Presenting Your Research: Effective Poster Presentations

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Goals of the poster

- To get researchers interested in the topic
- To clearly explain your research without overwhelming the reviewer with minutia
- To receive feedback on your research
- To get researchers to appreciate your research
- To leave them with a clear “take home message”

Organization

- Important!! Before you start, determine allowed poster size and **adhere to the instructions**
- Determine your "take home message" before you start working on the content of your poster (*if you have no data – make the research question your take home message*)
- The rest of the poster should be set up to build up to and support your "take home message"
- Know your audience and design a poster that is appropriate for your audience (general audience vs. specialist audience)

Poster – Organization

- **Title:**
  - state your overall conclusion
  - this should be your “take home” message
- **Introduction:**
  - minimum amount of background information
  - **briefly** introduce the topic
  - provide a rationale and hypothesis for the study
  - set up overall approach
  - models can help explain the question being addressed
- **Methods:**
  - use flow charts/diagrams
  - sometimes best to include along side the data presentation
  - do not include experimental details – just include what is required to explain the data

Poster – Organization

- **Results:**
  - use meaningful titles of poster figures/slides
  - logically arranged – one section should flow to the next
  - more is not always better. You need just enough to convince the reviewer
- **Conclusions and/or model:**
  - link conclusions to your original question
  - a model is always best for conveying message
  - no more than three conclusions
- **Acknowledgements:**
  - acknowledge funders
  - acknowledge individuals who helped with the work

Poster – Appearance

- Legible
  - text needs to be readable from at least one meter
  - limit the amount of text
- Readable
  - use a common font throughout using bold or color for emphasis
  - use a simple color palette (UA templates are available)
- Organized
  - should flow logically
- Succinct
  - effective use of figures
  - clear take home message

ICAM-1 and B7 potentially plays a role in FasL expression when signals are week but not strong leading to different kinetics of expression

Clueless Student and Evenworse Supervisor

Department of Medical Microbiology and Immunology, University of Alberta

INTRODUCTION

Fas ligand (**FasL**), also known as CD178 or CD95L, is expressed on CTLs and functions by engaging the death receptor Fas (CD95) on target cells and triggering apoptosis. Fas is constitutively expressed on the surface of many cells, with cells of liver, heart, lung, kidney, and ovary expressing the highest levels. Because Fas is so ubiquitously expressed, the expression of FasL on CTLs must be tightly regulated. We previously demonstrated that CTLs undergo two waves of FasL cell surface expression after TCR engagement. The first wave, detectable by 15 min, is from a pre-existing pool of FasL, and the second wave requires new protein synthesis and peaks at ~2 h after TCR stimulation. However, the biological significance of the two waves of FasL expression remains unknown.
Fas and FasL are known to play important regulatory roles in the immune system. Initial studies suggested that FasL was largely dispensable for viral clearance in the relatively few systems that were examined. However, more recent studies suggest that FasL may be important for clearing persistent infections and may contribute, along with the perforin pathway, to the shaping of the diversity of escape variants of influenza. Although FasL is not required for clearance of viruses that induce hepatitis in mice, it appears to contribute to viral pathogenesis because of significant bystander killing of hepatic cells. Thus, FasL may contribute to virus clearance or pathogenesis, particularly in chronic infections.

Previous studies have suggested that FasL-mediated target cell killing has a lower signaling threshold for activation compared with degranulation, although the source of FasL (stored or de novo) was not specifically examined. For instance, a self-derived peptide was shown to selectively activate the FasL pathway, and a low threshold signal preferentially allows for FasL-mediated killing. We demonstrated that signaling for FasL expression may be finely tuned as a weak TCR signal, in the form of cross-linked, anti-CD3, elicited stored FasL translocation without subsequent FasL synthesis; however, if a strong stimulus were provided as plate-bound anti-CD3, de novo-synthesized FasL was expressed with little or no stored FasL cell surface expression.

In the current study, we quantitatively compared the signaling strength required for stored FasL translocation, de novo FasL cell surface expression, and degranulation by CTL. These studies revealed that stored FasL translocation has a lower threshold of activation than de novo FasL synthesis and degranulation. Furthermore, we provide evidence to suggest that the stored, translocated FasL mediates highly specific CTL-mediated killing, whereas the de novo-synthesized FasL induced significant bystander killing. These data imply that FasL from these two sources may perform distinct roles in CTL-mediated responses.

