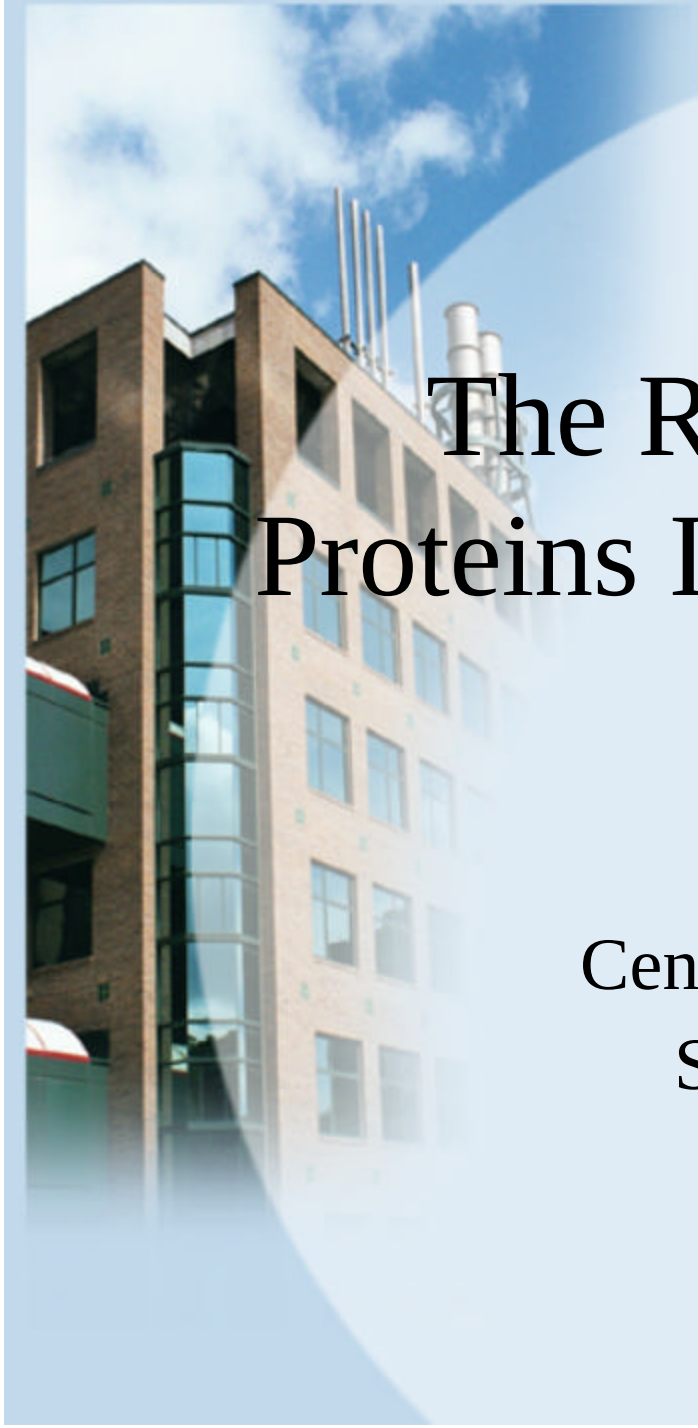
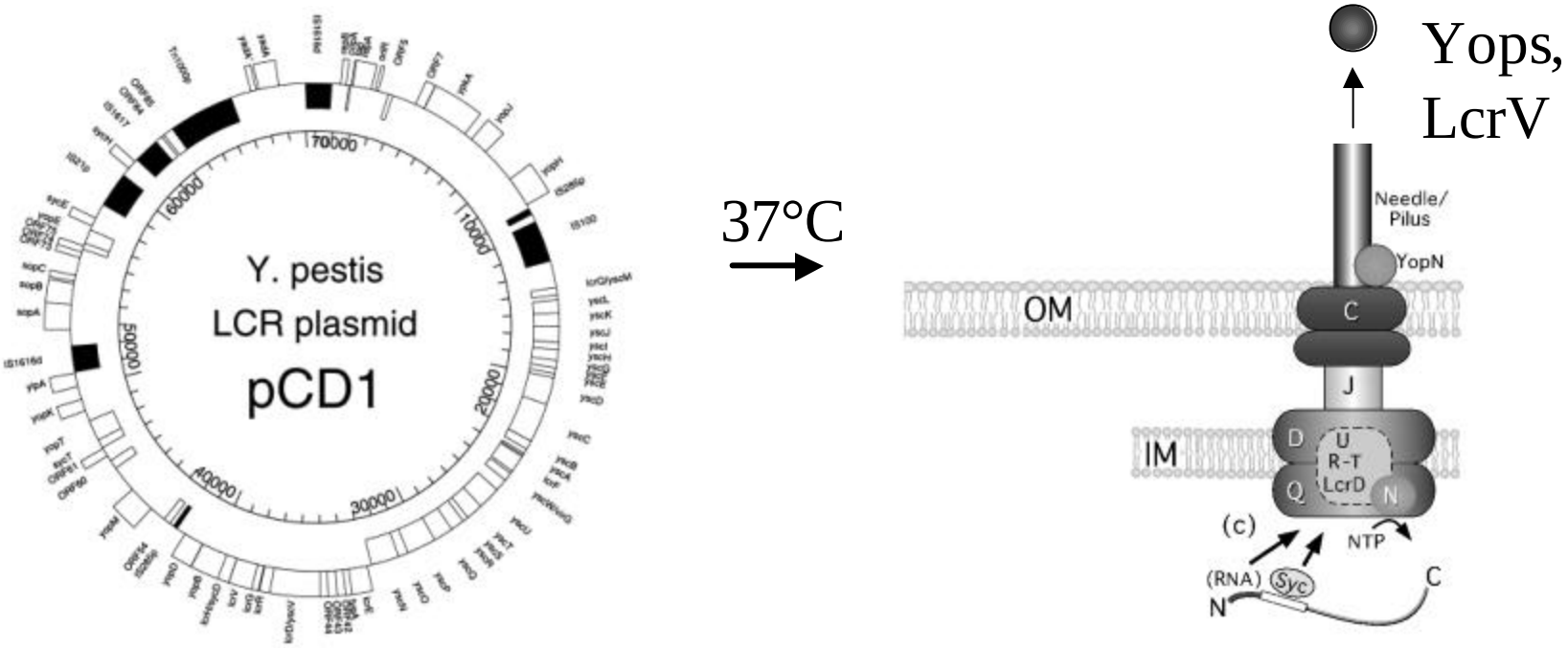


STATE UNIVERSITY OF NEW YORK
STONY BROOK
SCHOOL OF MEDICINE
Center for Infectious Diseases

The Role of Yop Effector Proteins In Disease Pathogenesis

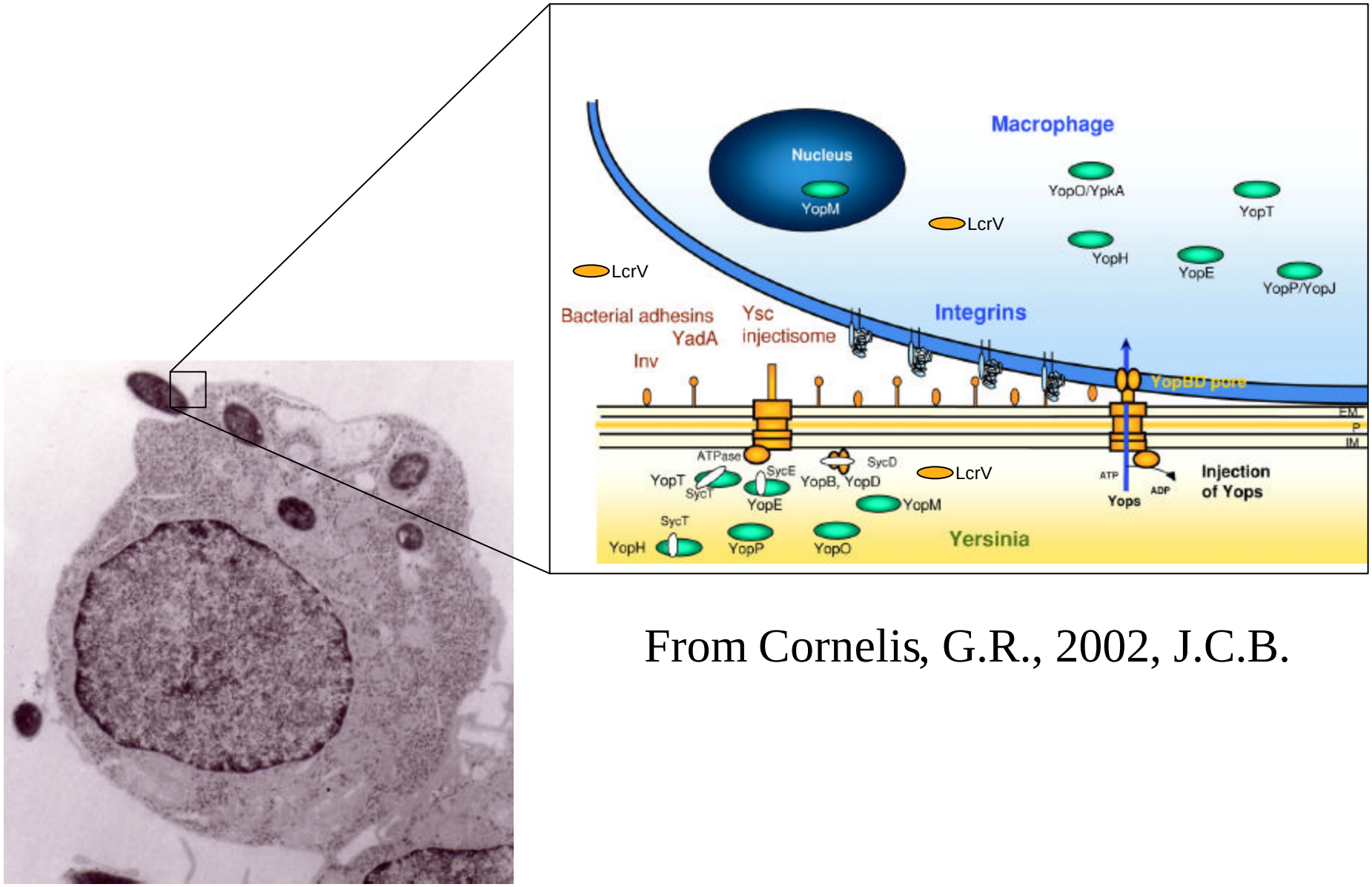
James Bliska, Ph.D.
Center for Infectious Diseases
Stony Brook University

