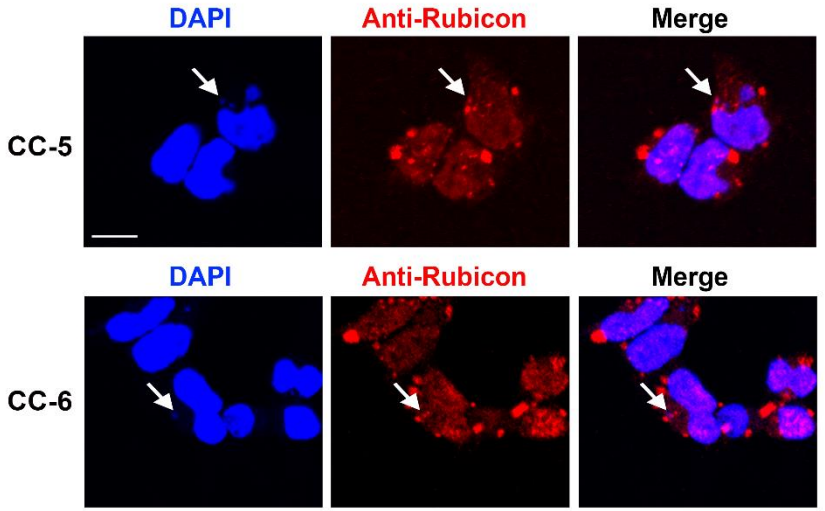


B

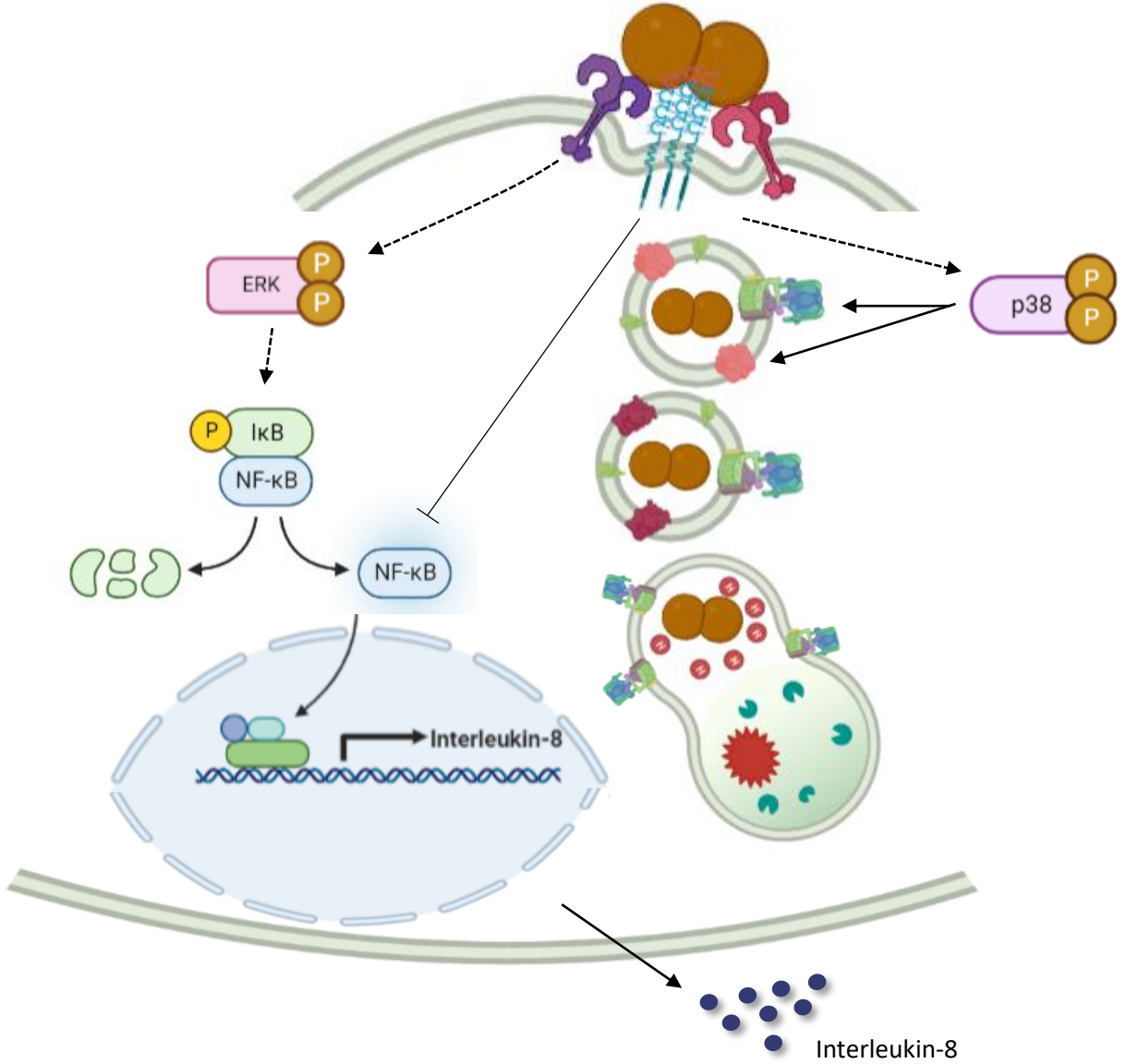


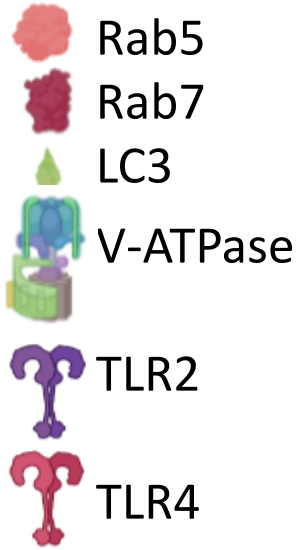


C



The CEACAM1-*A. baumannii* signaling pathway



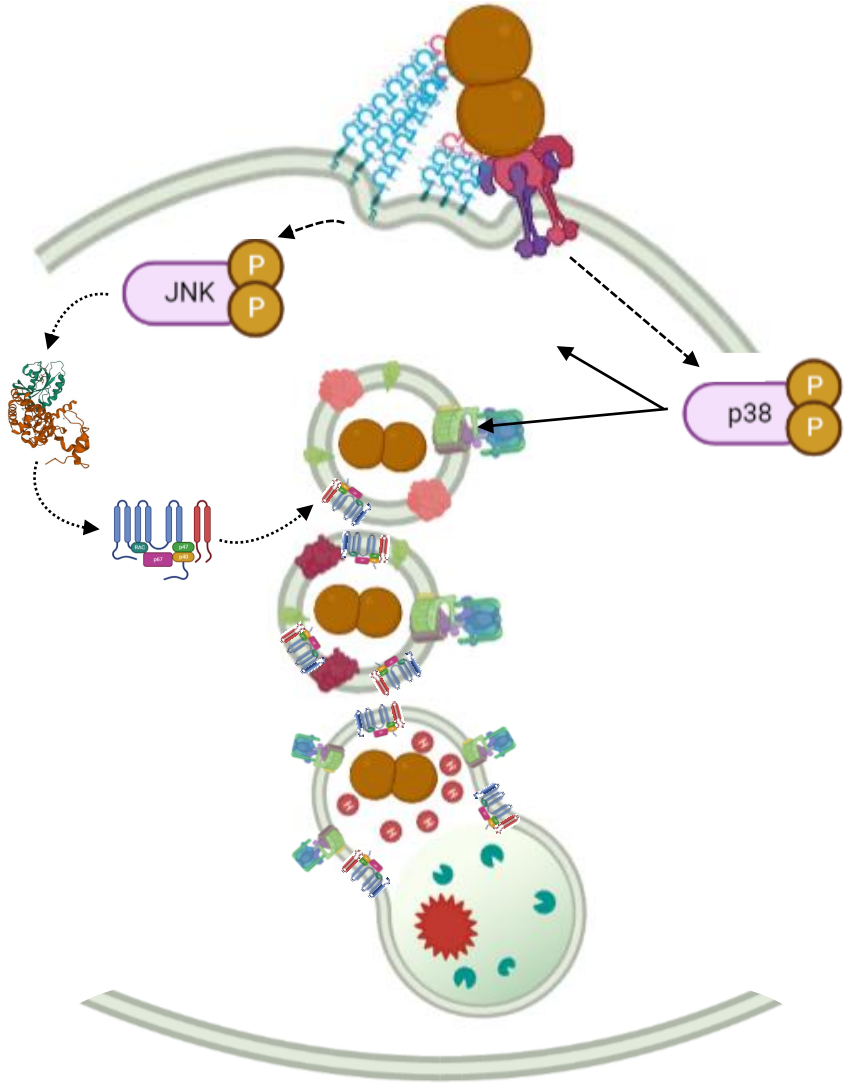


mSystems
RESEARCH ARTICLE
Host-Microbe Biology

Acinetobacter baumannii Targets Human Carcinoembryonic Antigen-Related Cell Adhesion Molecules (CEACAMs) for Invasion of Pneumocytes

Cecilia Ambrosi,[a] Daniela Scribano,[b,c] Meysam Sarshar,[d,e,f] Carlo Zagaglia,[b] Bernhard B. Singer,[g] Anna Teresa Palamara[a,d]

The CEACAM5-CEACAM6-*A. baumannii* signaling pathway



Rab5
Rab7
LC3
V-ATPase
TLR2
TLR4
RUBICON
NOX2