RESULTS

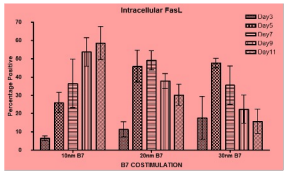

Figure 1.

B7 costimulation augments and maintains GrB expression. Early FasL expression is induced by B7 in a dose dependent manner. However, late FasL expression is not maintained by mid and high B7 costimulation.

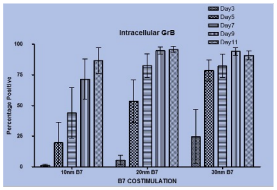

Figure 3.

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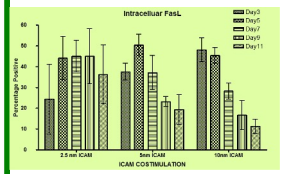

Figure 4

ICAM costimulation augments and maintains GrB expression. Early FasL expression is augmented by ICAM. FasL expression, however, is not maintained long term under mid and high ICAM costimulation conditions.

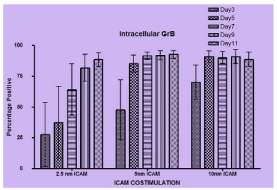

Figure 5

ICAM costimulation augments and maintains GrB expression. Early FasL expression is augmented by ICAM. FasL expression, however, is not maintained long term under mid and high ICAM costimulation conditions.

RESULTS

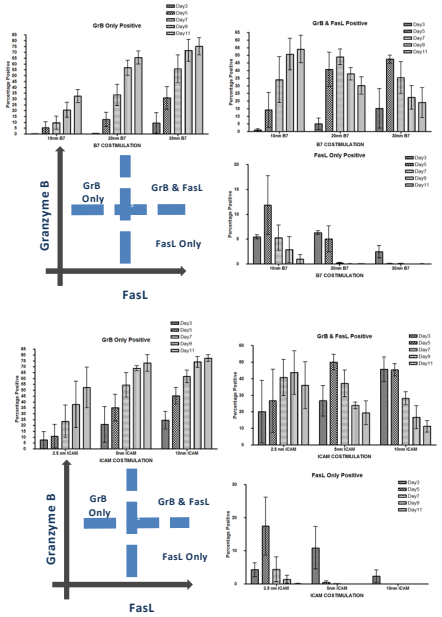

Figure 2.

B7 and ICAM costimulation induces FasL only expressing T cells early after activation (Day 3 & 5). After Day 5, FasL expression diminishes whereas granzyme B expression noticeably increases and is maintained until Day 11..

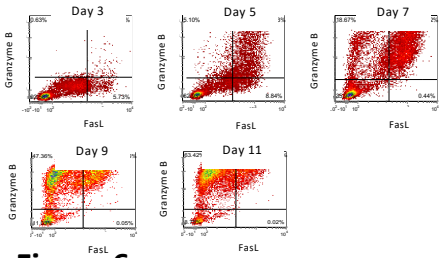

Figure 6.

B7 and ICAM costimulation induces FasL only expressing T cells early after activation (Day 3 & 5). After Day 5,

RESULTS

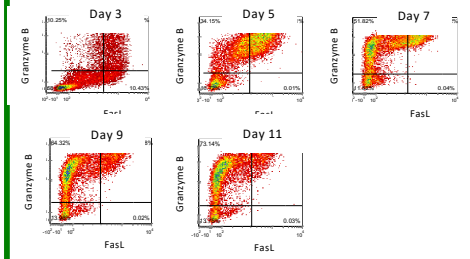

Figure 7

Mid level B7 & ICAM costimulation induces a time-dependent expression of early FasL only expressing, intermediate FasL/GrB double expressing and late GrB only expressing T cel.

CONCLUSIONS

- Low concentrations of B7 has not effect on FasL expression when TCR concentration is low
- High B7 concentration can enhance FasL and Granzyme B concentration when anti-TCR is low
- ICAM-1 always enhances FasL expression at any concentration
- ICAM-1 enhances Granzyme B expression at any concentration.
- I had difficulty in measuring surface de novo FasL expression.
- B7 and ICAM-1 augment FasL and Granzyme B expression in activated naïve CD8 T cells.
- FasL is expressed early after stimulation and then reduced
- Granzyme B is expressed after FasL expression and is maintained