pCD1 encodes a type III protein secretion system (TTSS)



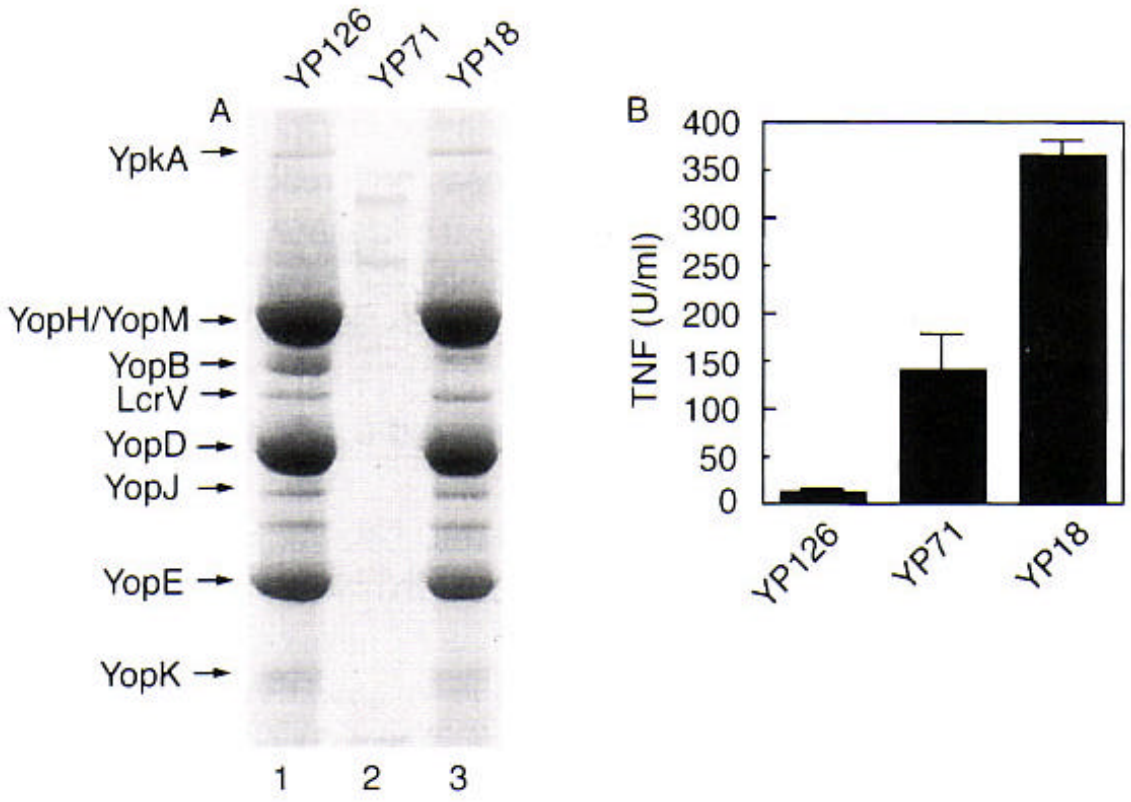
Adapted from:
Cornelis, Nature Reviews, 2002
Thanassi and Hultgren, Curr. Opin. Cell. Biol., 2000