AMERICAN SOCIETY FOR MICROBIOLOGY | mSystems
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Acinetobacter baumannii Targets Human Carcinoembryonic Antigen-Related Cell Adhesion Molecules (CEACAMs) for Invasion of Pneumocytes

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Do cell lines represent a reliable model to study host-microbe interactions?

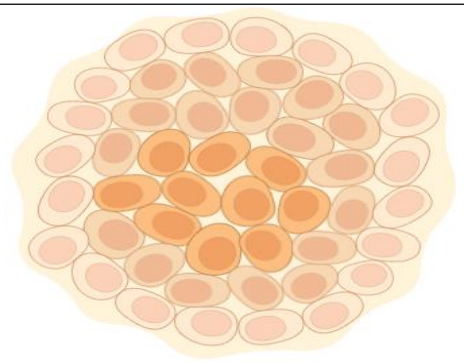
- Relatively inexpensive
- Widely available
- Unlimited proliferation
- Little maintenance
- Minimal technical expertise
- Storable
- Easy to sequence
- Allow high-throughput assays

- Genetic and phenotypic change
- Cross-contaminations
- Lack of differentiation
- Cancerogenic cells
- Unknown cancerogenic origin
- Aging
- Lack of tissue architecture/complexity
- Not sufficiently clinically predictive

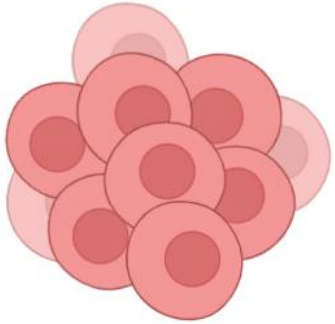
Capes-Davis 2019. doi: 10.1021/acs.chemrestox.9b00215

Transition from Monolayers to Organoids

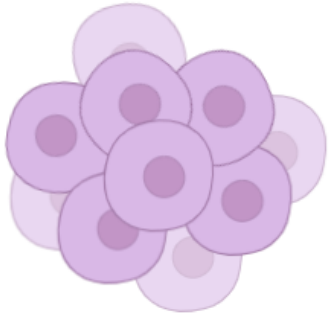
Organoids are three-dimensional structures that self-organize *in vitro*, recapitulating the microarchitecture and physiology of the tissue of origin, hence, are also called «mini-organs»



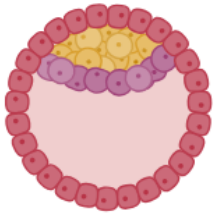
Embryonic stem cells (ESCs)



Induced pluripotent stem cells (iPSCs)