ACKNOWLEDGEMENTS

Canadian Cancer Society / Société canadienne du cancer

Ostergaard Lab Members
&
Kane Lab Members

Costimulation Regulates FasL Expression in Cytotoxic T Lymphocytes

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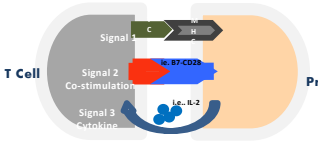
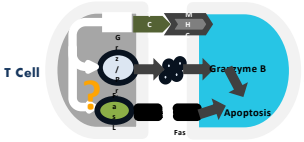
Department of Medical Microbiology and Immunology, University of Alberta

INTRODUCTION

Naïve CD8 T cells require and receive three signals during their activation: T cell receptor (TCR) recognition of MHC-peptide complex, a costimulatory signal and a cytokine signal.



Activated CD8 T cells utilize Fas ligand (FasL) and granzyme/perforin degranulation to induce target cell apoptosis.



Although FasL was thought to be stored in secretory lysosomes together with granzyme and perforin, our lab's observations suggest otherwise. These observations further suggest differential cellular signaling requirements for FasL and GrB expression. Furthermore, given the importance and diversity of costimulatory molecules present during naïve CD8 T cell activation, we believe that costimulatory molecules augment FasL expression in CD8 T cells in a GrB-independent manner.

HYPOTHESIS

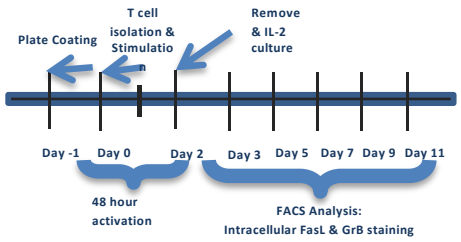
Costimulatory signals provided during naïve CD8 T cells activation augments FasL expression and FasL expression is independent of GrB.

OBJECTIVES

The aim of this study is to determine the role of the costimulatory molecules B7 and ICAM on FasL and GrB expression patterns under sub-optimal TCR stimulation using plate bound anti-CD3 antibody and plate bound recombinant B7 and recombinant ICAM.

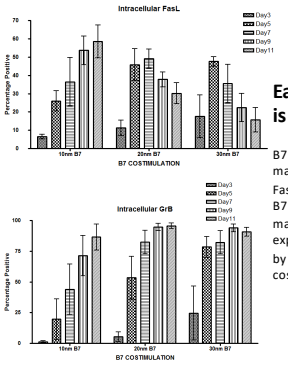
METHODS

24 well plates were coated with sub-optimal levels of anti-CD3 antibody for sub-optimal TCR stimulation plus low, medium or high levels of recombinant B7 or recombinant ICAM.



Naïve CD8 T cells were isolated and cultured in the coated wells for 48 hours. At the end of Day 2, the cells were transferred to a new plate, removed from additional TCR and costimulatory molecule stimulation, and cultured with low levels of IL -2 as a survival factor. Intracellular FasL and GrB were examined by flow cytometry everyone second day starting from Day3.

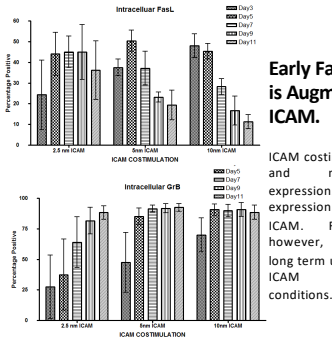
RESULTS



Early FasL Expression is Augmented by B7.

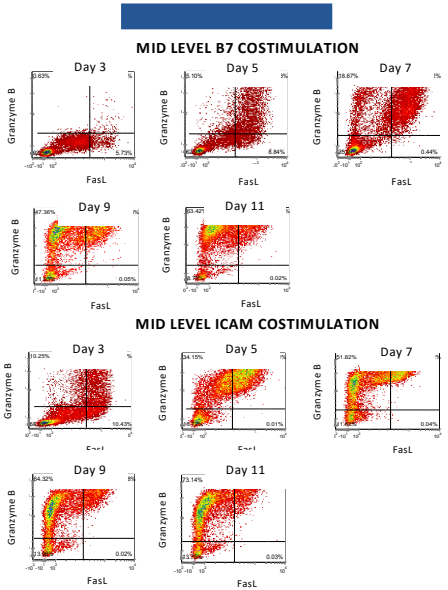
B7 costimulation augments and maintains GrB expression. Early FasL expression is induced by B7 in a dose dependent manner. However, late FasL expression is not maintained by mid and high B7 costimulation.

