Pfeifer's Lab Journal Club

Tufts University
Chemical and Biological Engineering
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Delivery of protein to the cytosol of macrophages using Escherichia coli K-12

Higgins et al. 1999

www.taipeitimes.com/.../2005/08/23/2005041006, www.berekely.edu

Outline

- Background
- Experiments & Results
- Discussion

Background

Importance of Protein Delivery

- Induction of cell-mediated immunity
- Vaccine design
- Efficient delivery

  - Generation of transfected phenotypes
  - Study of protein functions and localization

www.answers.com/topic/stamp-ctc-polio-vaccine-jpg

Existing Methods

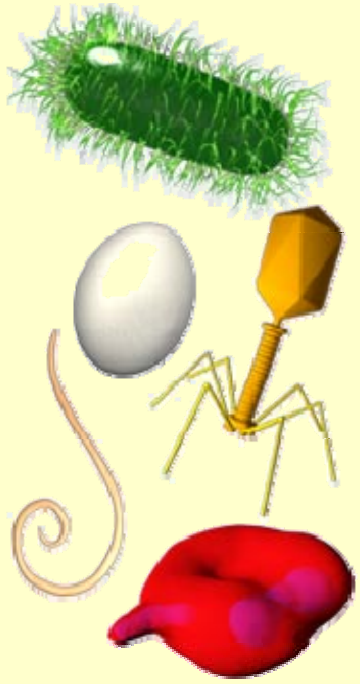
- **Mechanical techniques**:
  - Electroporation
  - microinjection
- **Fusion Methods**:
  - Vesicles
  - Liposomes
- **Chemical treatments**:
  - ATP
  - EDTA
- **External addition w/ pore-forming toxins**:
  - α-toxin of *S. aureus*

Disadvantages:

- Protein to be delivered needs laborious purification
- Methods limited to *in vitro* experiments

Biological Vectors

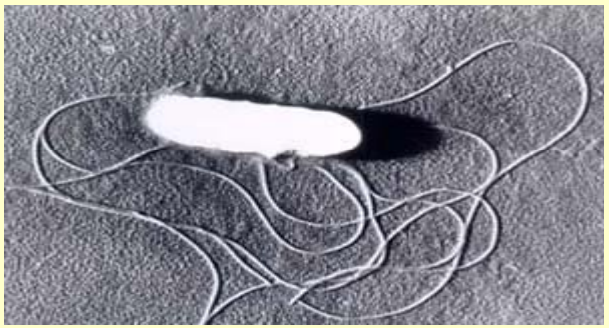
- Retroviruses
  - Delivery and expression of DNA in host cells
- Bacteria
  - Delivery of antigenic proteins
  - Much work done on DNA delivery to mammalian cells



www.sdnhm.org/.../teachers/background.html

Listeria monocytogenes

- A Gram+ bacterial pathogen
- Replicates in mammalian cells cytosol
- Common model pathogen for cell-mediated immunity study
- Internalize in host → contain in phagosomes → lyse phagosomes (mediated by LLO) → get into cytosol



www.bact.wisc.edu/themicrobialworld/Listeria.html

Listeriolysin O (LLO)

- Pore-forming cytolysin
- Secreted by several Gram+ bacteria
- Previous works:
  - Co-encapsulated protein delivery to macs: LLO encapsulated in pH-sensitive liposome
  - Purified LLO and foreign protein into mammalian cells (*in vitro, in vivo*)

Disadvantage: Need to purify LLO & protein

E. coli expressing cytoplasmic LLO

- Efficient delivery of co-expressed proteins to macs' cytosol
- Experiments:
  - Large active protein: *E. coli* β-gal
  - Rapid delivery: chicken ovalbumin (OVA)
    - OVA not toxic to *E. coli*
    - Readily expressed in high levels
  - Efficient delivery: *E. coli*/LLO OVA delivery to MHC-I
  - Process & present time