Model of type III secretion

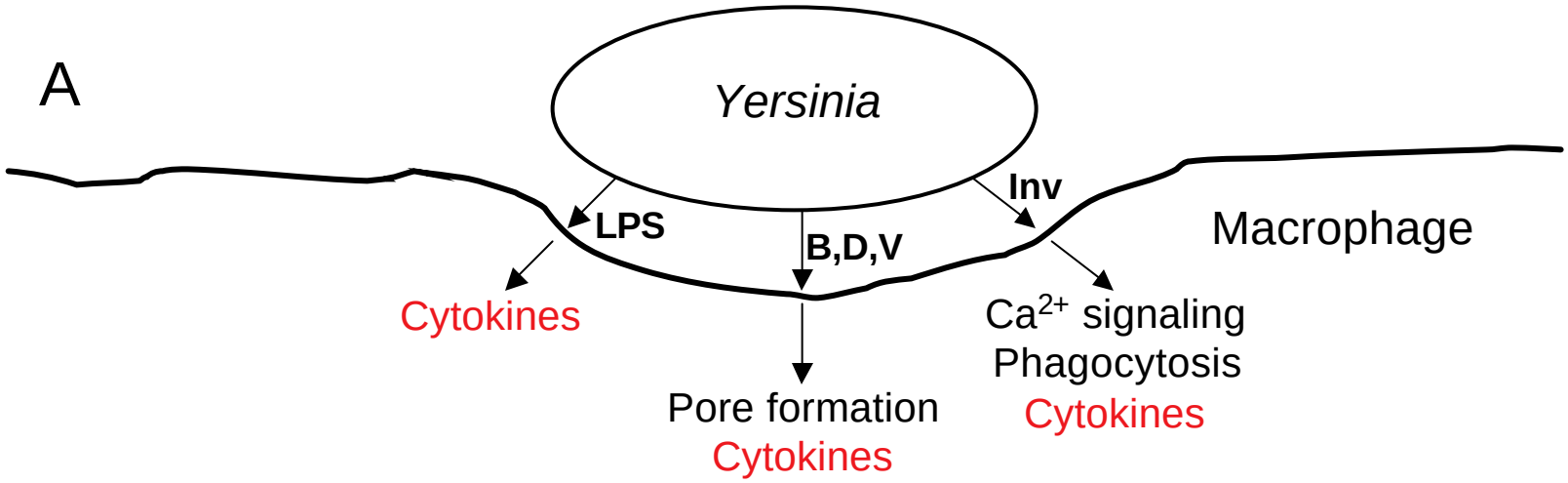


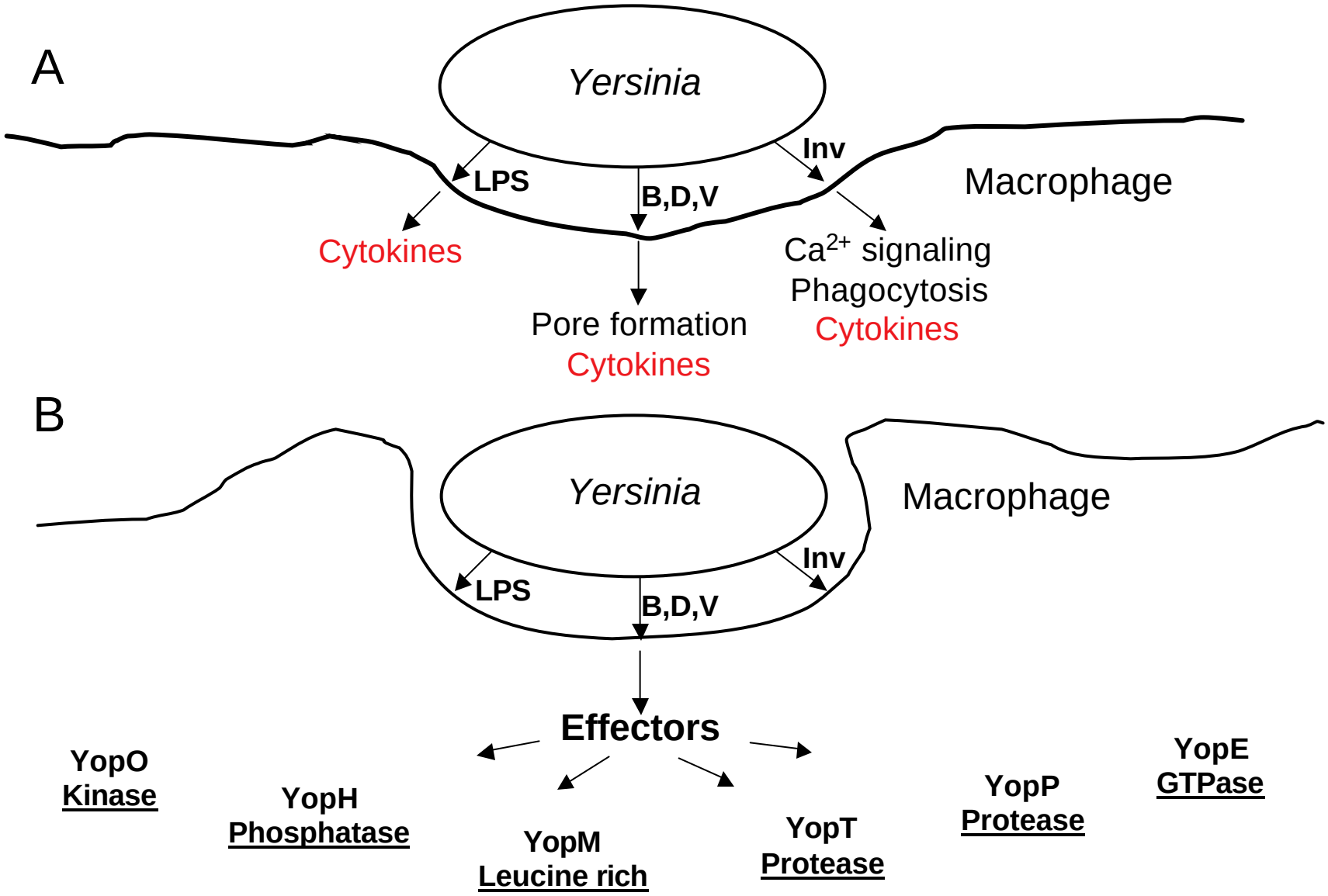
From Cornelis, G.R., 2002, J.C.B.

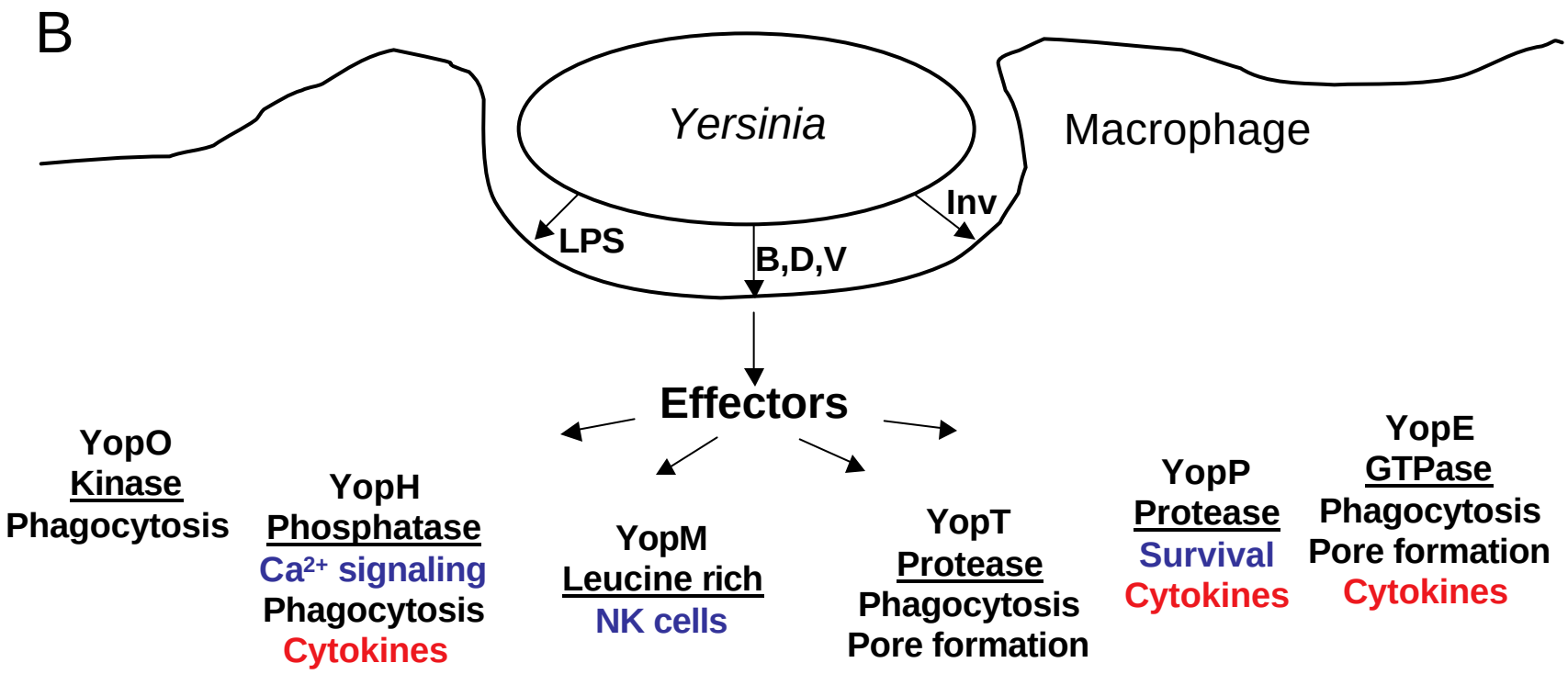
Type III secretion counteracts TNFα production in macrophages



