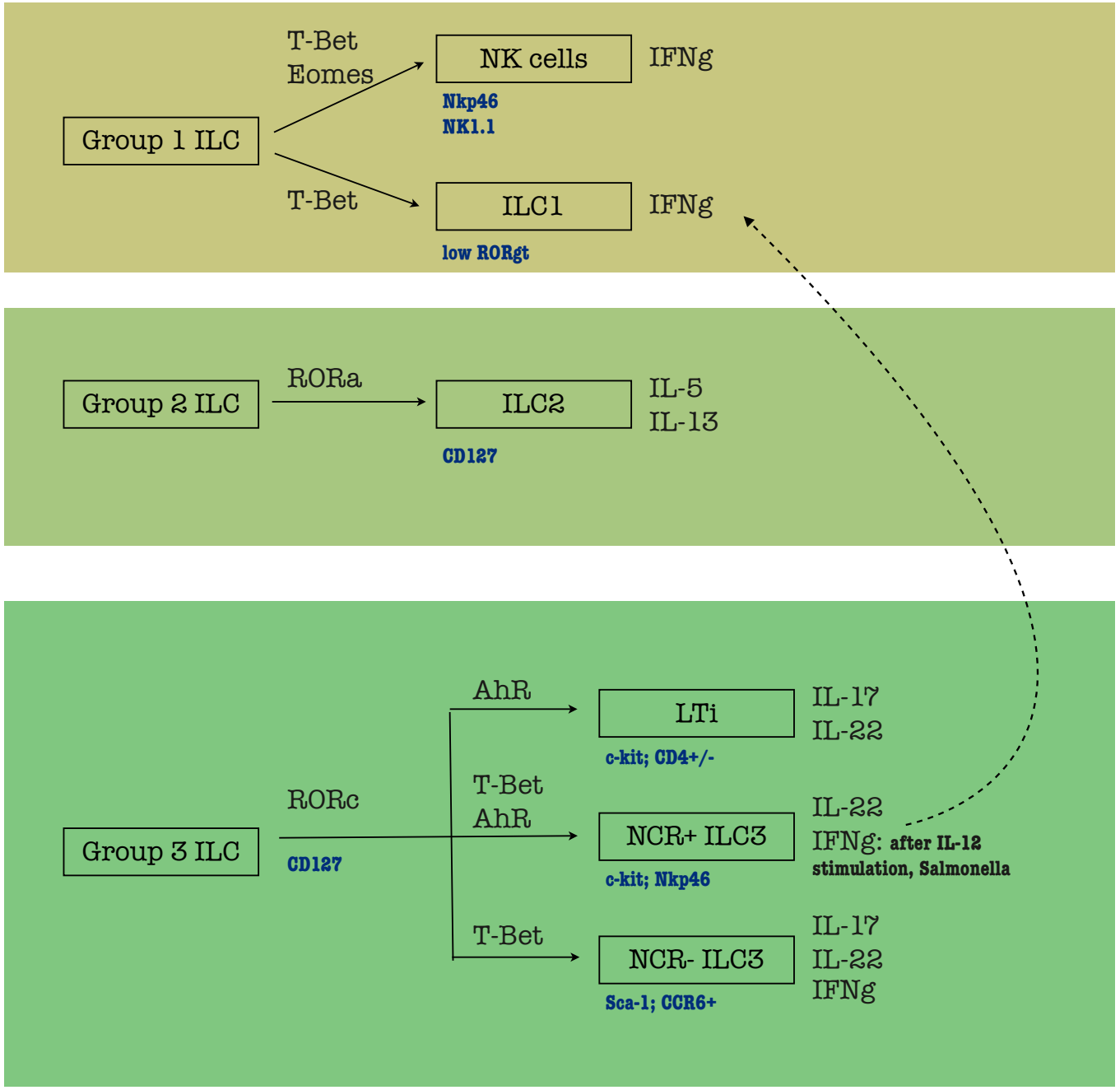
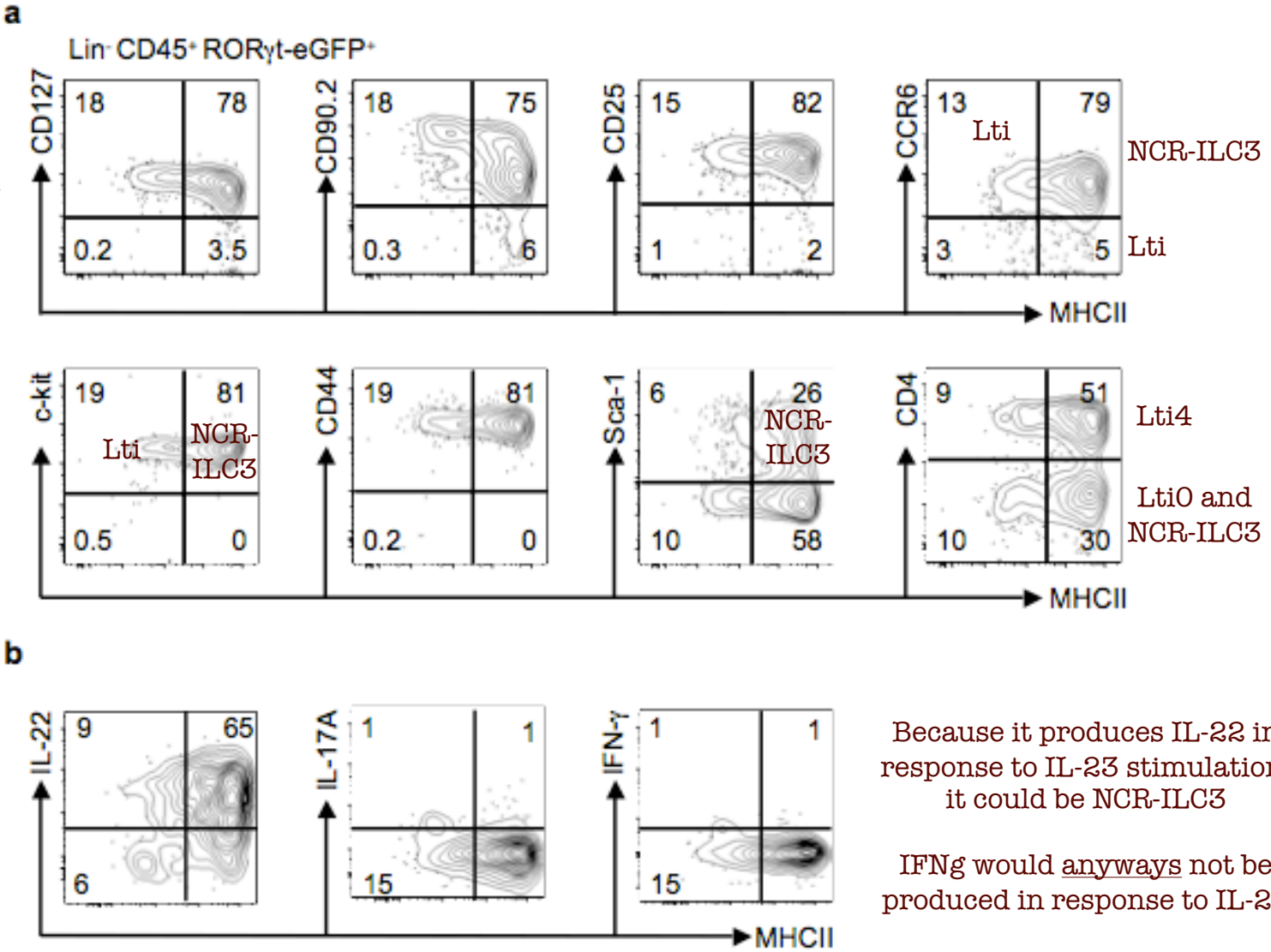