Experiments & Results

Expression of LLO & Target Proteins

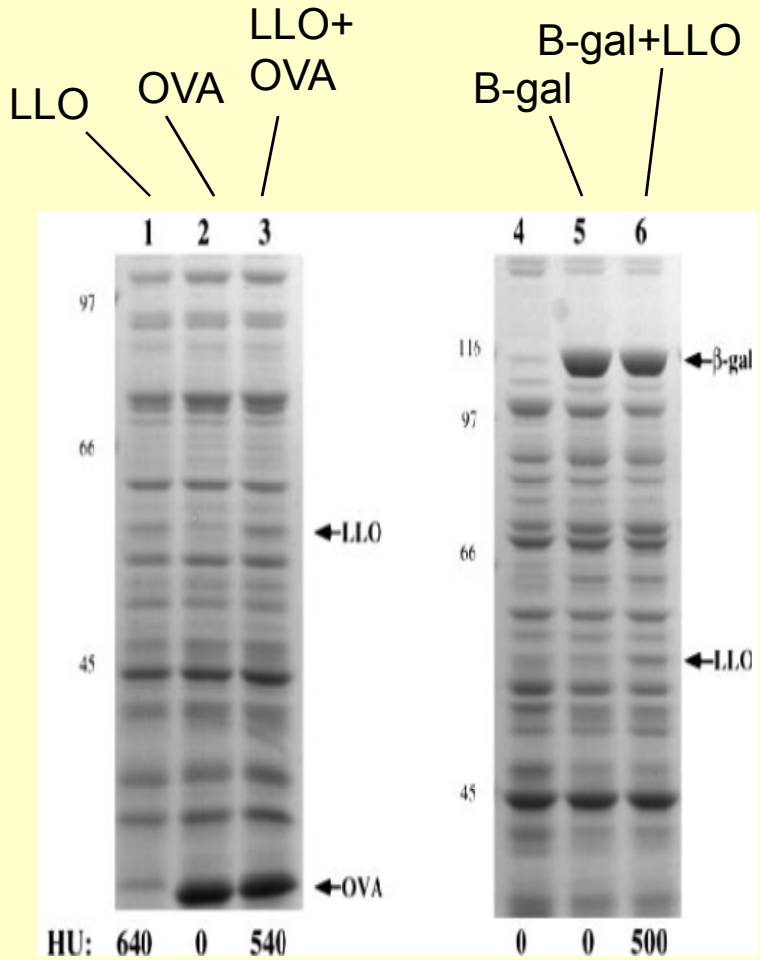
- *Hly* gene encodes mature cytoplasmic LLO in *E. coli* (vector: pACYC184➔pDP3615 (*tet* promoter))
- OVA (32 kDa) + LLO
- β-gal + LLO
- After IPTG induction:

  both proteins expressed

  ~20% of total E. coli protein
- All LLO strains:

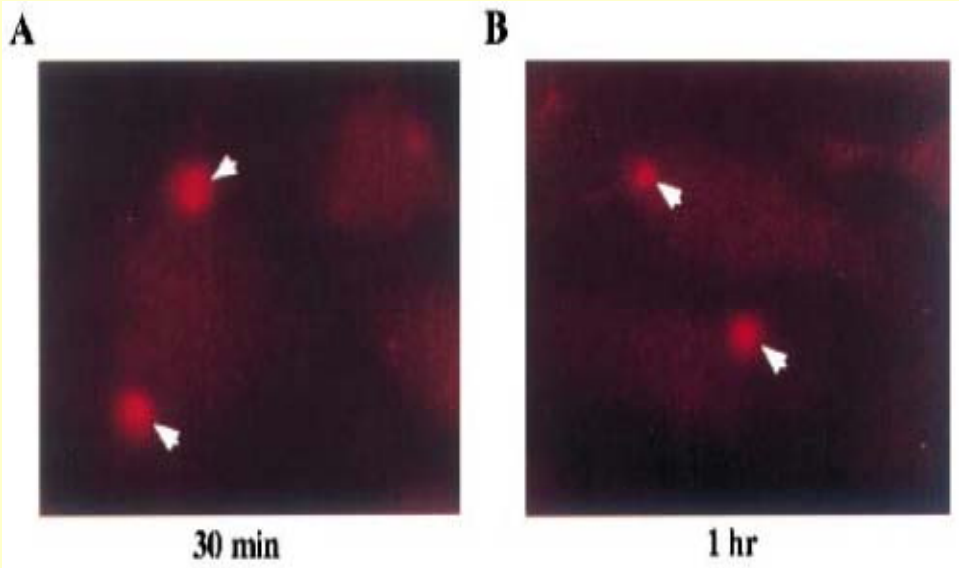
  500-600 HU activity

  - LLO contained within E. coli

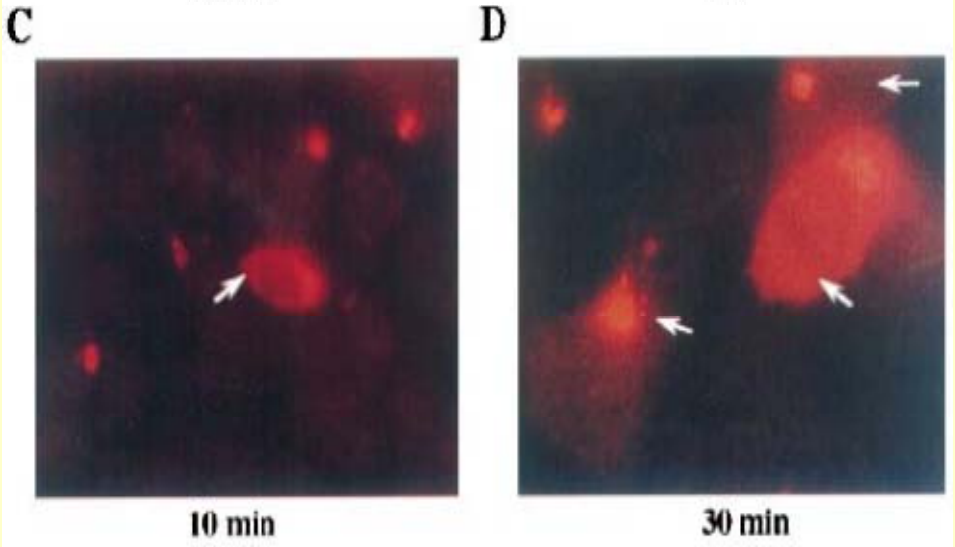


Protein Delivery to Macs' Cytosol

- OVA only:
  - Contained in phagosome even after 1hr



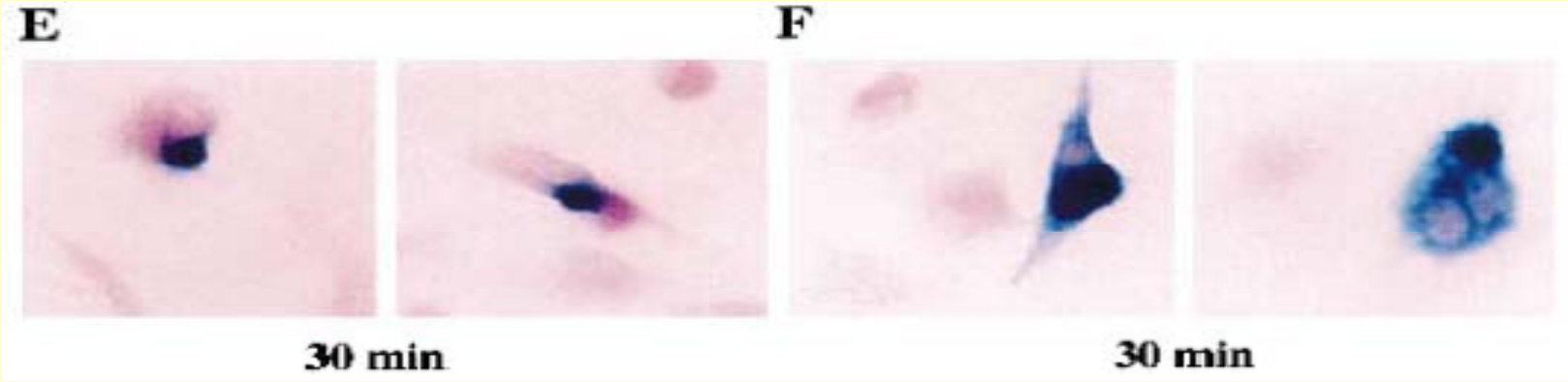
- OVA + LLO:
  - Release into cytosol withing 30 min or earlier
  - Release true for at least 50% of macs uptaken bacteria



Protein Delivery to Macs' Cytosol

- Cytosolic release after *E. coli* lysis ➔ possible degradation and/or inactivation of target protein