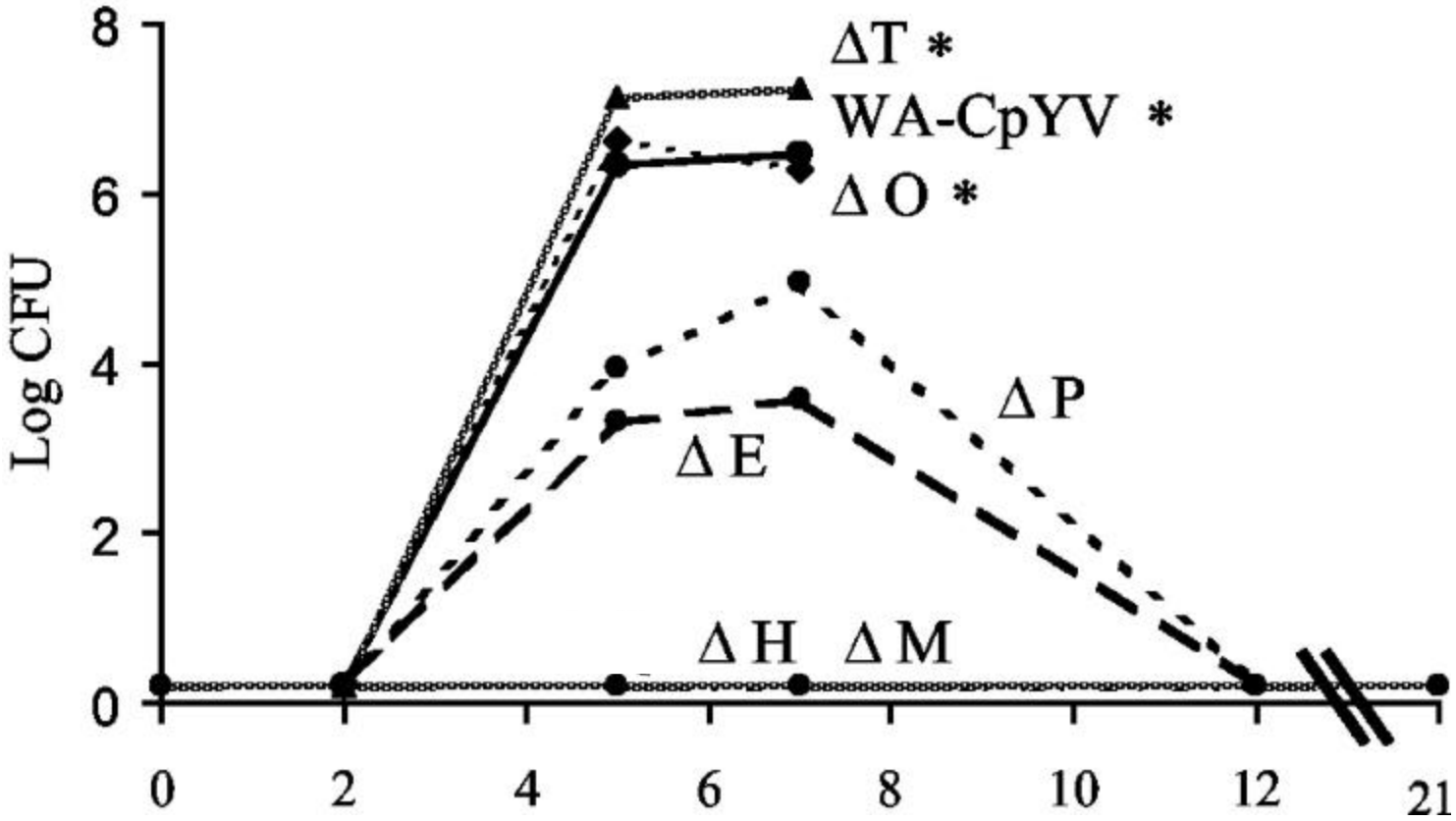
Palmer et al. 1998


A
Yersinia
LPS
B,D,V
Inv
Macrophage
Cytokines
Pore formation
Cytokines
Ca2+ signaling
Phagocytosis
Cytokines


A
Yersinia
LPS
B,D,V
Inv
Macrophage
Cytokines
Ca2+ signaling
Phagocytosis
Cytokines
Pore formation
Cytokines
B
Yersinia
Macrophage
LPS
B,D,V
Inv
Effectors
YopO
Kinase
YopH
Phosphatase
YopM
Leucine rich
YopT
Protease
YopP
Protease
YopE
GTPase


B
Yersinia
Macrophage
Inv
LPS
B,D,V
Effectors
YopO
Kinase
Phagocytosis
YopH
Phosphatase
Ca2+ signaling
Phagocytosis
Cytokines
YopM
Leucine rich
NK cells
YopT
Protease
Phagocytosis
Pore formation
YopP
Protease
Survival
Cytokines
YopE
GTPase
Phagocytosis
Pore formation
Cytokines

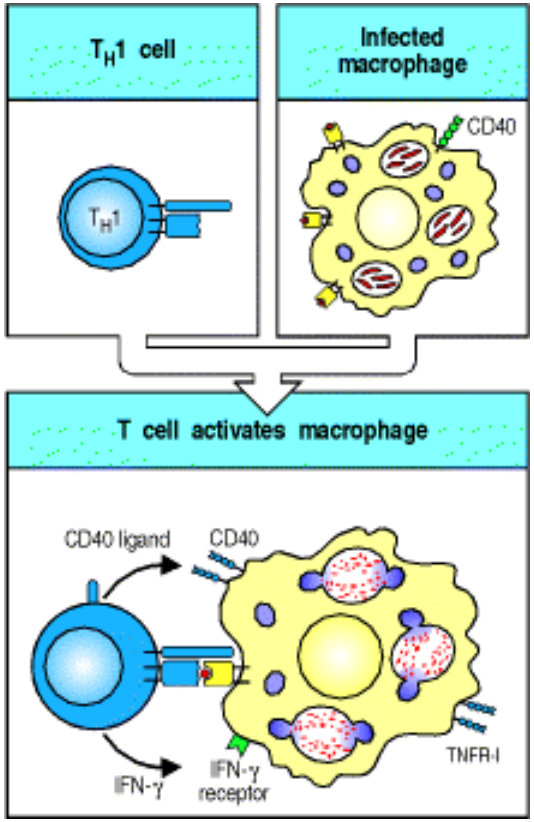
Role of Yops in colonization of mouse spleen by *Y. enterocolitica*



Trülzsch 2004, Infect Immun

A T_H1-directed immune response is protective against *Yersinia* infections

- IL-12, IFNγ and TNFα are required for protection
  - Nakajima, 1993
  - Bohn, 1996
- IL-12
  - Secreted by dendritic cells and macrophages
  - Activates T_H1 cells and NK cells to secrete IFNγ
- IFNγ
  - Activates macrophages
- TNFα
  - Pleotrophic; activates macrophages



From Immunobiology. C. Janeway, Ed.

IFNγ and TNFα promote protection against plague

Nakajima and Brubaker, 1993

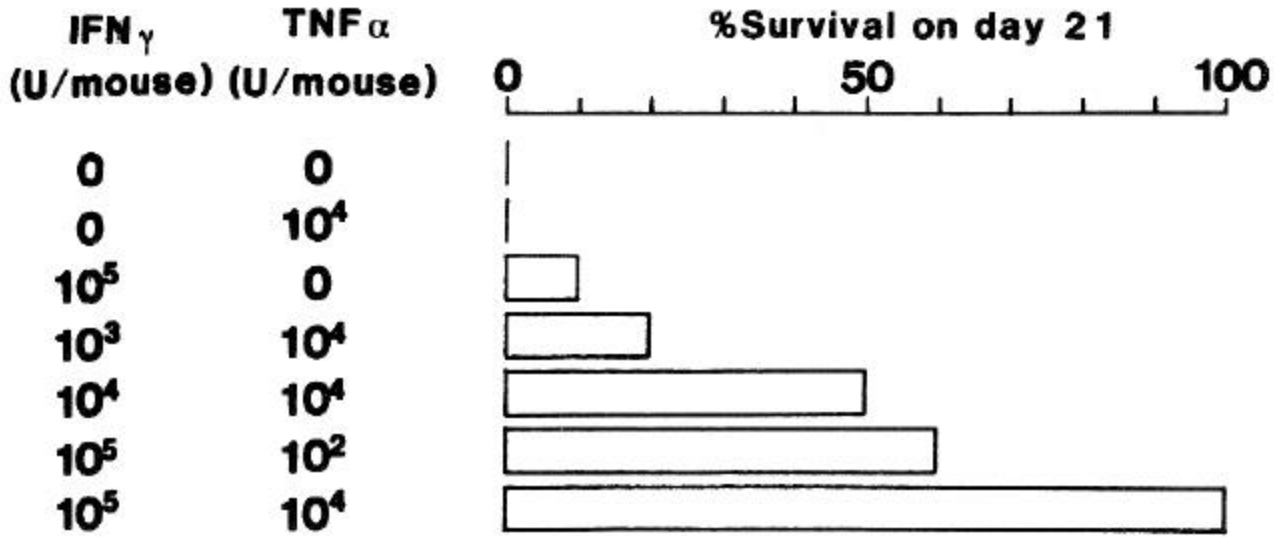
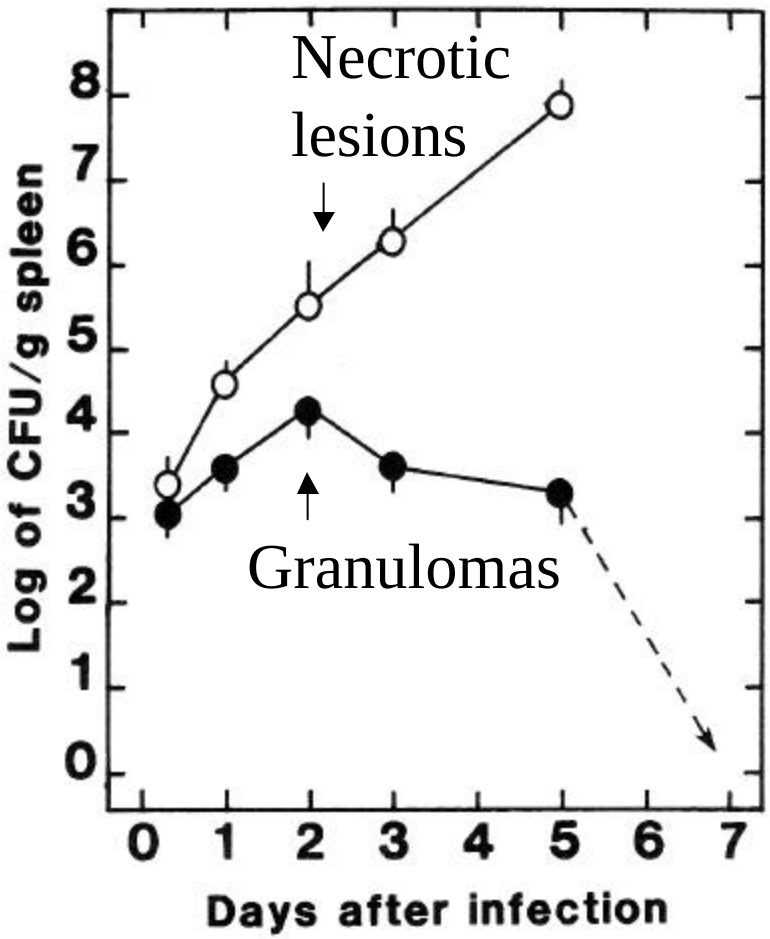


FIG. 1. Protection of C57BL/6 mice by combinations of intraperitoneally injected mouse recombinant IFN-γ and TNF-α against intravenous challenge against 10 MLD of Lcr^+ cells of *Y. pestis* KIM. IFN-γ and TNF-α were injected 6 and 2 h, respectively, before infection.

