

In situ analysis of perforin expression in SIV-specific CD8 T cells in tissues from rhesus macaques vaccinated with live-attenuated SIV Δ nef and challenged with SIVmac251

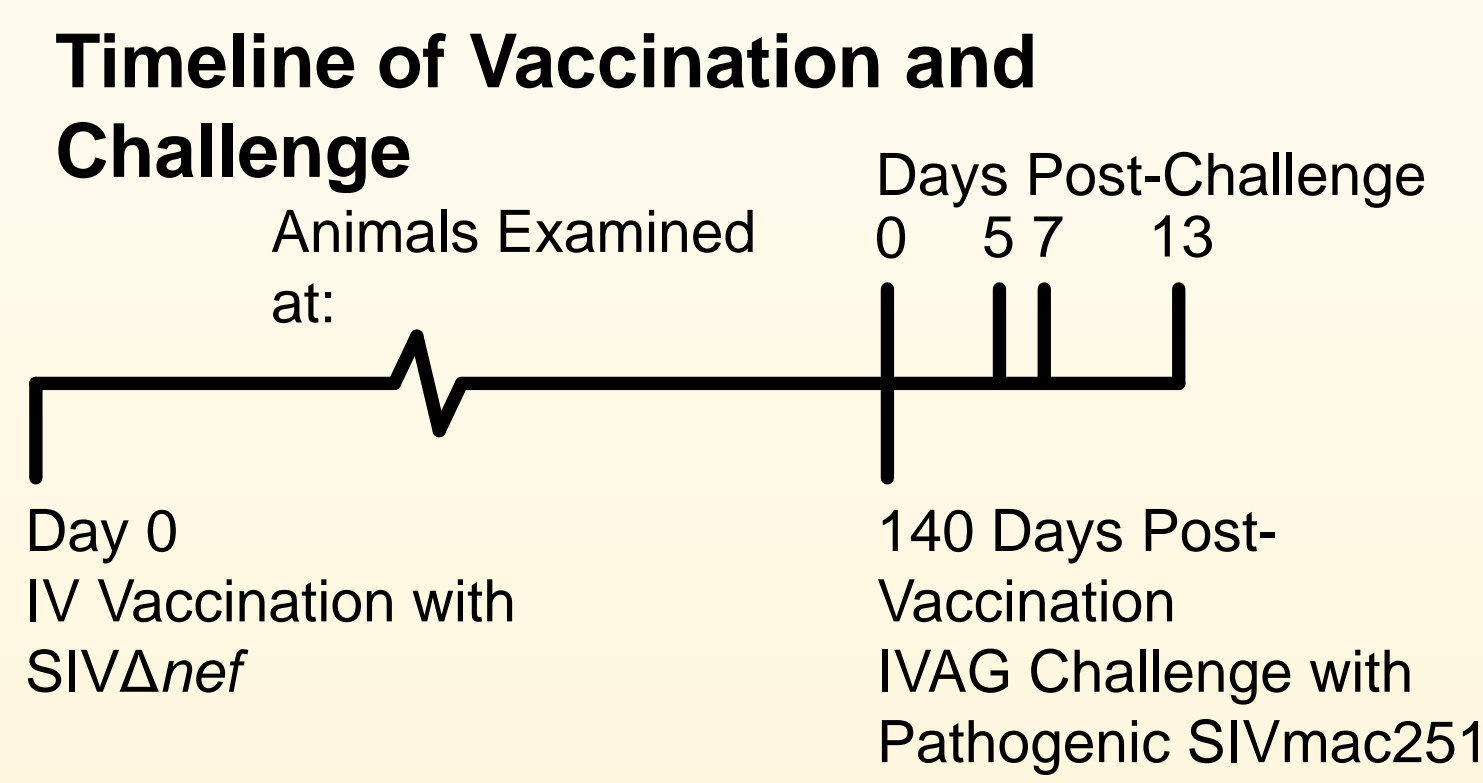
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Introduction

- Human immunodeficiency virus (HIV) was identified as the cause of acquired immunodeficiency syndrome (AIDS) in the 1980s, and since then it has proliferated into one of the most vexing pandemics of its time, if not all of human history. As of today, there is still no known cure for HIV, although numerous strategies are currently being pursued to confer immunity.
- Simian immunodeficiency virus (SIV) infections in rhesus macaques are an excellent model for HIV infection. Currently, the best vaccines to date have been live-attenuated SIV vaccines in rhesus macaques.
- A robust and rapid response by CD8⁺ T cells has been associated with the immunity provided by live, attenuated SIV vaccines. To characterize this response and identify correlates of protective immunity, I examined the expression of perforin, a cytolytic protein integral to the cytotoxic activity of CD8⁺ T cells, within virus-specific CD8⁺ T cells taken from tissues from rhesus macaques.

Methods

- Adult, female Rhesus macaques were immunized with live, virulence-attenuated SIV Δ nef and subsequently challenged with pathogenic SIVmac251.