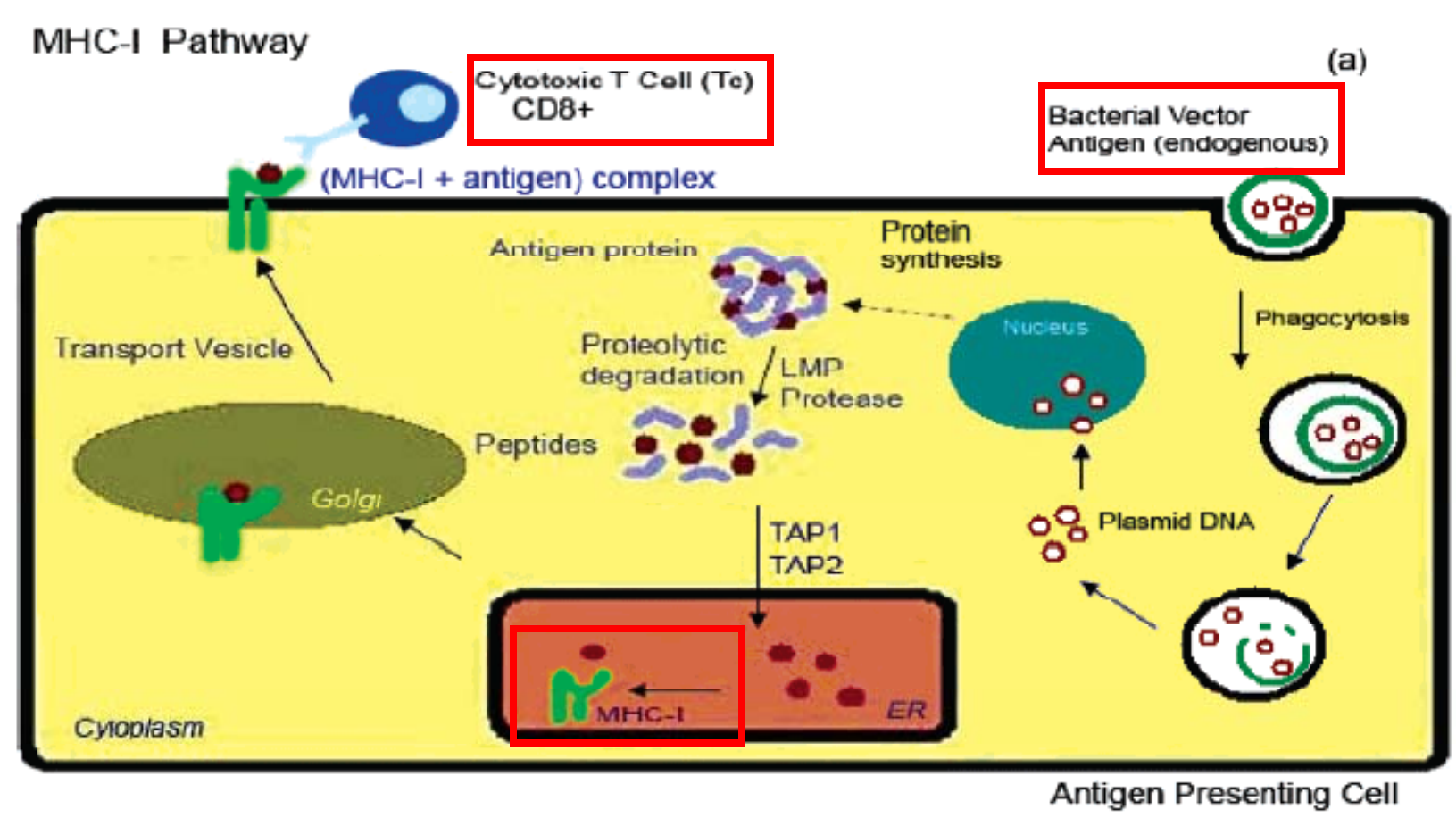
- β-gal (116 kDa) has enzymatic activity ➔ X-gal staining