Priming with IFNγ+TNFα prevents bacterial growth in spleens

Nakajima and Brubaker, 1993



○ No cytokines

● IFNγ+TNFα

A Paradox:

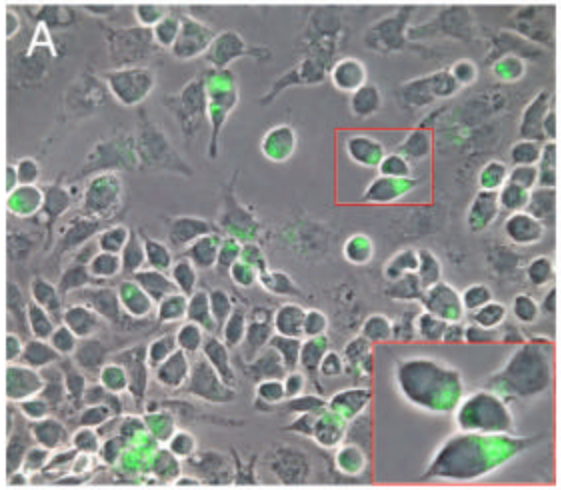
- How can activated macrophages protect if yersiniae are exclusively extracellular pathogens?

Observations:

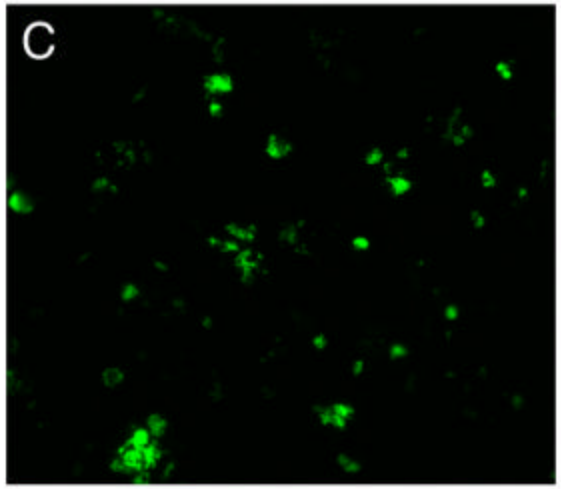
- Yersiniae are not fully antiphagocytic at early stages of infection
  - Cavanaugh, 1959
- Yersiniae do not kill macrophages by apoptosis at low multiplicities of infection
  - Goguen, 1986
- Yersiniae can survive and replicate in naïve macrophages
  - Cavanaugh, 1959
  - Straley, 1984

Pathogenic *Yersinia* survive and replicate in macrophages

Phase+GFP



GFP

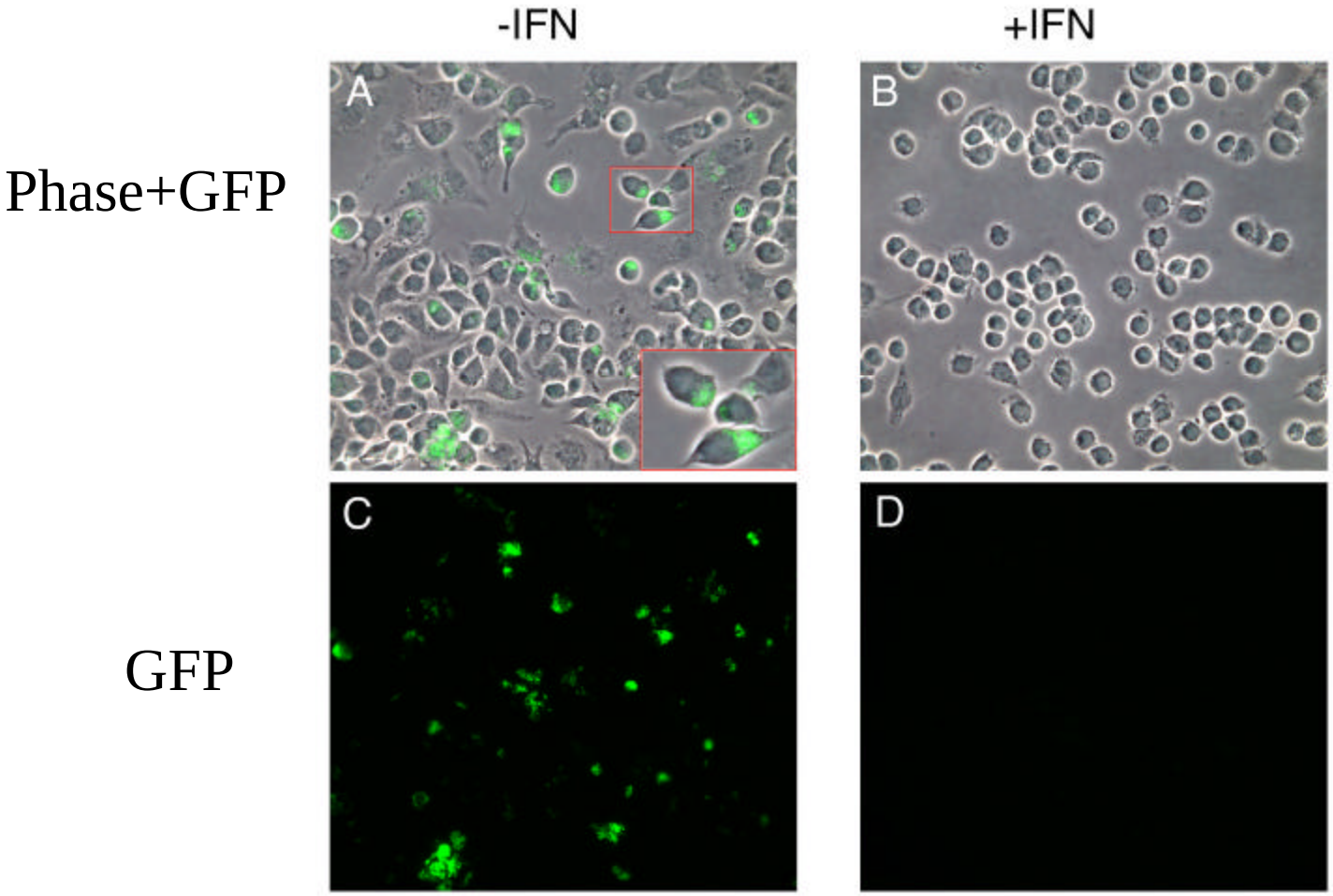


Pujol and Bliska, 2003, 2004; Grabenstein et al. 2004

A solution to the paradox:

- Activated macrophages are protective because they can eliminate intracellular yersiniae and drive a T_H1 response
- Yops and LcrV prevent production of activated macrophages by counteracting proinflammatory cytokine production