Group 1 ILC
T-Bet
Eomes
NK cells
IFNg
Nkp46
NK1.1
T-Bet
ILC1
IFNg
low RORgt
Group 2 ILC
RORa
ILC2
IL-5
IL-13
CD127
Group 3 ILC
RORc
CD127
AhR
LTi
IL-17
IL-22
c-kit; CD4+/-
T-Bet
AhR
NCR+ ILC3
IL-22
IFNg: after IL-12 stimulation, Salmonella
c-kit; Nkp46
T-Bet
NCR- ILC3
IL-17
IL-22
IFNg
Sca-1; CCR6+

Per definition: all Group 3 ILCs should express CD127 and CD90!



Because it produces IL-22 in response to IL-23 stimulation, it could be NCR-ILC3

IFNg would anyways not be produced in response to IL-23

Sciencexpress

Role of Tissue Protection in Lethal Respiratory Viral-Bacterial Coinfection

Amanda M. Jamieson,[1,2]*†‡ Lesley Pasman,[1]‡ Shuang Yu,[1] Pia Gamradt,[2] Robert J. Homer,[3,4] Thomas Decker,[2] Ruslan Medzhitov[1]*

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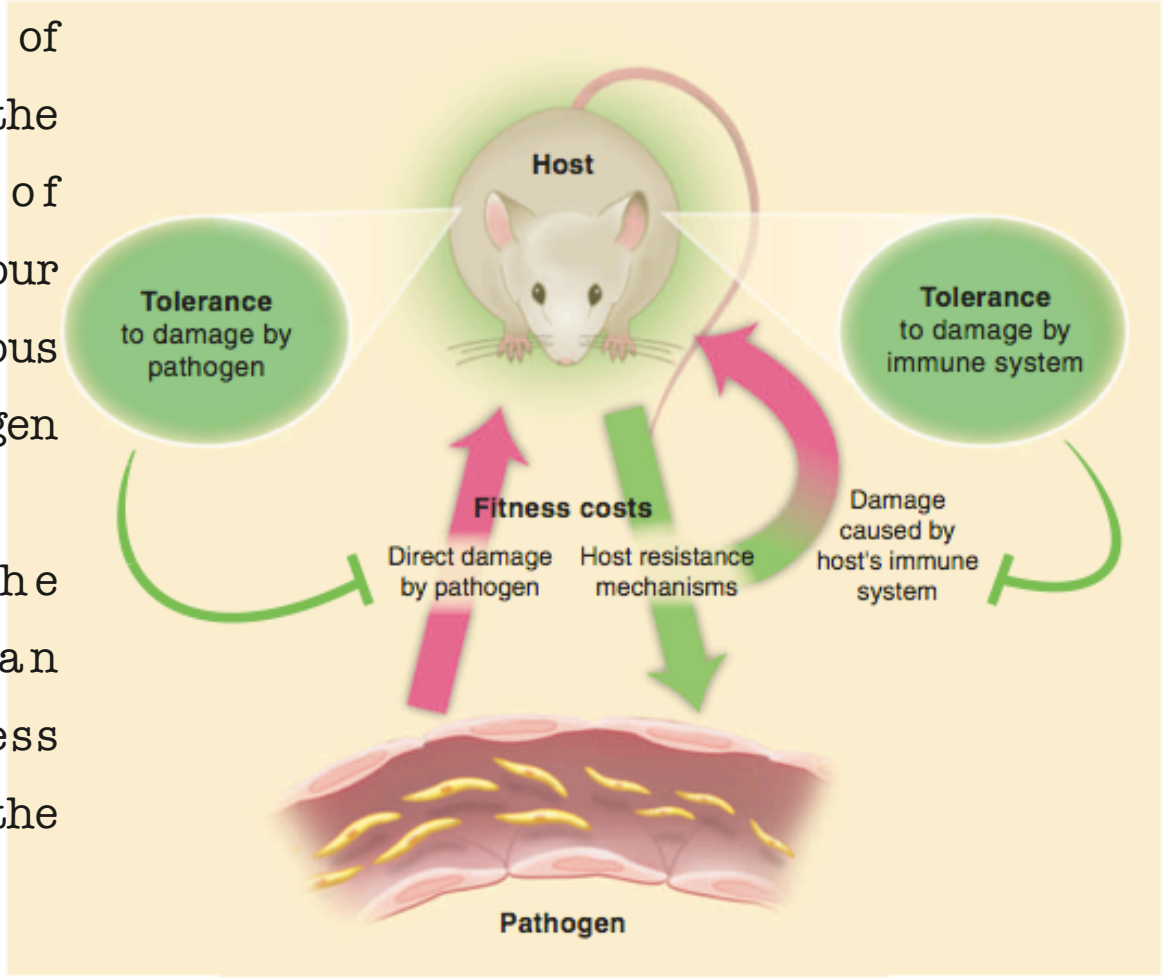
‡These authors contributed equally to this work.

Introduction of the notion of "disease tolerance" into the conceptual tool kit of immunology will expand our understanding of infectious diseases and host pathogen interactions.

Tolerance reduces the negative impact of an infection on host fitness without directly affecting the pathogen burden



Medzhitov, R., Schneider, D. S., & Soares, M. P. (2012). Disease tolerance as a defense strategy. *Science (New York, NY)*, *335*(6071), 936–941. doi:10.1126/science.1214935

host defense = immune **resistance** + disease **tolerance**

infectious disease outcome =

pathogen virulence

+

immune resistance

+

tissue protection/repair

What is the contribution of disease tolerance to infectious disease outcome?