β-gal

β-gal + LLO

Reminder: MHC-I Pathway; Antigen Presentation to CD8+ T Cells

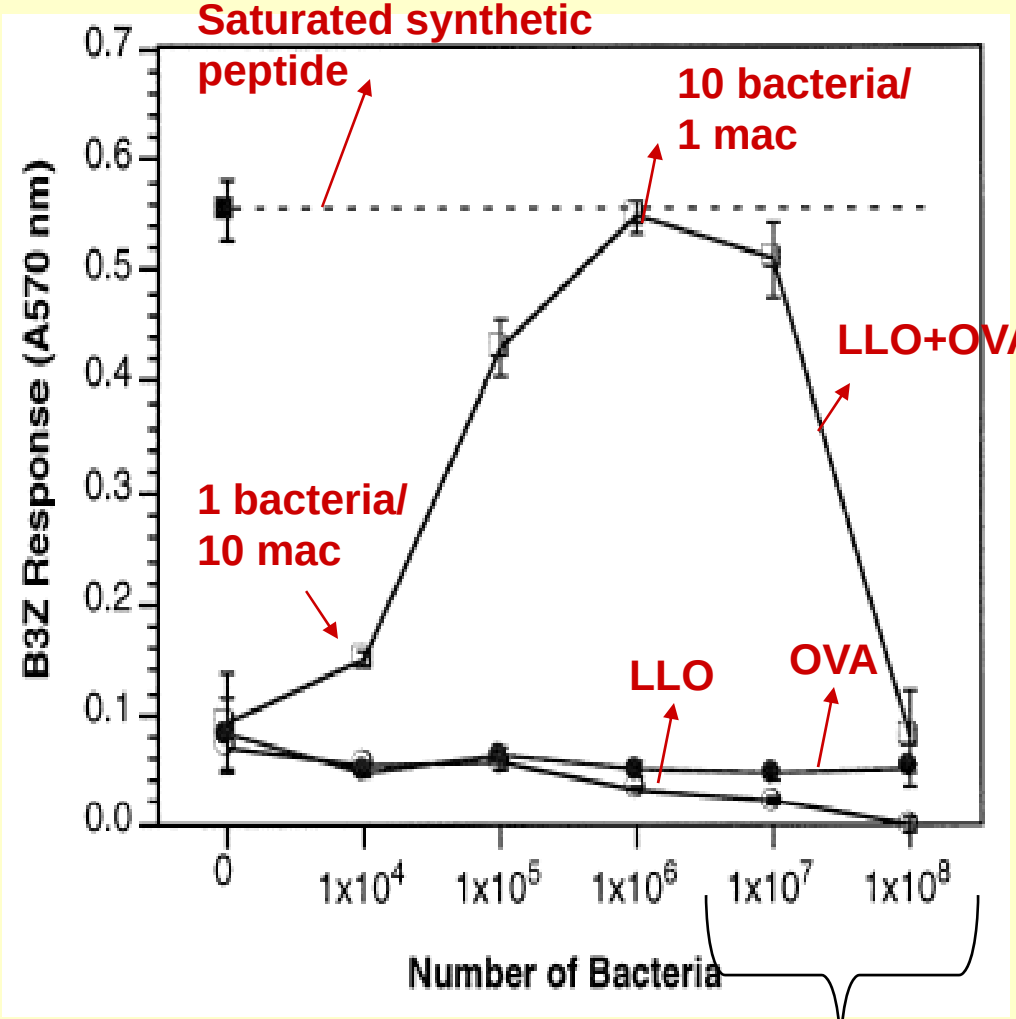


Parsa S, Pfeifer B: Molecular Pharmaceutics. Vol. 4, No.1, 2006.

Antigen Processing & Presentation

- T cell hybrid: B3Z
  -Upon proper presentation prompts B-gal synthesis

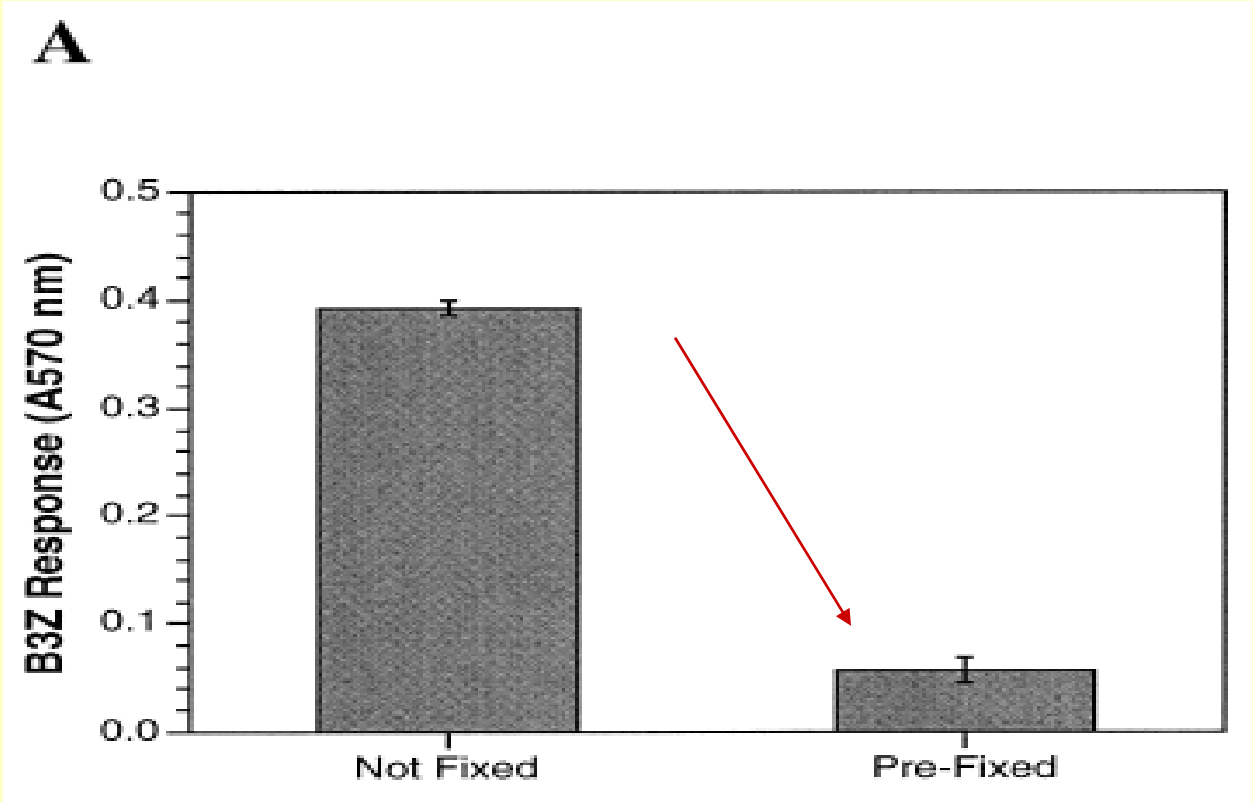
- Measure amount of MHC-epitope combo on macs surface