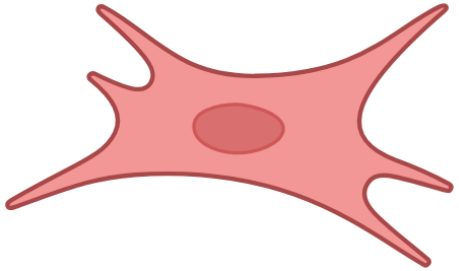


Adult stem cells (ASCs)



5-6 day old human blastocyst

Thompson et al., 1998



Reprogramming differentiated adult cells

Takashi & Yamanada, 2006, 2007

Somatic stem cells

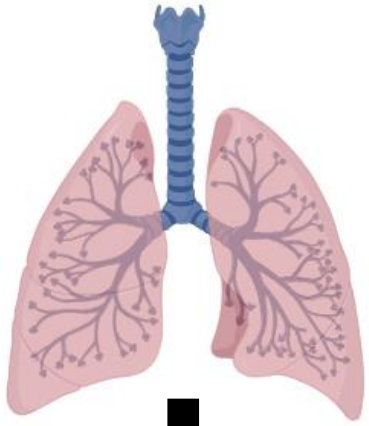
Sato et al., 2009

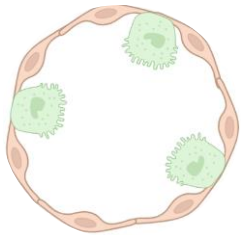
Am J Physiol Cell Physiol. 2020 Jul 1; 319(1): C151–C165.
Published online 2020 May 27. doi: 10.1152/ajpcell.00120.2020: 10.1152/ajpcell.00120.2020
Making Cell Culture More Physiological

A brief history of organoids

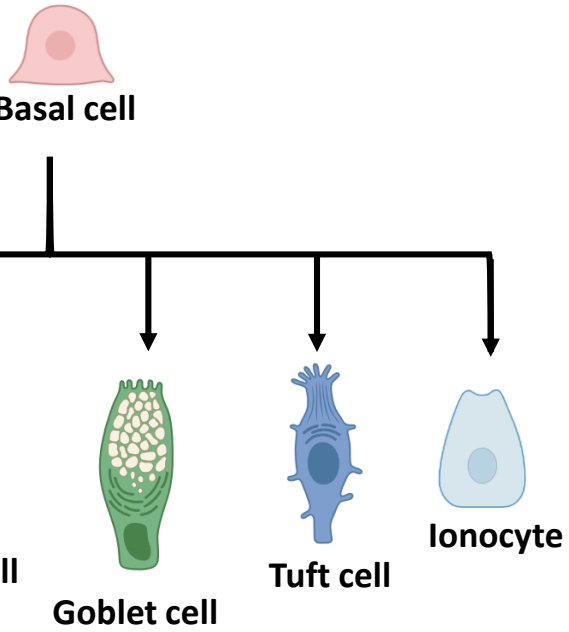
Claudia Corro,* Laura Novellasdemunt,* and Vivian S.W. Li#