- Complications from secondary bacterial infections are a leading cause of morbidity and mortality associated with influenza virus infection

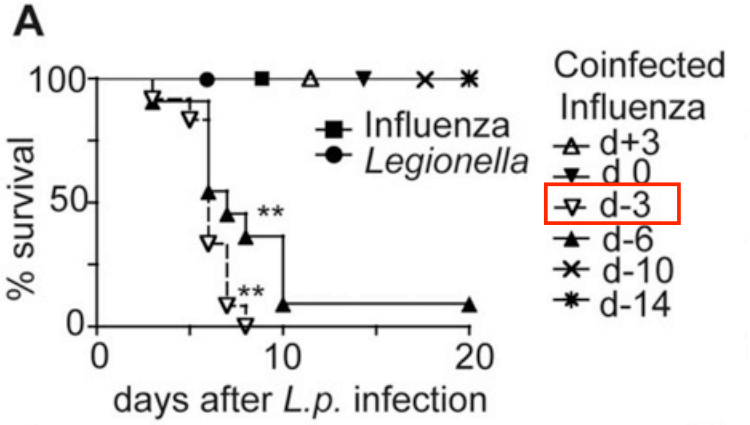
- Influenza virus can suppress the immune response to a bacterial infection

Amanda M Jamieson, et al (2010). Influenza virus-induced glucocorticoids compromise innate host defense against a secondary bacterial infection. Cell host & microbe, 7(2), 103.)

- **Other causes??**

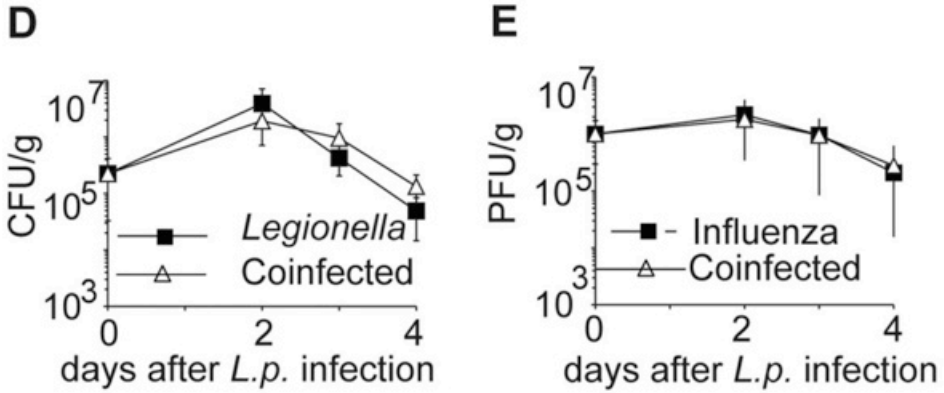
Is mortlaity due to pathogen burden?

L. pneumohphila strain lacking the flaA gene

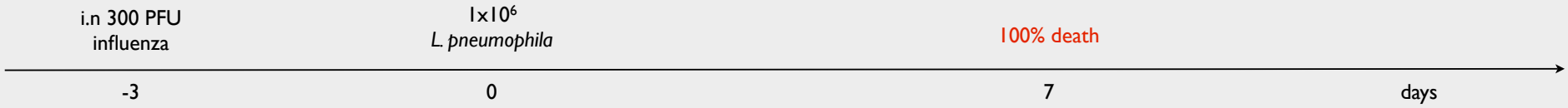


Lung

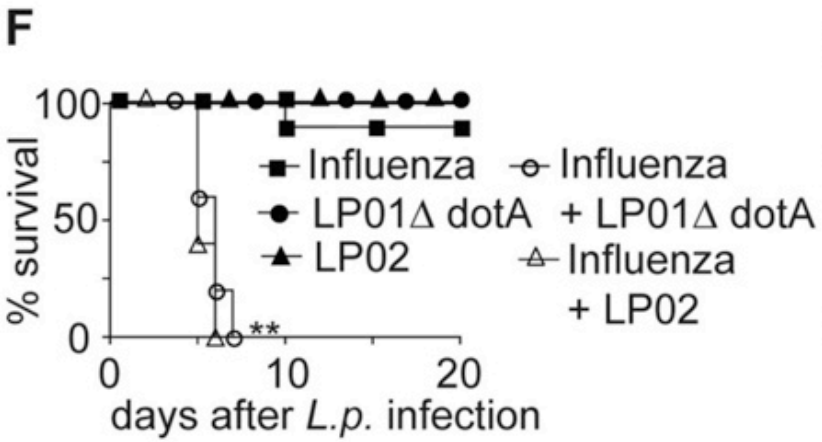
day 0 = 1 h after infection

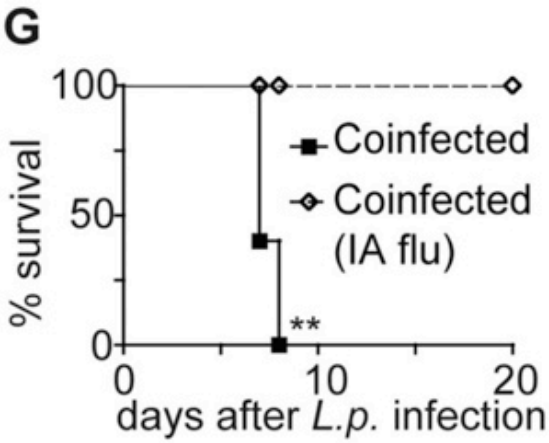


Lethal synergy is not due to impaired resistance to the pathogens



Is mortality due to pathogen virulence?





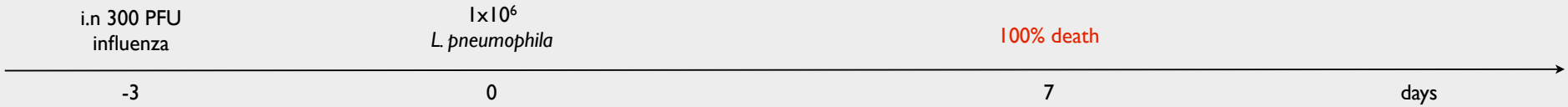
dotaA _ lacks the dot/Icm type IV secretion system