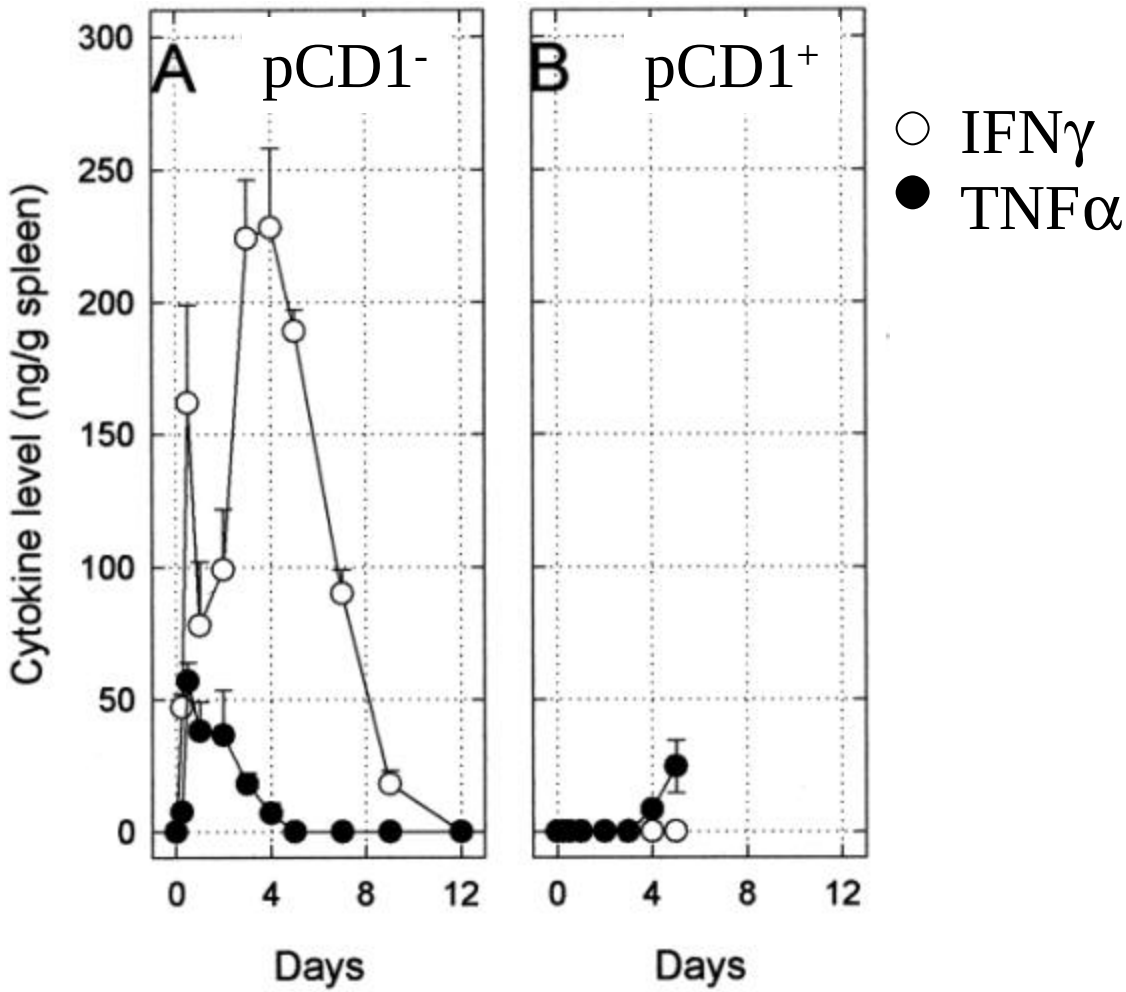
IFNγ priming prevents intracellular replication

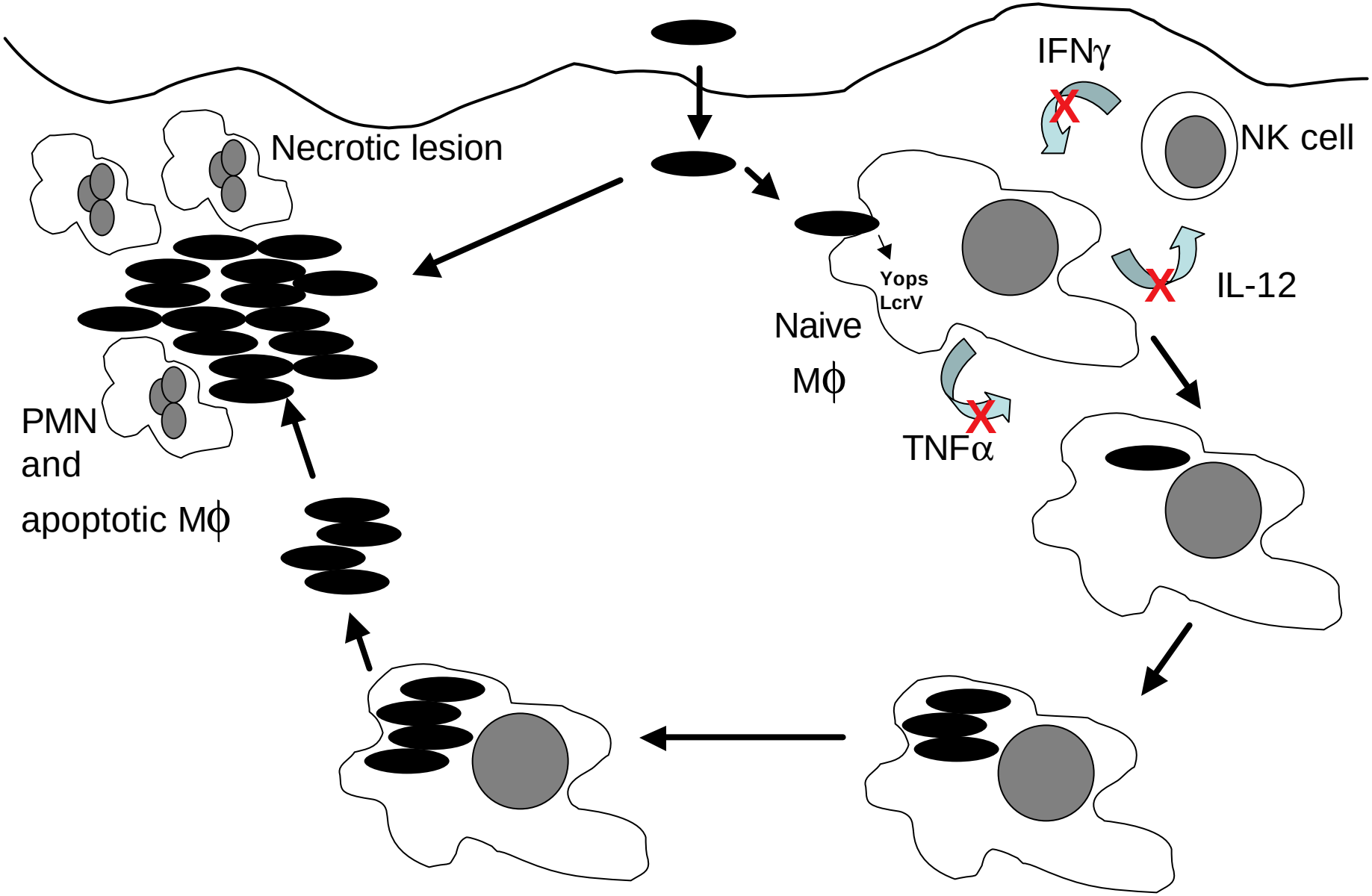


Pujol and Bliska, unpublished

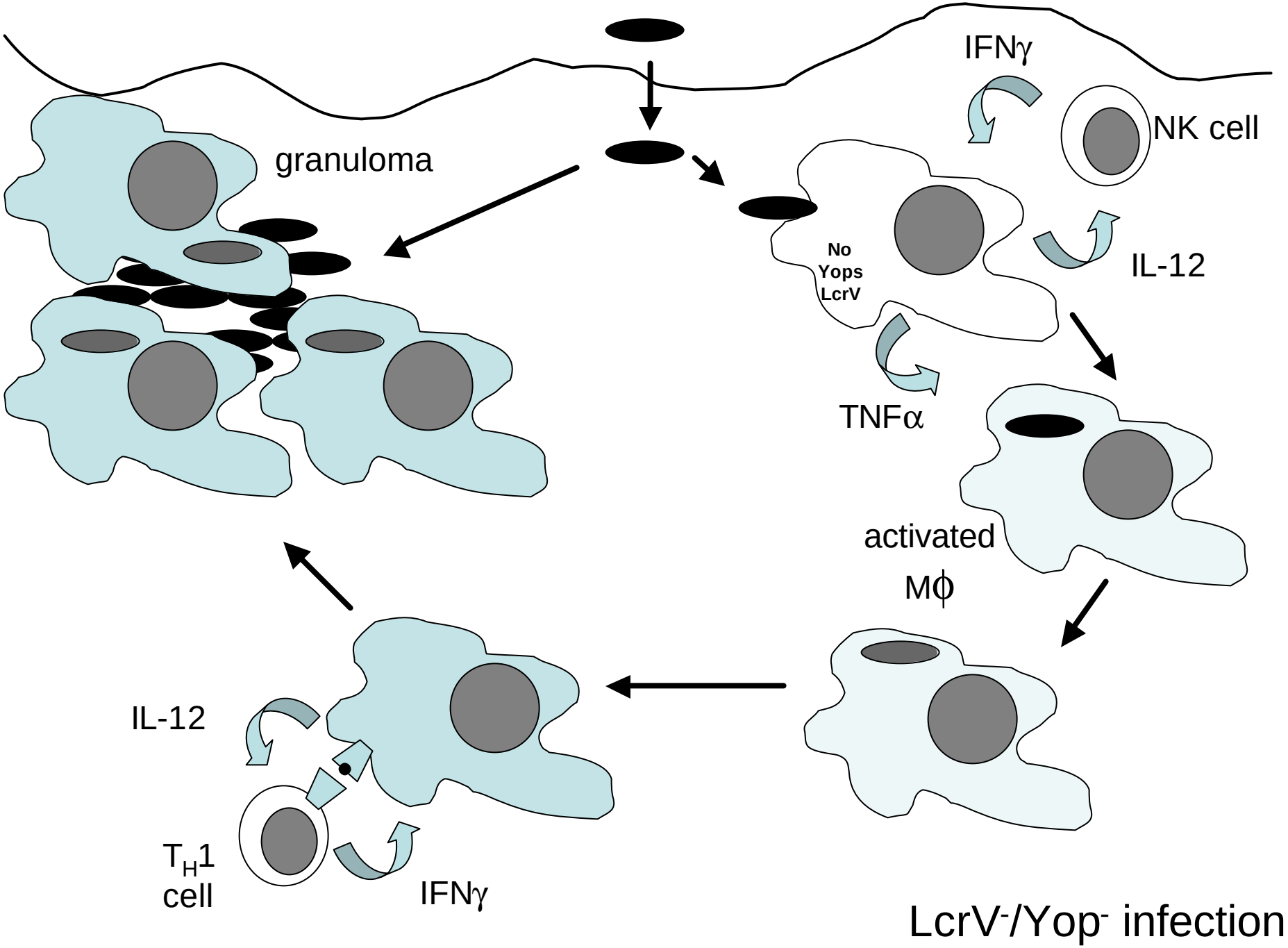
The virulence plasmid counteracts cytokine production in vivo