The airway and alveolar organoids

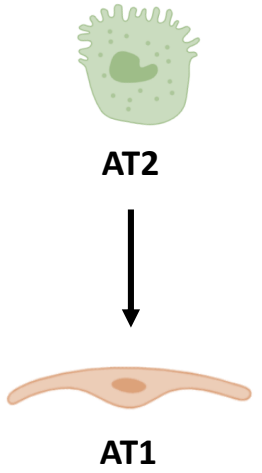




Airway Organoids
or proximal respiratory tract



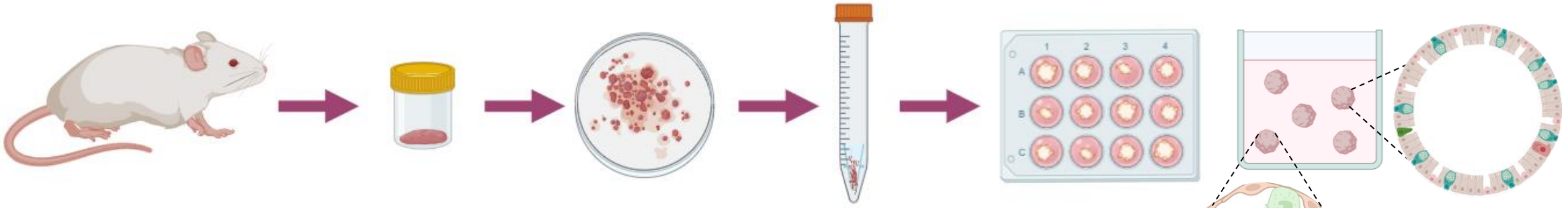
Alveolar Organoids

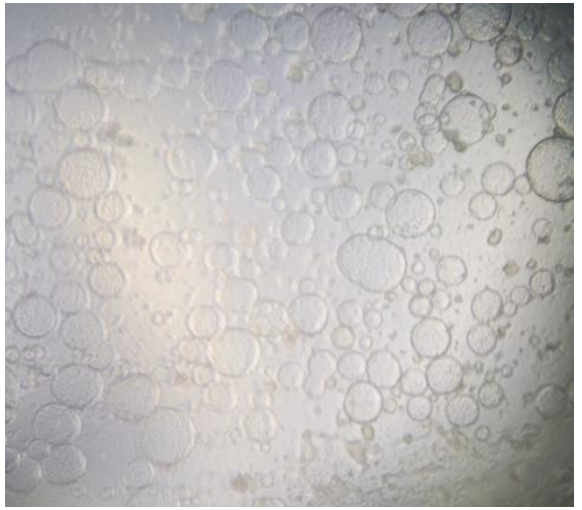


Unlike the gut epithelium, the lung epithelium is only slowly self-renewing under non-damage conditions

van der Vaart & Clevers. 2021. Journal of internal medicine, 289(5), 604-613.
Demchenko et al., 2022- *Cell and Tissue Research*, *390*(3), 317-333.

Setting up the mouse airway/alveolar organoid model





Unpublished results

The Air Liquid Interface (ALI) culture system

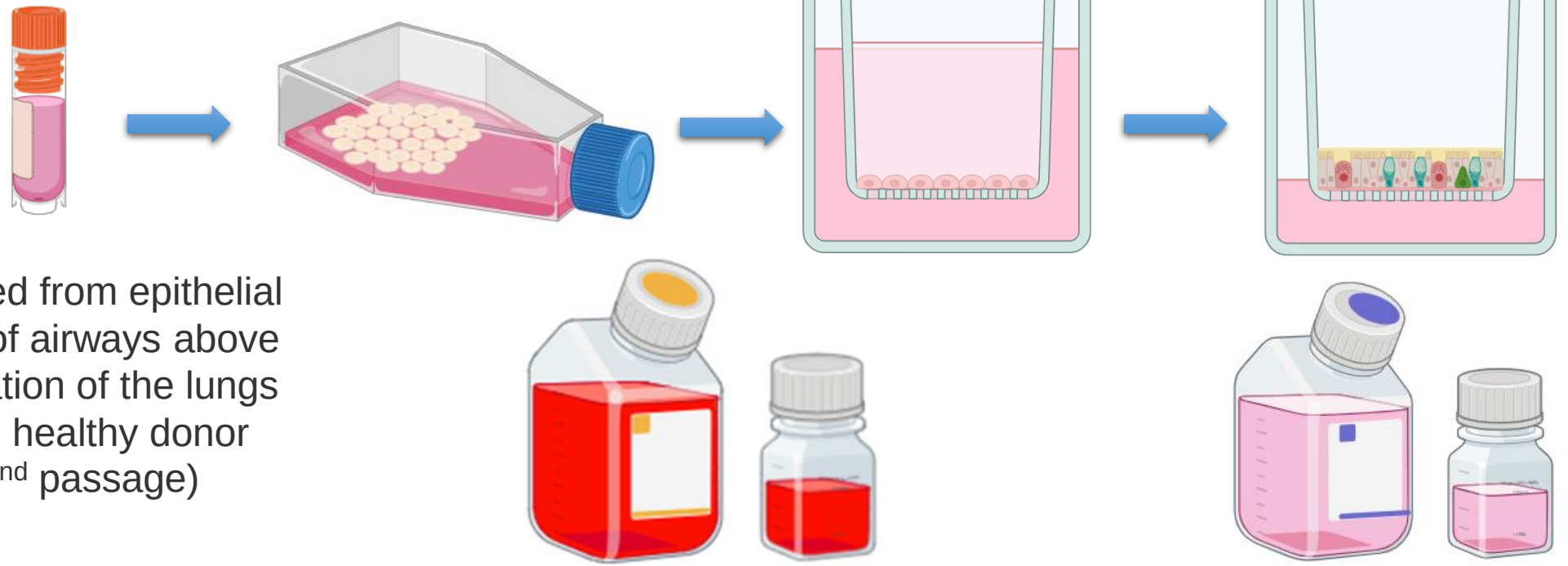
Commercial Normal Bronchial Human Epithelial cells (NBHE)