LP02: thymidine auxotroph

Even severely attenuated bacteria leads to mortality

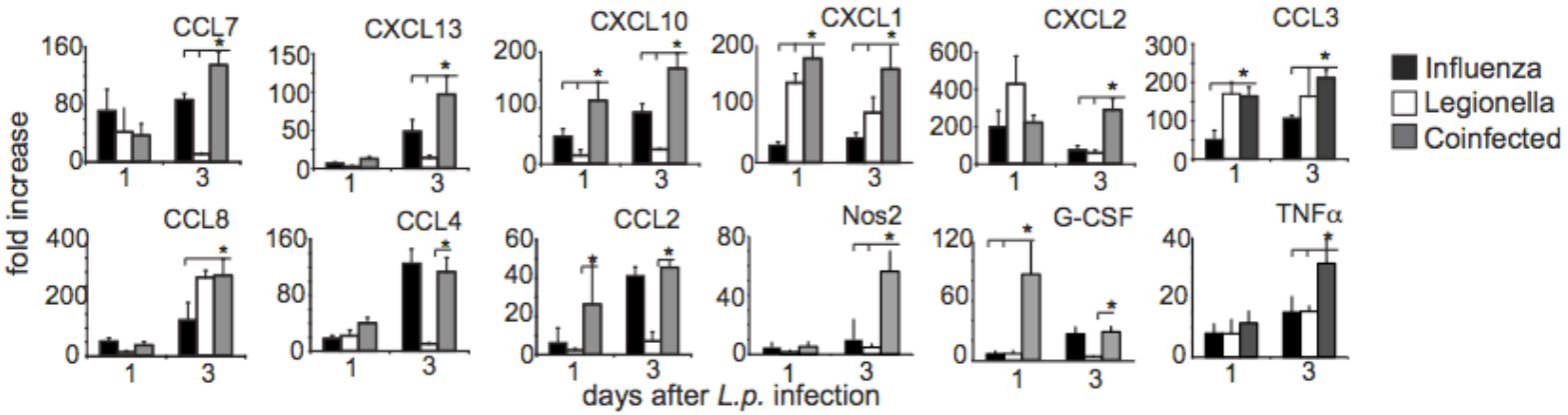
IA flu = formalin inactivated

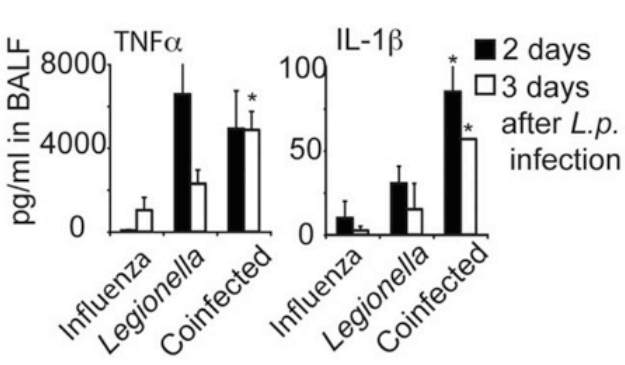
A productive viral infection is necessary to make the host sensitive to a secondary bacterial infection

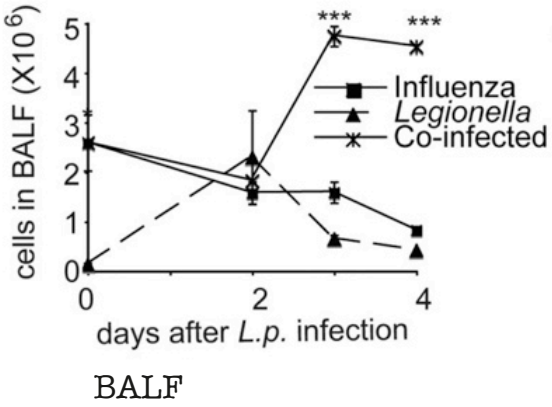


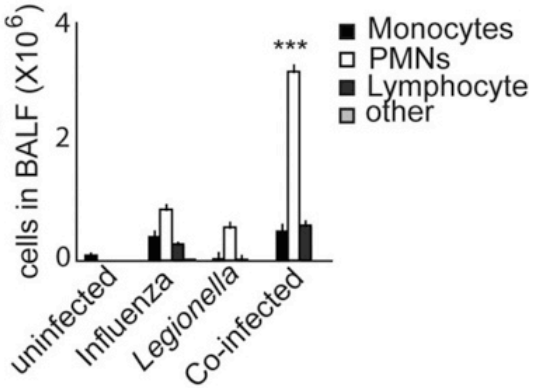
Is mortality due to excessive inflammatory responses?