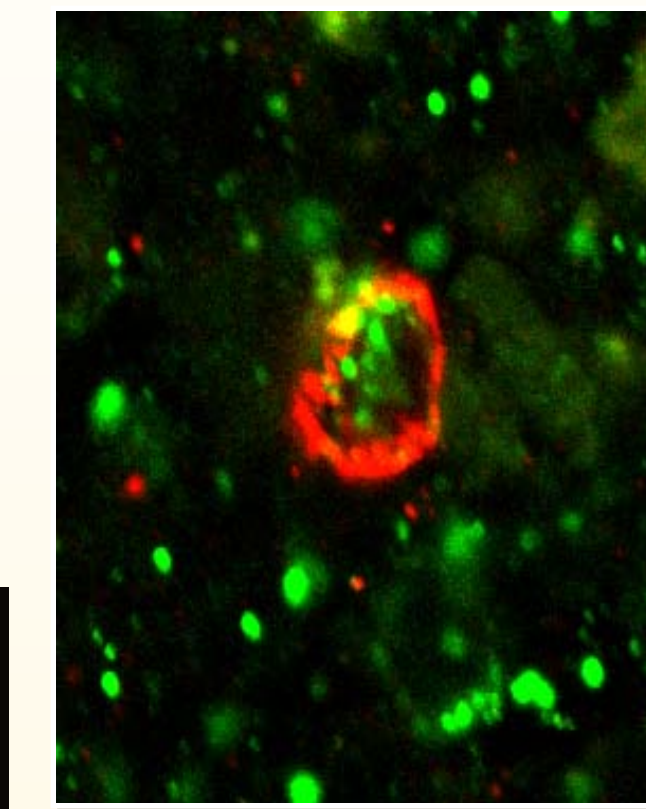
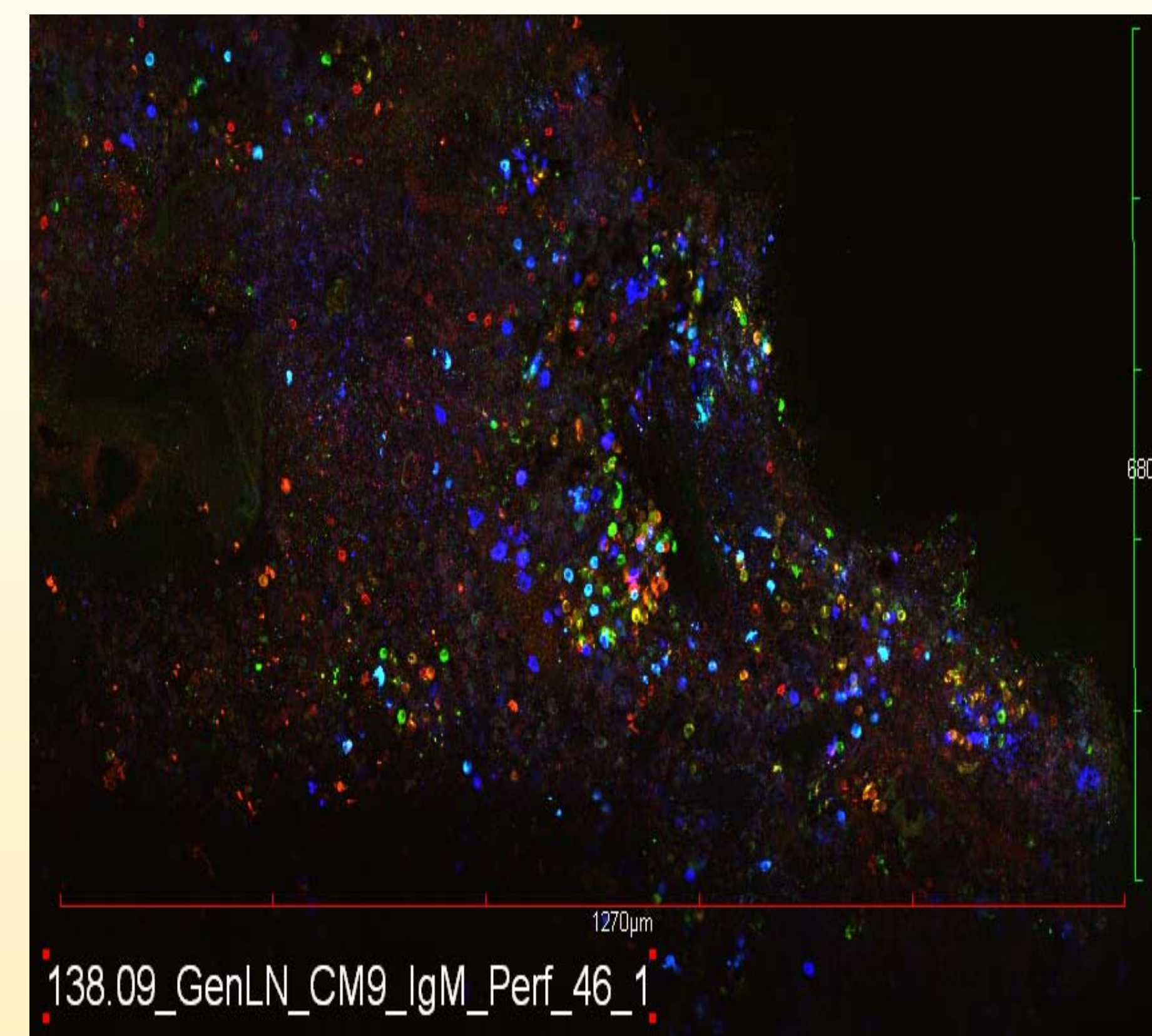