Isolated from epithelial lining of airways above bifurcation of the lungs of an healthy donor (2nd passage)

Expansion in flask

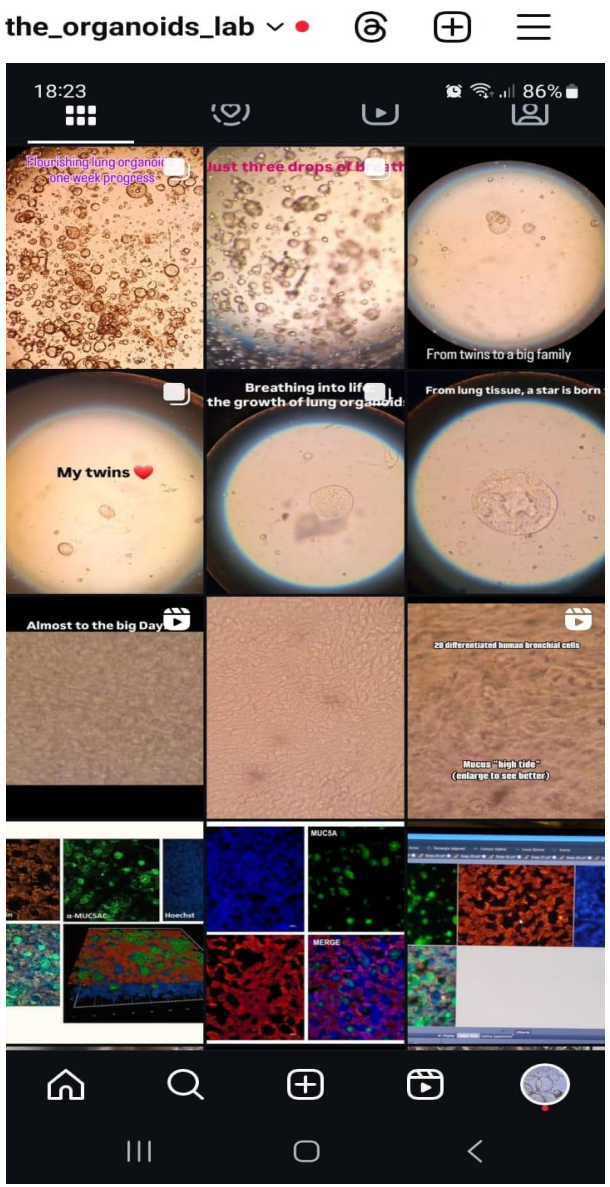
Expansion in inserts

Differentiation in inserts



Stay connected! Follow us on Instagram for the latest updates!

the_organoids_lab



Take home messages

The study of host–pathogen interactions contributes to:

•**Revealing how microbes attach to, invade, and exploit host cells** to evade immunity and find new therapeutic strategies

•**Pioneering the field of cellular immunology**, showing that control of intracellular infections relies on cell-mediated rather than humoral immunity