Gene expression in lungs

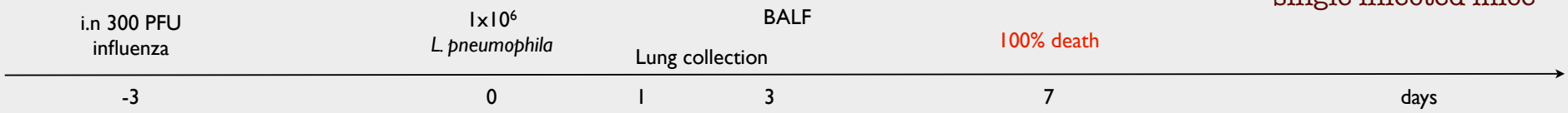




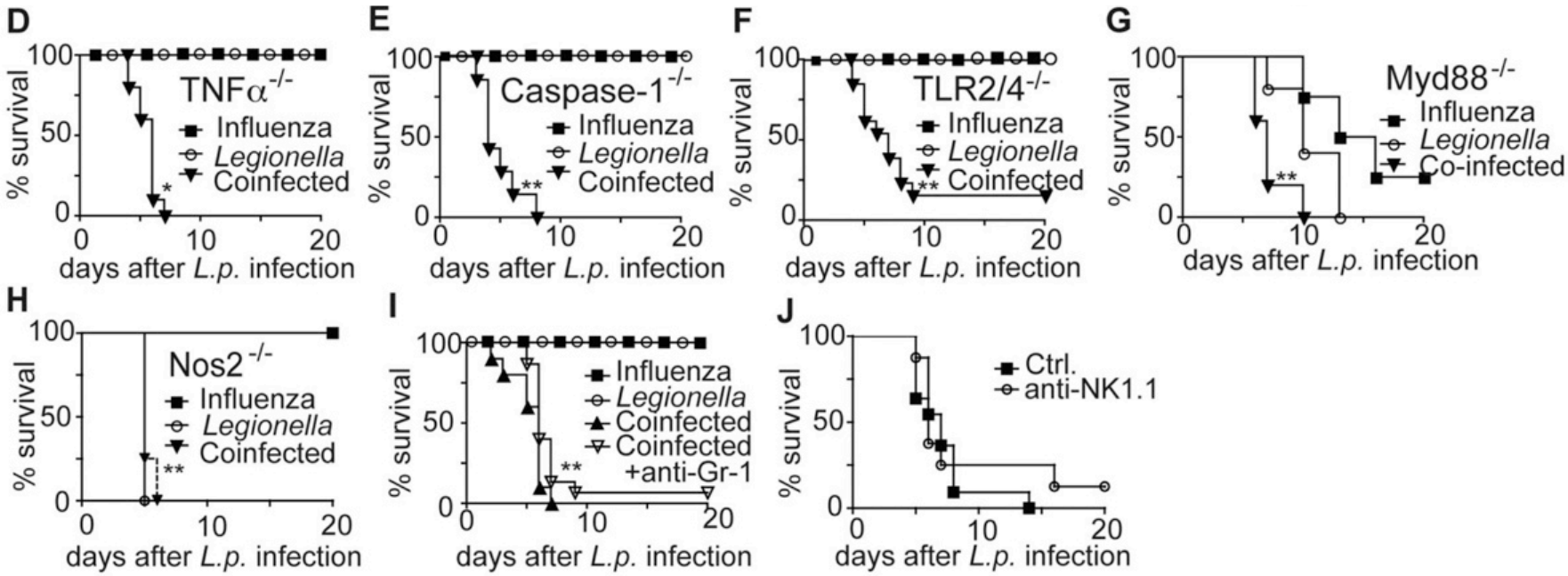




Some inflammatory genes/proteins are expressed/produced at higher levels in coinfected compared to single infected mice



Are increased neutrophil infiltration and inflammatory mediators sufficient to lead to death?



Supp: also susceptible Rag2$^{-/-}$ and IFNαr1$^{-/-}$

Lethal outcome is not solely due to excessive inflmmatory response or immunopathology

i.n 300 PFU influenza

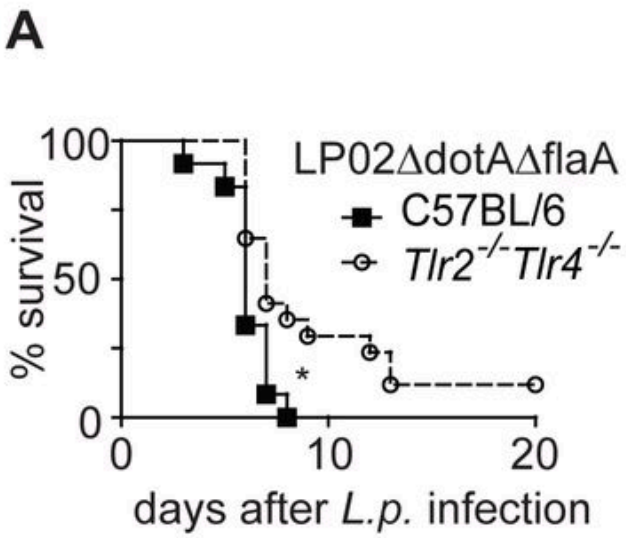
1x10^6 *L. pneumophila*

100% death

-3 0 7 days

Combining host immunodeficiency and bacterial attenuation

Neither bacterial growth nor virulence, nor host immune responses were individually required to cause lethality



: lacks flagellin and is unable to replicate and secrete effectors.

The only immune stimulators left are TLR2/4 agonists

Small increase in survival but most mice still succombed from coinfection

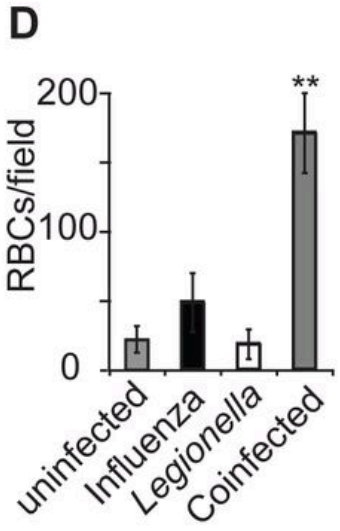
i.n 300 PFU influenza

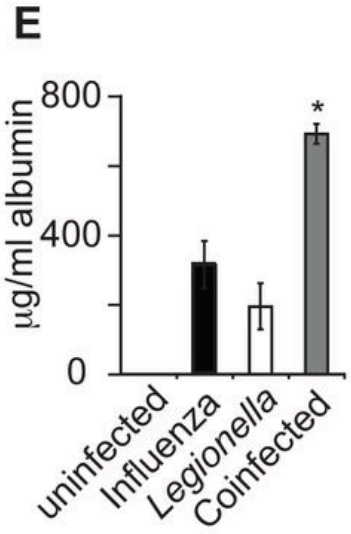
1×10^6 *L. pneumophila*

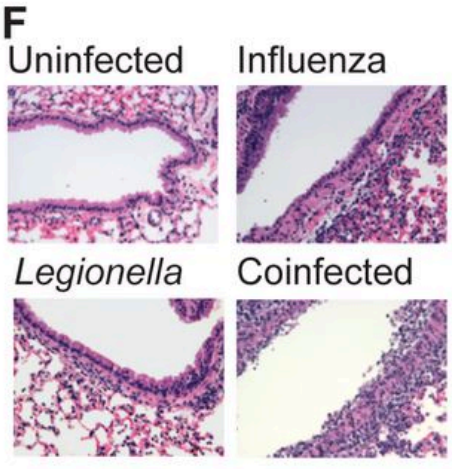
100% death

-3 0 7 days

Is mortality due to failed tolerance to tissue damage?