RESULTS



Early FasL Expression is Augmented by ICAM.

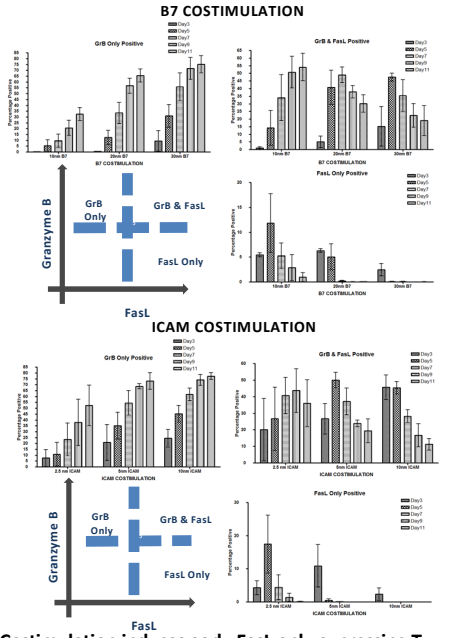
ICAM costimulation augments and maintains GrB expression. Early FasL expression is augmented by ICAM. FasL expression, however, is not maintained long term under mid and high ICAM costimulation conditions.



Mid level B7 & ICAM costimulation induces a time-dependent expression of early FasL only expressing T cells.

FasL only expressing cells are visible early after activation. By day 5 & 7 after ICAM and B7 costimlation, respectively, a substantial population of FasL/Gr double expressing cells are present. On day 9 and 11, the expression of FasL is reduced resulting a high percentage of GrB only expressing T cells.

RESULTS



Costimulation induces early FasL only expressing T cells followed by late GrB only expressing T cells.

B7 and ICAM costimulation induces FasL only expressing T cells early after activation (Day 3 & 5). After Day 5, FasL expression diminishes whereas granzyme B expression noticeably increases and is maintained until Day 11..

CONCLUSIONS

- B7 and ICAM-1 augment FasL and Granzyme B expression in activated naïve CD8 T cells.
- FasL is expressed early after stimulation and then reduced
- Granzyme B is expressed after FasL expression and is maintained