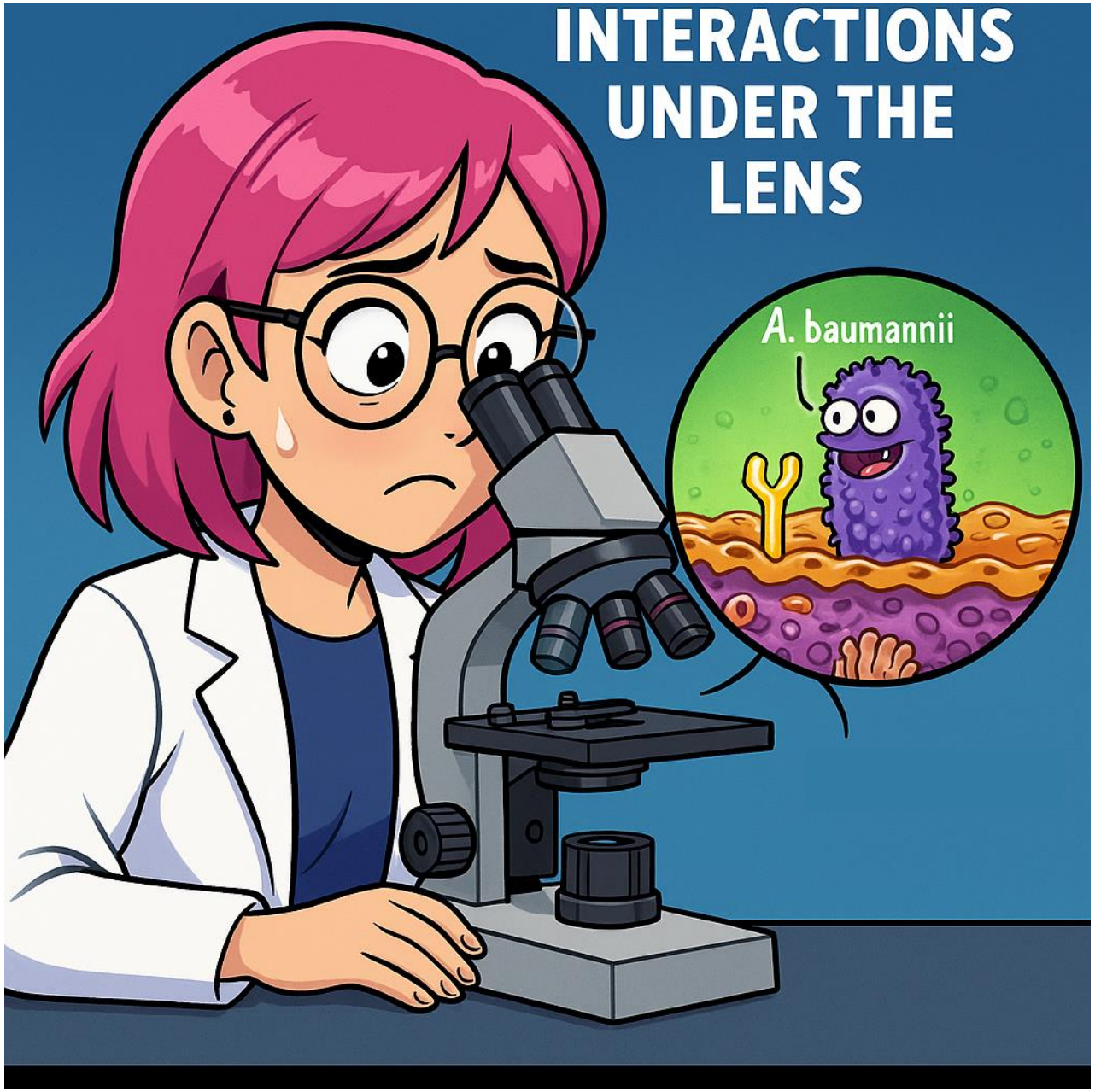
•**Leading to key discoveries in immune system biology**, including antigen-presenting pathways, T cell subsets, and cellular receptors

•**Establishing the discipline of cellular microbiology**, highlighting how pathogens hijack host signaling, cytoskeletal structures, and cell death pathways for their survival

•**Enabling the dissection of fundamental cellular processes** such as signal transduction, intracellular trafficking, and host–pathogen metabolism

•**Uncovering the co-evolution of intracellular pathogens and host cells** through the development of tightly linked metabolic interactions

•**Providing insights on new strategies for managing inflammatory diseases and cancers,** with broader applications in oncology and chronic conditions.



Thank you for your attention

Università San Raffaele
Roma