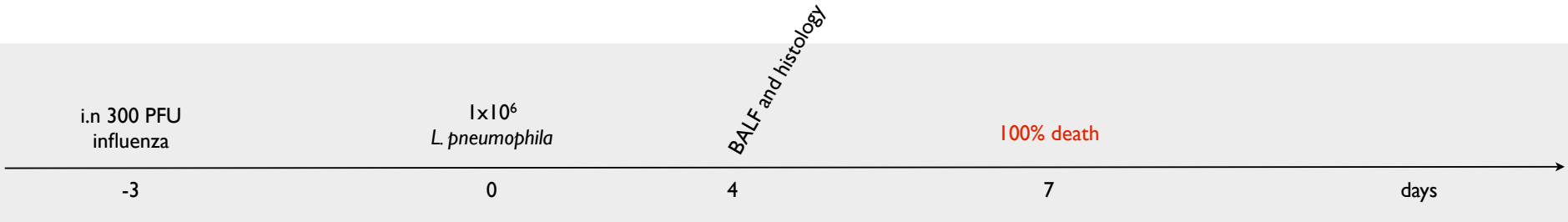






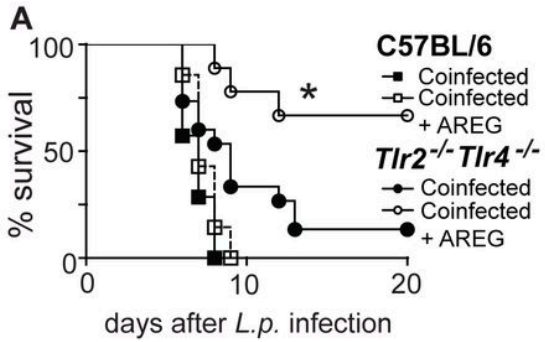
BALF

Damage in lung epithelila-capillary barrier

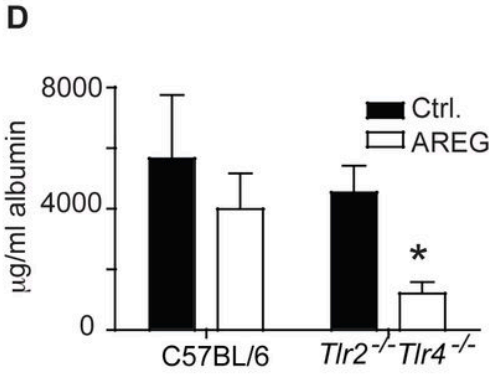


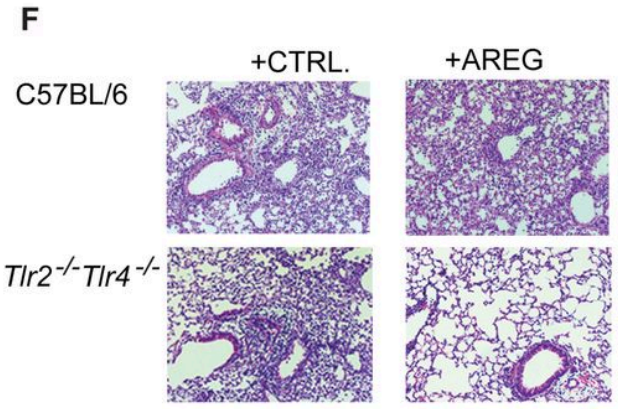
Is mortality due to failed tolerance to tissue damage?

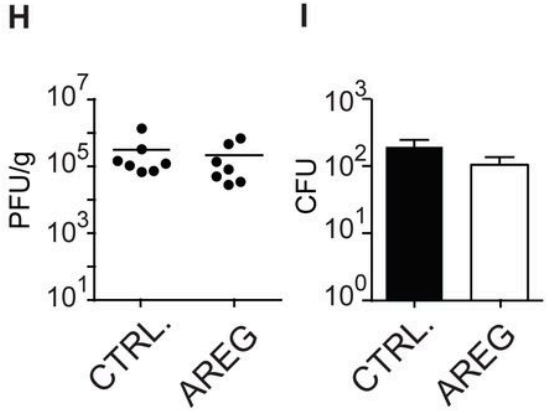
Amphiregulin is an epithelila growth factor family member. Contributes to tissue homeostasis in the lung during influenza infection.



The reason AREG administration did not rescue C57BL/6 mice is likely because the disease is too severe in this case







Viral and bacterial burden remained unchanged

i.n 300 PFU influenza

1x10⁶ *L. pneumophila*

I.P. daily with 10 g of AREG

100% death

-3 0 7 days

Conclusions

- Lethal synergy of inflenza virus and bacteria coinfection can result from loss of tolerance to infection-induced tissue damage

- Morbidity and mortality of coinfection can be independent of pathogen burden or excessive inflammatory response

- Promoting tissue repair can in principle rescue coinfected animals from morbidity and mortality even without affecting pathogen burden

- **2 distinctive host defense strategies: resistance and tolerance**

nature medicine

Inflammatory monocytes regulate pathologic responses to commensals during acute gastrointestinal infection

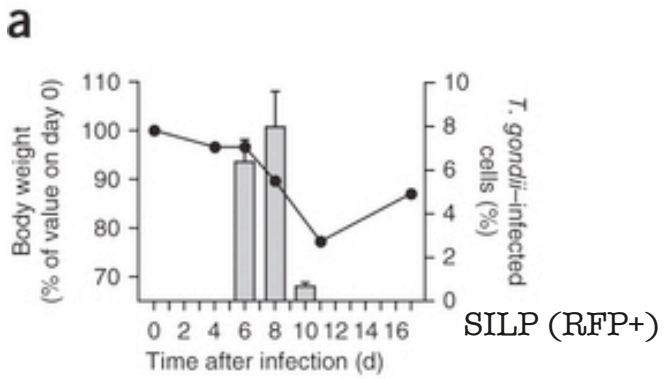
John R Grainger[1], Elizabeth A Wohlfert[1], Ivan J Fuss[2], Nicolas Bouladoux[1], Michael H Askenase[1,3], Fanny Legrand[4], Lily Y Koo[5], Jason M Brenchley[6], Iain D C Fraser[7] & Yasmine Belkaid[1]

- During acute intestinal inflammation Ly6C^{hi} inflammatory monocytes and neutrophils infiltrate the intestine

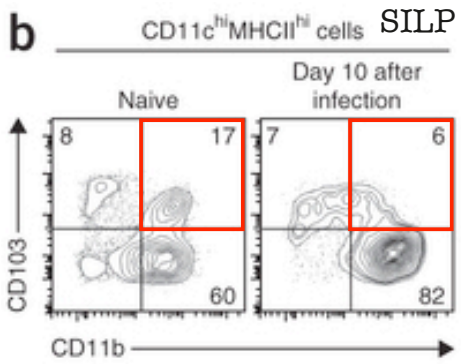
- Neutrophils are involved in pathogen clearance but also collateral tissue damage (ROS, superoxides, proteases and cytokines)

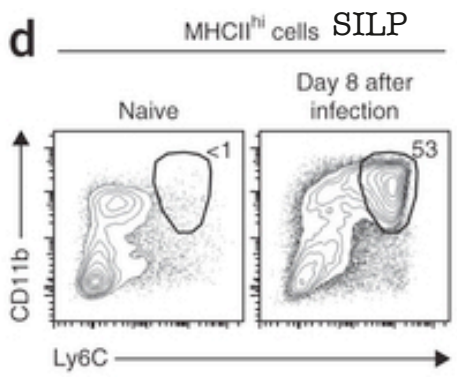
Are there regulatory mechanisms during acute intestinal inflammation that dampen the pathogenic potential of neutrophils?