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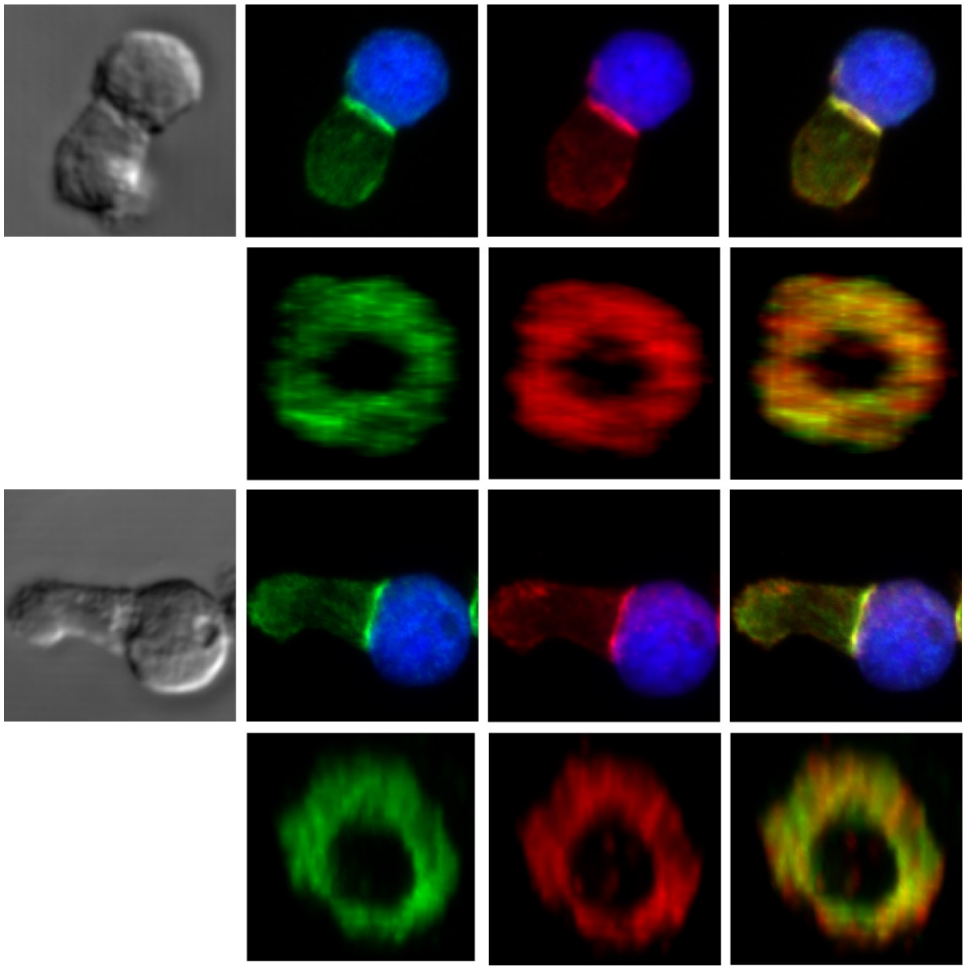
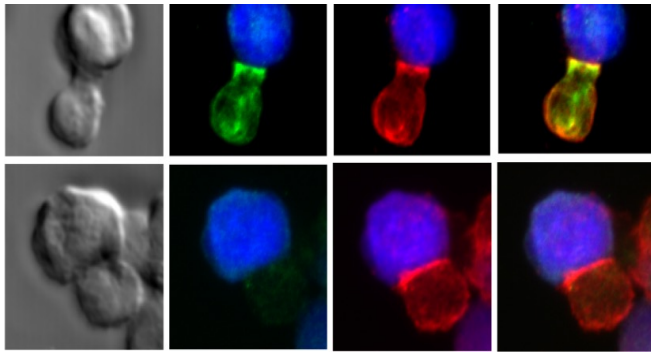
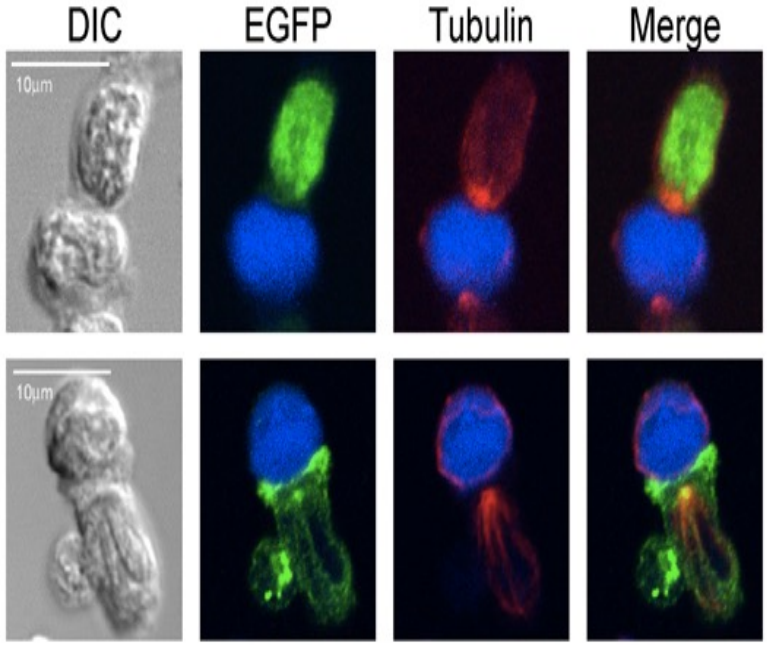
Reliance on figure legends to describe figures and confusing message for a single figure



CTL were mixed with EL4 target cells pulsed with SIINFEKL OVA peptide and incubated for 10 min at 37oC then fixed and stained with antibodies to lpxn (red) and LFA-1 (green). After staining cells where imaged by confocal microscopy with 100 nm sections and reconstructed. Shown is a projection image.