Processing & Presentation Time

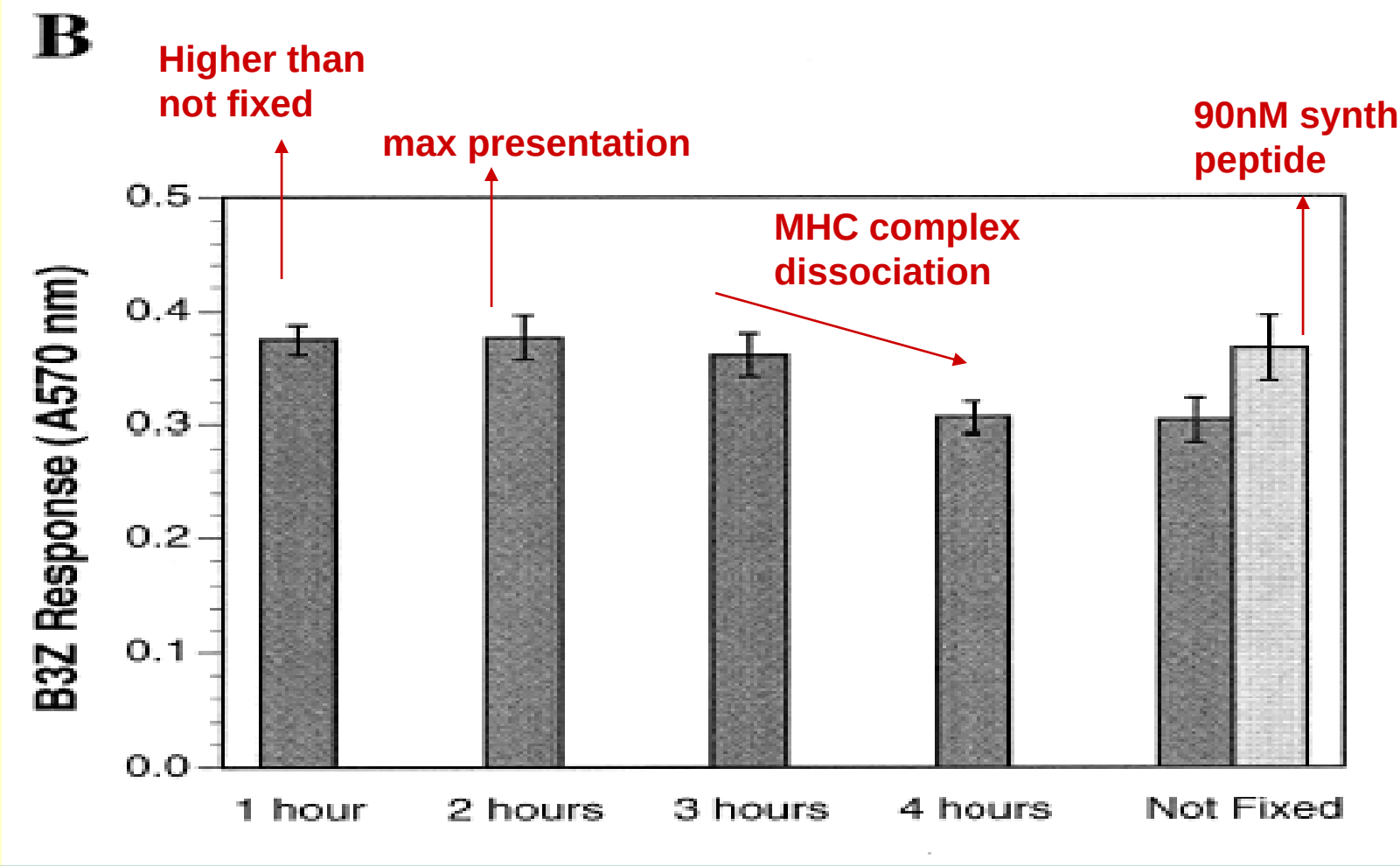
Fixation of macs w/ 1% paraformaldehyde:

- prevents phagocytosis of bacteria
- stablizes MHC-1/peptide complexes



Processing & Presentation Time

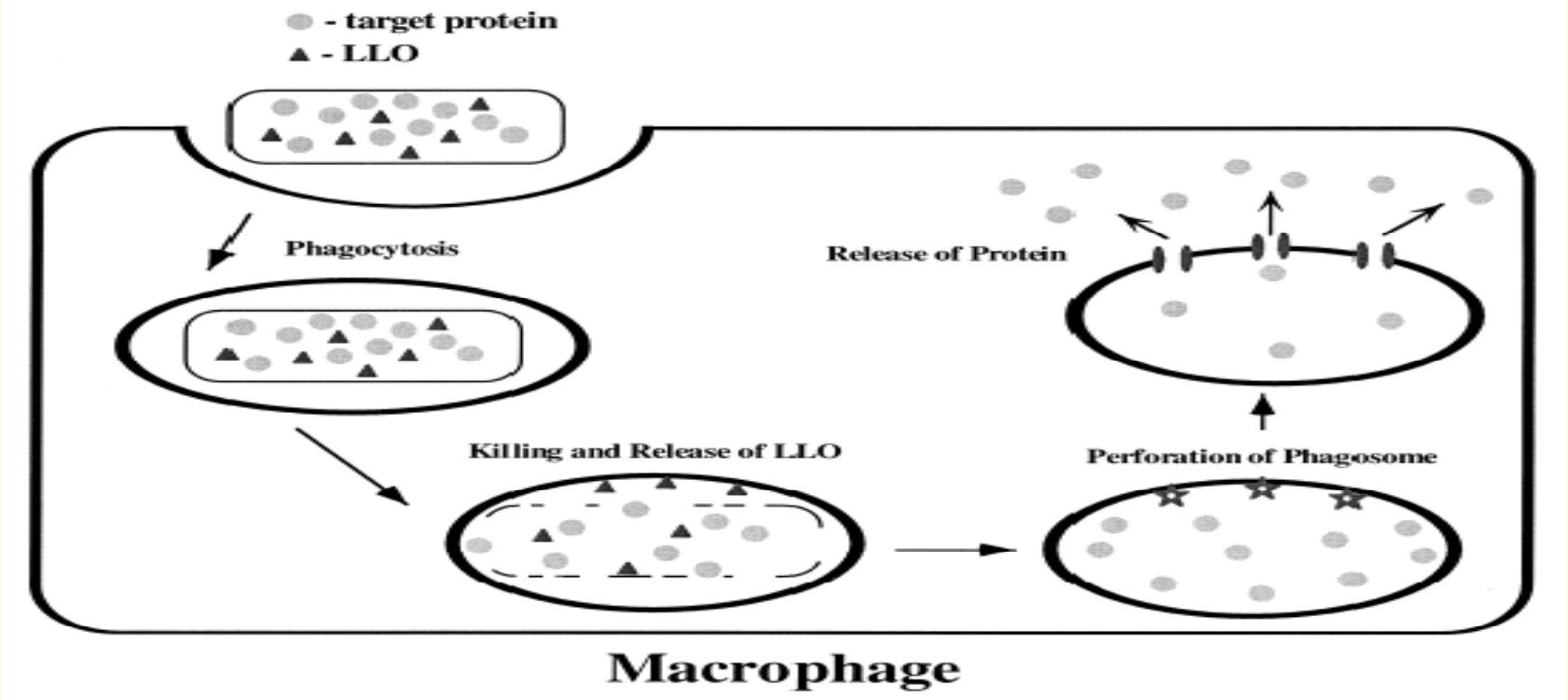
Paraformaldehyde fixation at different intervals:



No delay in processing & presentation using *E. coli*/LLO delivery system

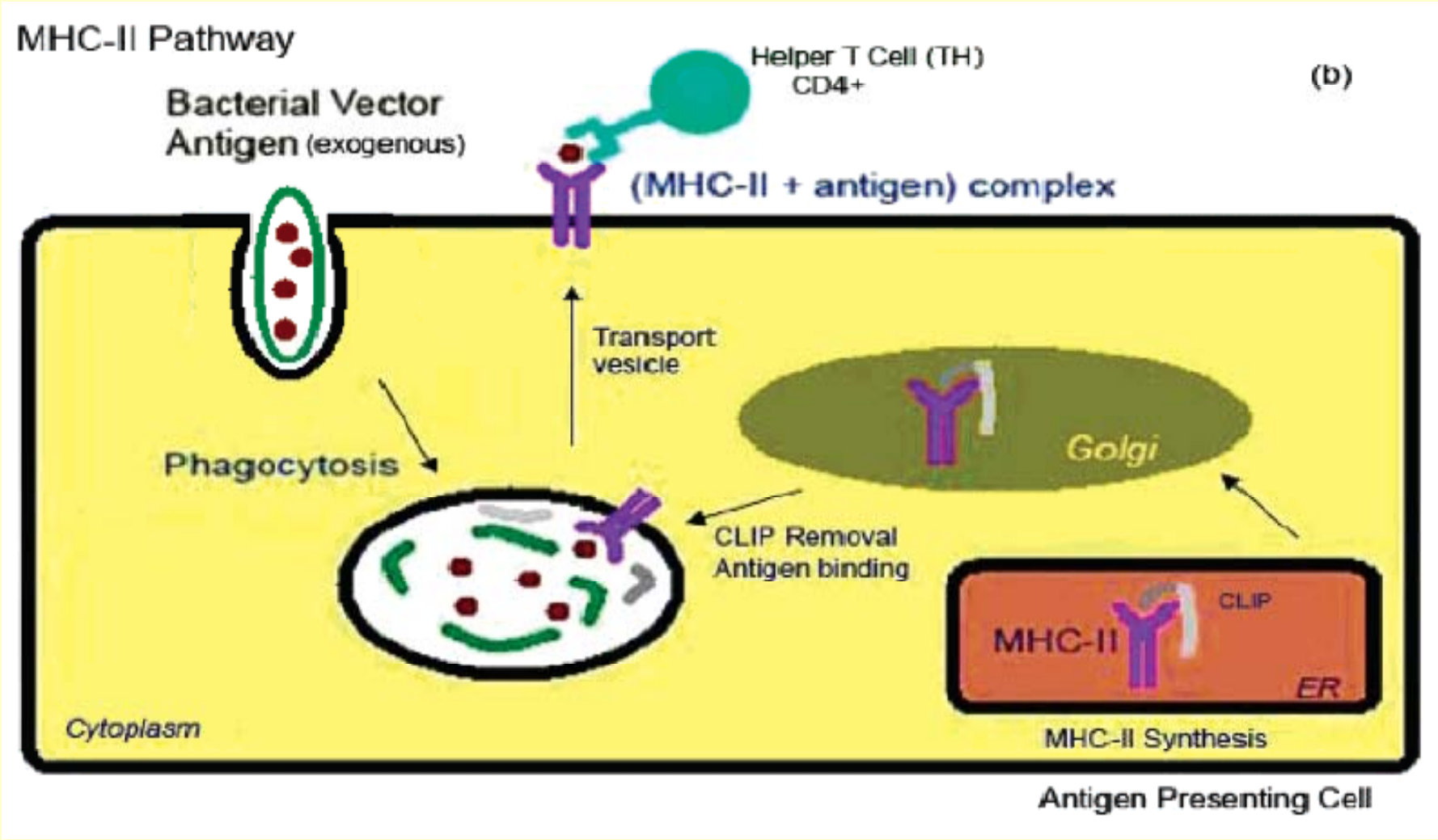
Discussion

- *E. coli*/cytoplasmic LLO deliver co-expressed proteins to cytosol of macrophages
  - efficient delivery
  - rapid delivery (first appear in cytosol in 10 min)
  - large, enzymatically active protein can be delivered retaining its activity
  - proposed mechanism:



Advantages

- LLO
  - Acidic pH optimum
  - 1x10^5 LLO molecule/ *E. coli* cell
  - Not toxic (degrade in cytosol)
    - Toxicity when 25 bacteria/ 1 macrophage
- *E. coli*/LLO vector
  - no protein purification needed; only protein expression in *E. coli*
  - High levels of protein can be delivered and produced (use of inducible promoters)
  - Bacteria killed: no intracellular growth in host cell
  - Potential for pathogen-specific protein & DNA delivery
  - *In vivo* experiments


MHC-II Pathway
Helper T Cell (TH)
CD4+
(b)
Bacterial Vector
Antigen (exogenous)
(MHC-II + antigen) complex
Transport
vesicle
Phagocytosis
Golgi
CLIP Removal
Antigen binding
CLIP
MHC-II
ER
Cytoplasm
MHC-II Synthesis
Antigen Presenting Cell