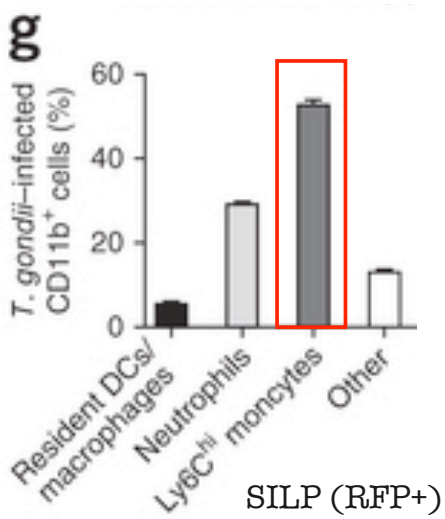
Mucosal *T. gondii* infection



SILP (RFP+)





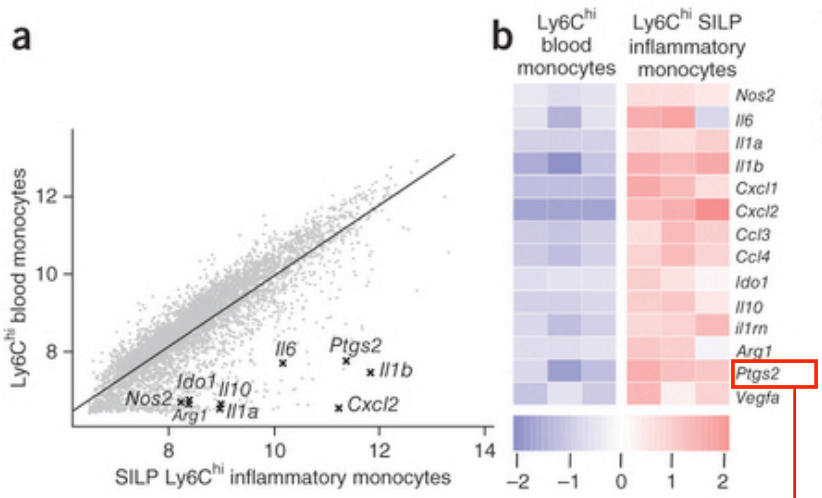


SILP (RFP+)

Following infection Ly6C^{hi} monocytes proportions increase and are the main population infected with T.gondii

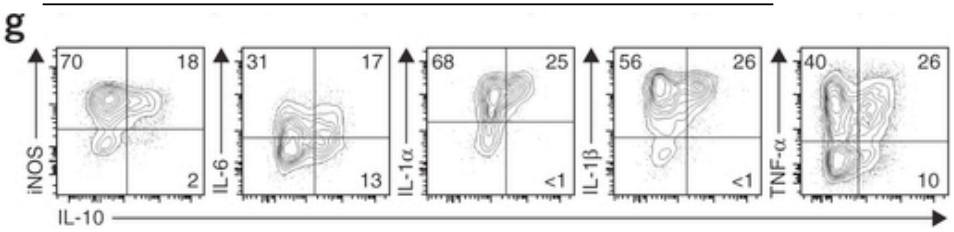
10 bradizoites RFP-expressing cysts (oral) - day 8-10 p.i

Do blood-and SILP-derived Ly6C^{hi} monocytes have different functional phenotyes?

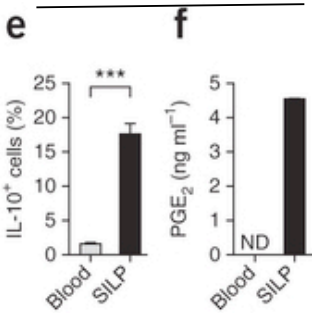


each = 1 biological sample (FACS-sorted monocytes)

Gated on Ly6C^{hi} CD11b^{hi} MHCHII^{hi}
No stimulation (only Brefeldin A)



FACS sort
- 18h culture - EIA



prostaglandin-endoperoxide synthase 2 encodes COX-2, a key enzyme in prostaglandin synthesis.

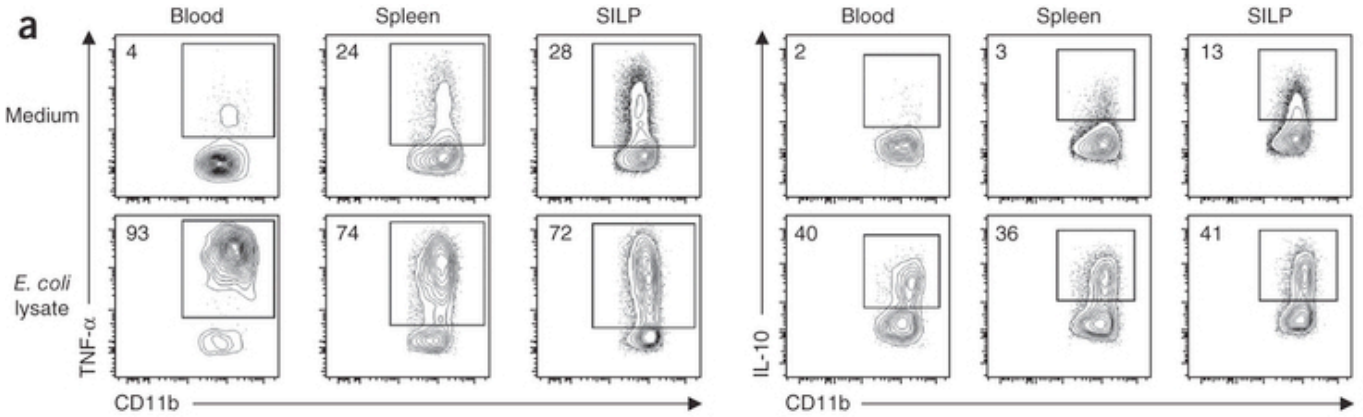
PGE2 can be produced by all cell types of the body. Epithelia, fibroblasts, and infiltrating inflammatory cells representing are the major sources.
PGE2 can exert suppressive effects on innate cells
Kalinski, P. (2012). Regulation of immune responses by prostaglandin E2. The Journal of Immunology, 188(1), 21–28. doi:10.4049/jimmunol.1101029

Inflammatory monocytes in the small intestine can also adopt regulatory functions

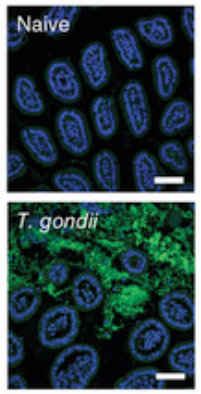
10 bradizoites cysts (oral) - day 8 p.i

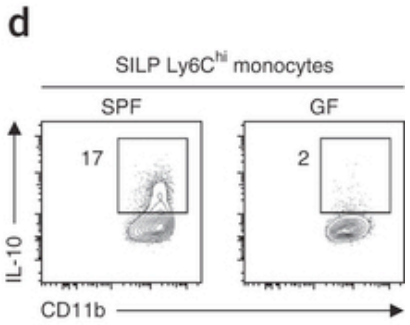
Can intestinal microbial products drive the regulatory phenotype of monocytes?