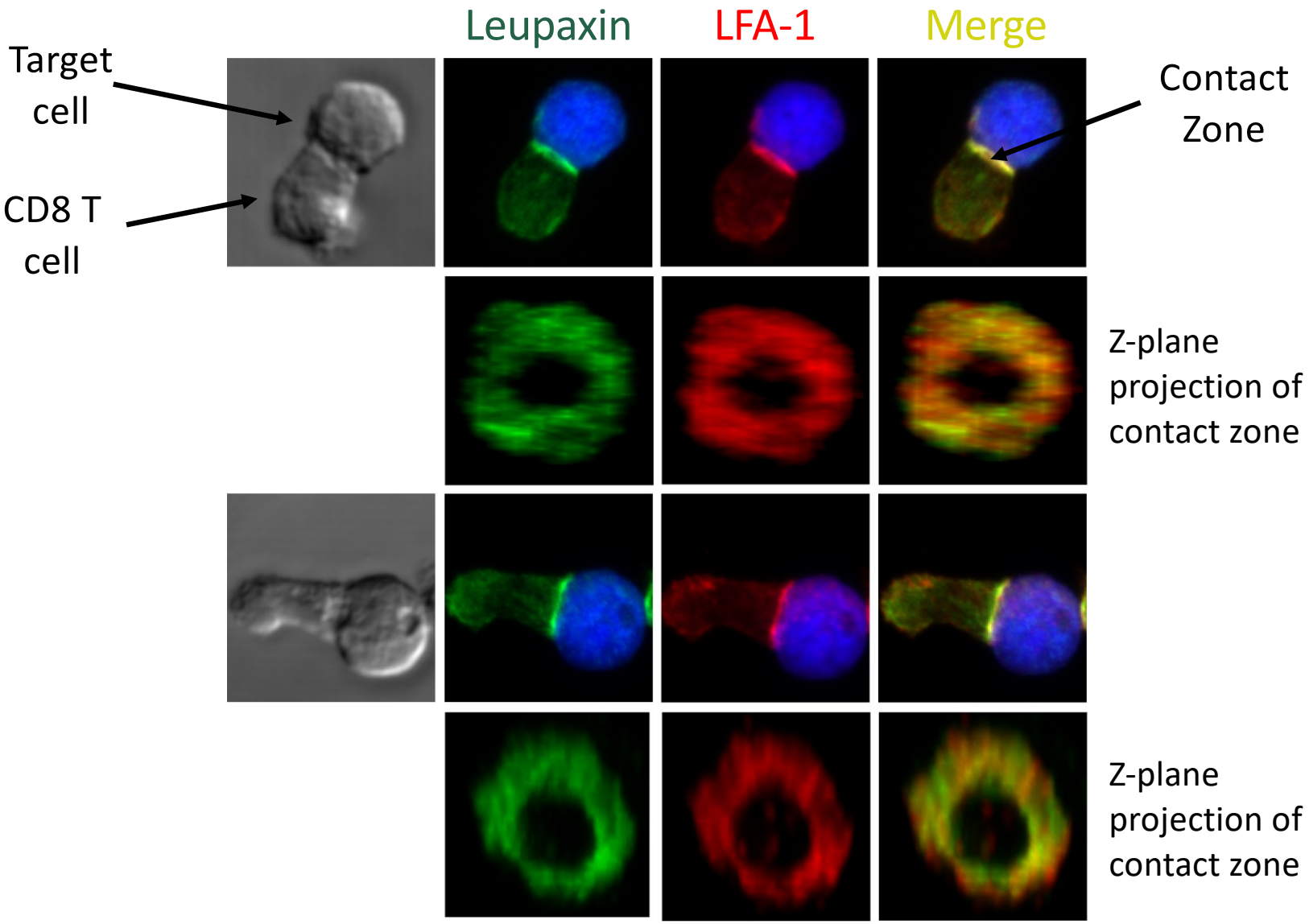


CTL from WT or LPXN KO OT-1 mice were mixed with EG7 target cells and incubated for 10 min at 37oC and transferred to coverslips and stained with leupaxin and paxillin . Images shown are from a single optical section.