Brubaker, 2003

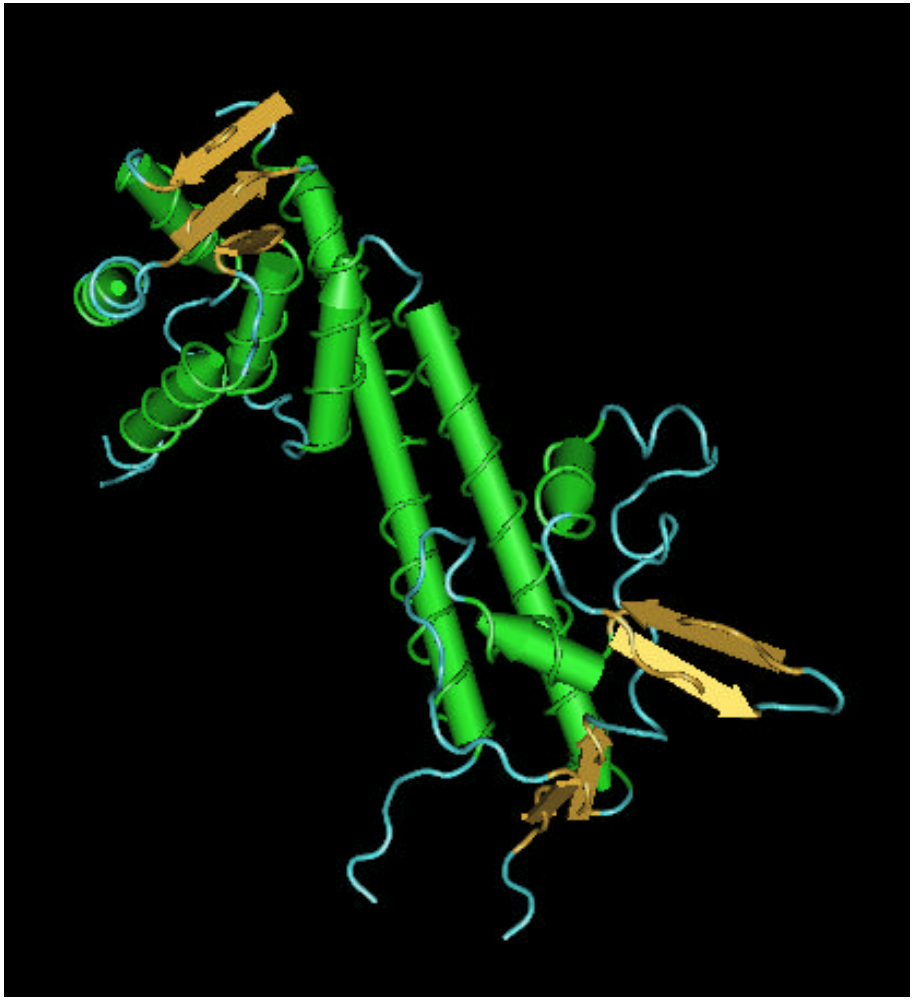




WT infection


IFNγ
NK cell
granuloma
No
Yops
LcrV
IL-12
TNFα
activated
Mϕ
IL-12
T_H1
cell
IFNγ
LcrV-/Yop- infection

LcrV is a multifunctional protein

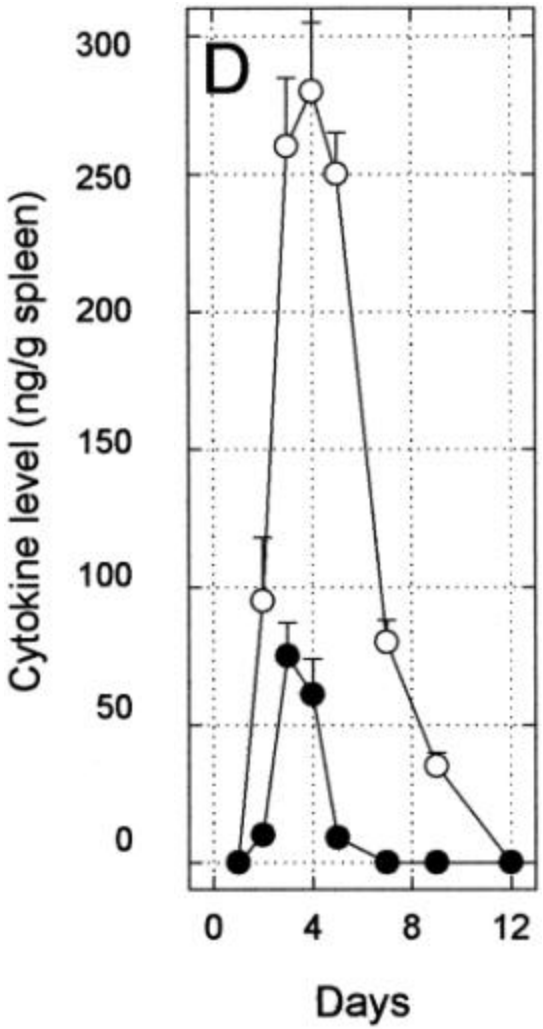


Structure from Derewenda et al, 2004

- Protective antigen
  - Burrows 1956
- Regulates TTSS
  - Price et al 1991
- Inducer of IL-10
  - Nakajima et al 1995
- Required for Yop translocation
  - Nilles et al 1998
  - Pettersson et al 1999
- TLR2 ligand
  - Sing et al 2002