Gated on Ly6C^{hi} CD11b^{hi} MHCHII^{hi}
Medium: no stimulation, only Brefeldin A



Ileum
FISH with eubacterial 16S probe





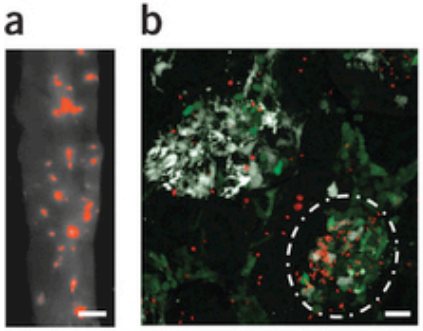
Ly6C^{hi} monocytes acquire the ability to produce IL-10 and PGE2 in response to commensal products

10 bradizoites cysts (oral) - day 8 p.i

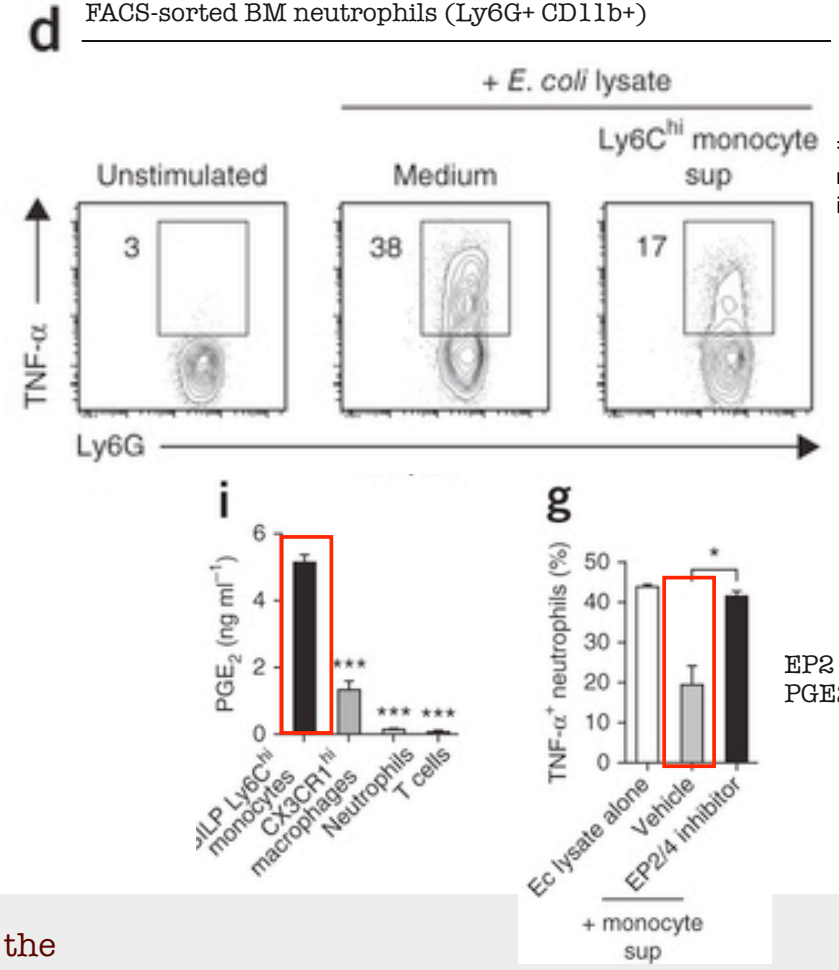
What cells types are being regulated by inflammatory monocytes?

Jejunum
Confocal microscopy
CD11c - white;
LysM - green
$CD11c^{YFP}$ x $LysMe^{GFP}$

Jejunum



Lysozyme is expressed by monocytes and neutrophils



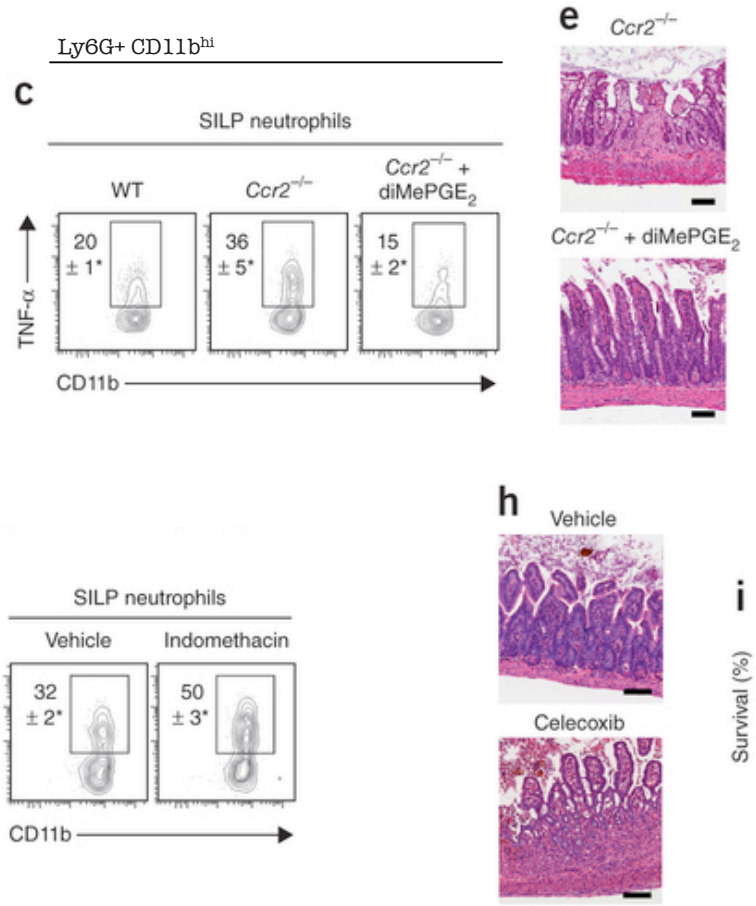
= FACS-sorted Ly6Chi monocytes cultured overnight in the presence of E. coli lysate

EP2 and EP4 are PGE2 receptors

in vitro, $Ly6C^{hi}$ monocytes regulate the inflammatory potential of neutrophils via PGE2

10 bradizoites cysts (oral) - day 8 p.i

Are neutrophils modulated by monocyte-derived PGE2 *in vivo*?



Indomethacin: a COX-1 and COX-2 inhibitor
Celecoxib: a specific COX-2 inhibitor

PEG2-derived from Ly6C^{hi} can limit lethal neutrophil-mediated immunopathology

10 bradizoites cysts (oral) - starting at day 6: daily injection of PEG2, indomethcin or celecoxib - readout day 8 p.i

Conclusions

- Commensals (?) trigger a regulatory programm (**PGE2**, IL-10, arginase and IDO) in Ly6C^{hi} inflammatory monocytes that limit neutrophilic pathogenic potential

- This dual phenotype (regulatory and inflammatory) endows monocytes to control parasite burden while limiting collateral damage to tissue