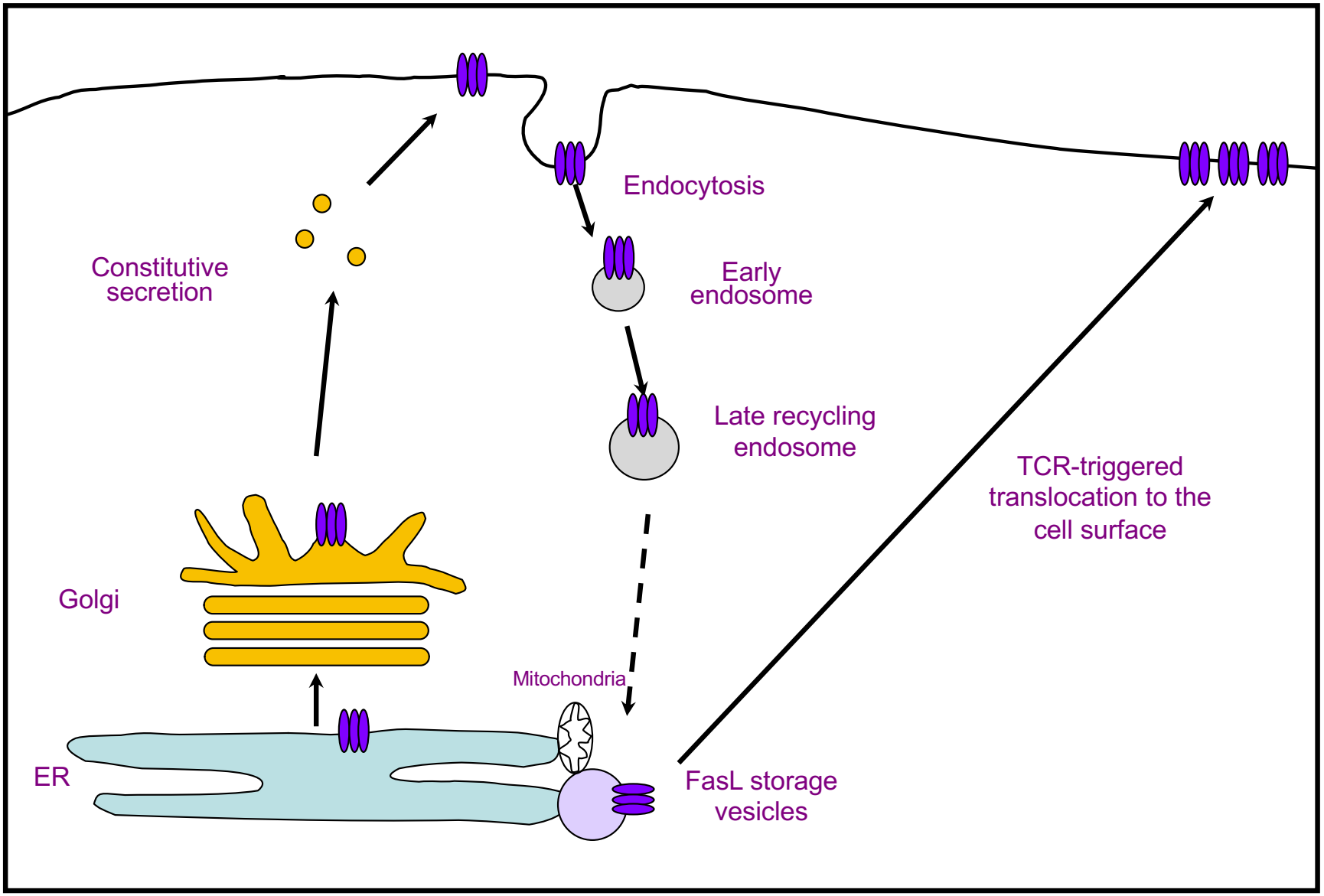


CTL transfected with LPXN-GFP or GFP were mixed with ELF4 target cells pulsed with SIINFEKL and incubated for 10 min at 37oC and transferred to coverslips and stained with leupaxin and paxillin . Images shown are from a single optical section.

Leupaxin localizes to the CD8 T cell contact zone during target cell adhesion



Proposed model for FasL trafficking in CTL



Conclusions

- FasL is translocated directly to the cell surface after synthesis then rapidly endocytosed into a storage vesicle
- The storage vesicle is in close proximity to the ER and mitochondria
- Upon TCR stimulation FasL is rapidly transported to the cell surface where it mediates killing

Presentation of the poster

- Think about what you want to say ahead of time
  - Don’t be overly rehearsed since that makes it appear as though you don’t really understand your material and you are just memorizing it
- Clearly identify the single important question/hypothesis being addressed
- Provide a strong rationale for the question/hypothesis
  - Why should the reviewer care?
- Address the question
  - Results show how question/hypothesis is addressed
  - Have no more than three conclusions
  - make your central take home message obvious to the reviewer

Presentation of the poster

- Be clear, focused and concise
- Don’t assume that your audience is an expert in your area
- Honor time and space limits
- Make it interesting
- Be knowledgeable on ALL material presented on the poster
  - If you included it on your poster, you need to be able to discuss it, even if you didn’t do the work
- Be enthusiastic!