Passive immunization with anti-LcrV restores cytokine production

Brubaker, 2003



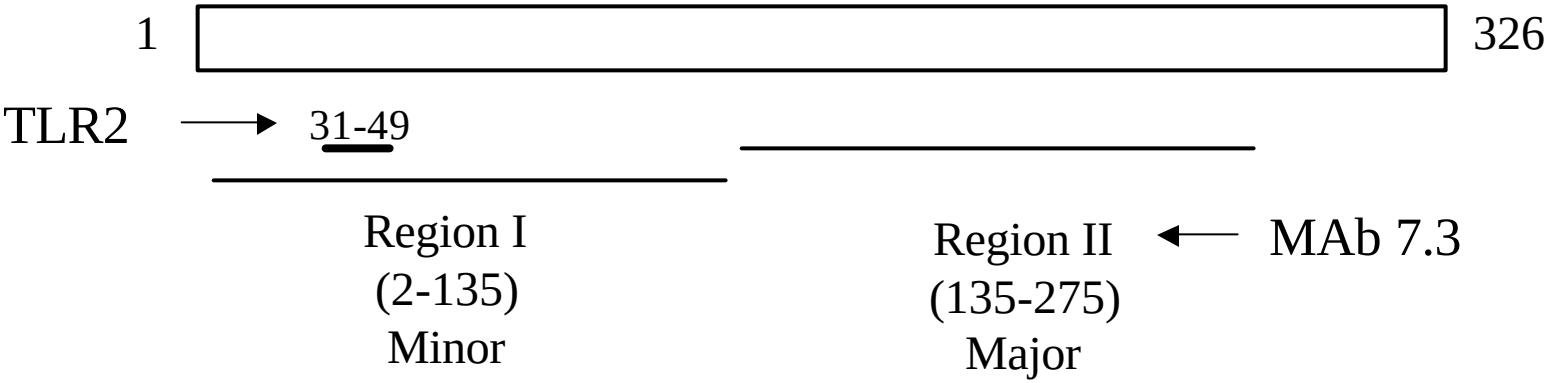
pCD1^{+}

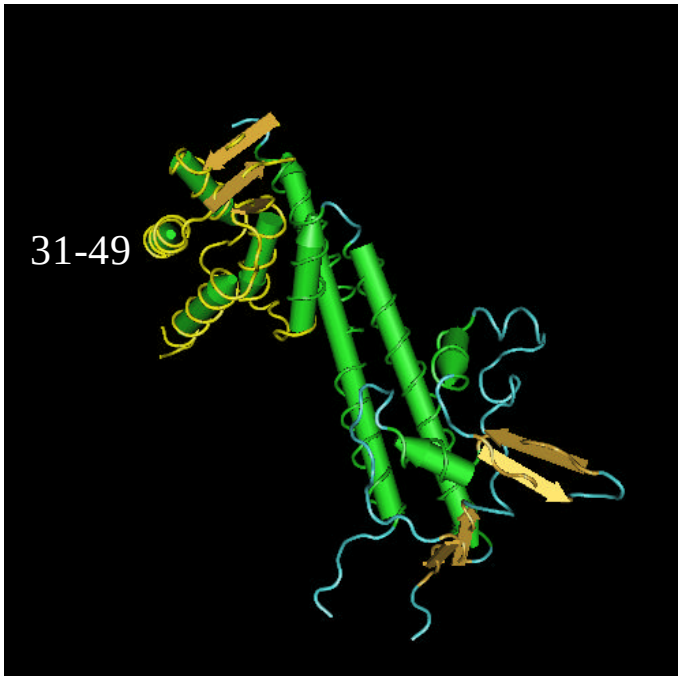
○ IFNγ

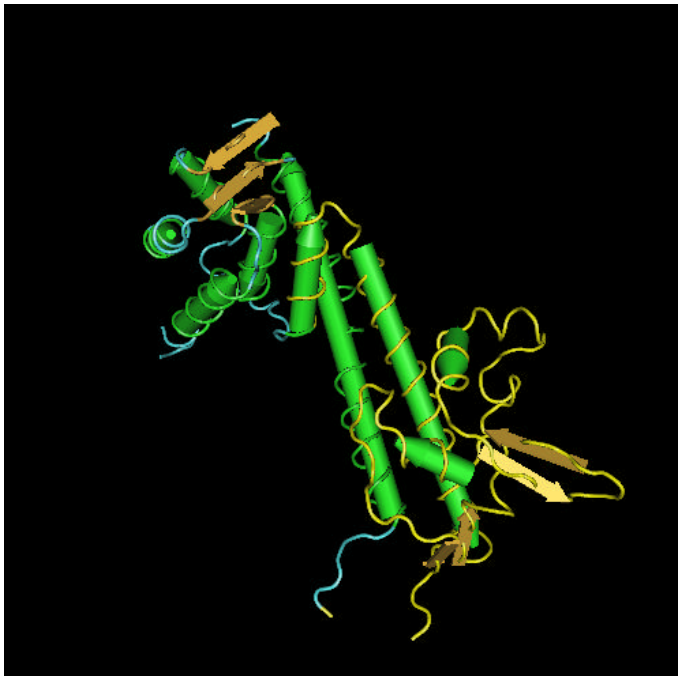
● TNFα

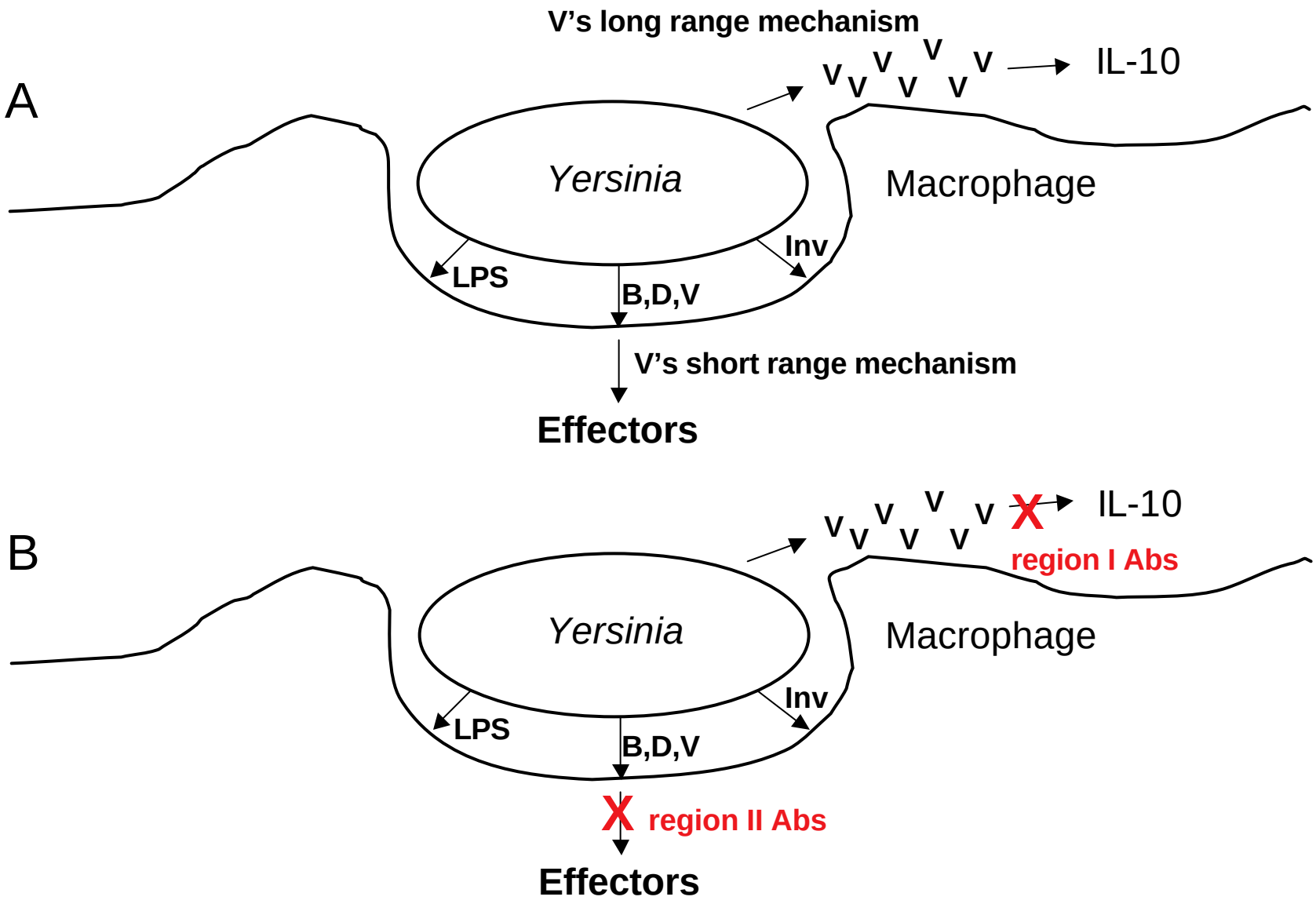
Functional regions of LcrV

Motin et al 1995, Hill et al 1997, Sing et al 2002








A
V's long range mechanism
V V V V V V
IL-10
Yersinia
Macrophage
Inv
LPS
B,D,V
V's short range mechanism
Effectors
B
V V V V V V
X
IL-10
region I Abs
Yersinia
Macrophage
Inv
LPS
B,D,V
X
region II Abs
Effectors

Summary

- Yops function in concert with LcrV to target several key immune response pathways in macrophages
- Yops and LcrV counteract proinflammatory cytokine production to prevent T_H1 response and macrophage activation
- Antibodies directed to region I and region II epitopes in LcrV neutralize distinct functions
  - Region I→IL-10 inducing activity
  - Region II →Yop translocation

Acknowledgements

- **Bliska Laboratory**

HaChung Chung
Jens Grabenstein
Maya Ivanov
Betty Noel
Celine Pujol
Ryan Rampersaud
Michelle Ryndak
Gloria Viboud
Yue Zhang

- **Past**

Deborah Black
Lee Montagna
Lance Palmer

- **Funding**

NIAID