- In genital lymph node tissue from animals sacrificed at varying days before and after challenge, CD8⁺ T cells that recognize the SIV proteins Tat and Gag were identified *in situ* using Mamu A*01/Tat tetramers, and perforin was detected using immunofluorescence.

Results

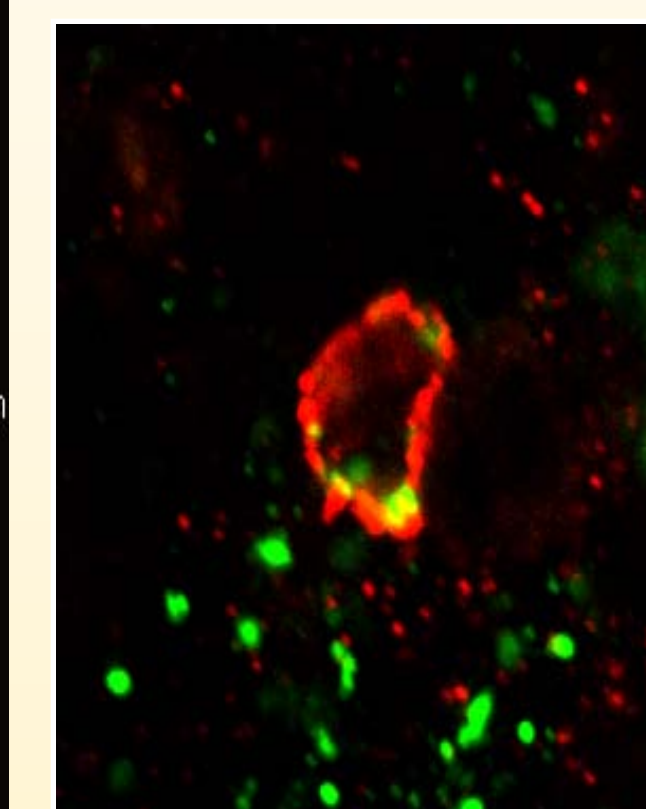
Example of Typical SIV Tetramer and Perforin Staining In Genital Lymph Node Tissue

Red: Tat/gag specific Tetramer Staining
 Green: Perforin Staining
 Blue: IgM staining



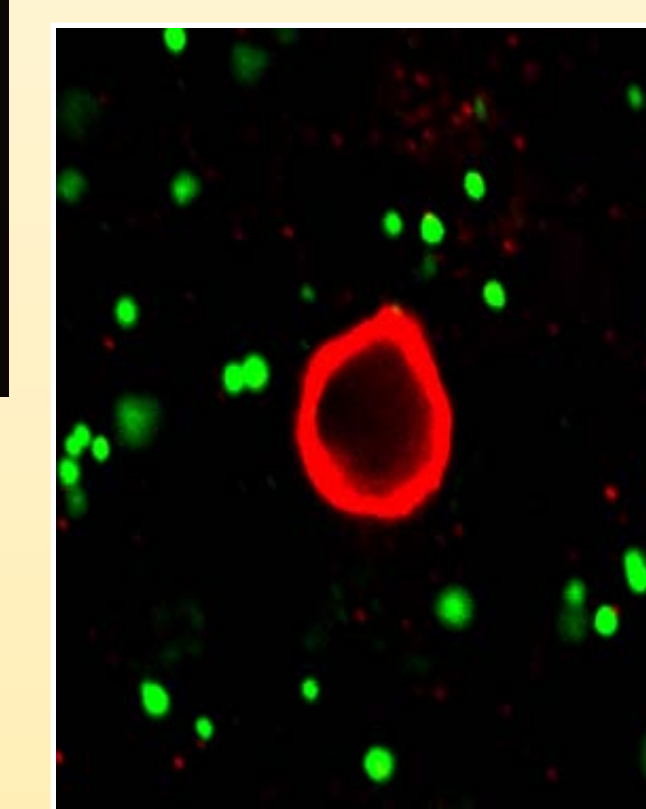
Perforin Medium or High Cells:

- Many cytotoxic granules filled with perforin, indicating high cytotoxic activity
- Possibly effector CD8⁺ T cells



Perforin Low Cells:

- Few (1-3) cytotoxic granules filled with perforin, suggesting low effector function
- Possibly effector memory CD8⁺ T cells (T_{EM} cells)



Perforin Negative Cells:

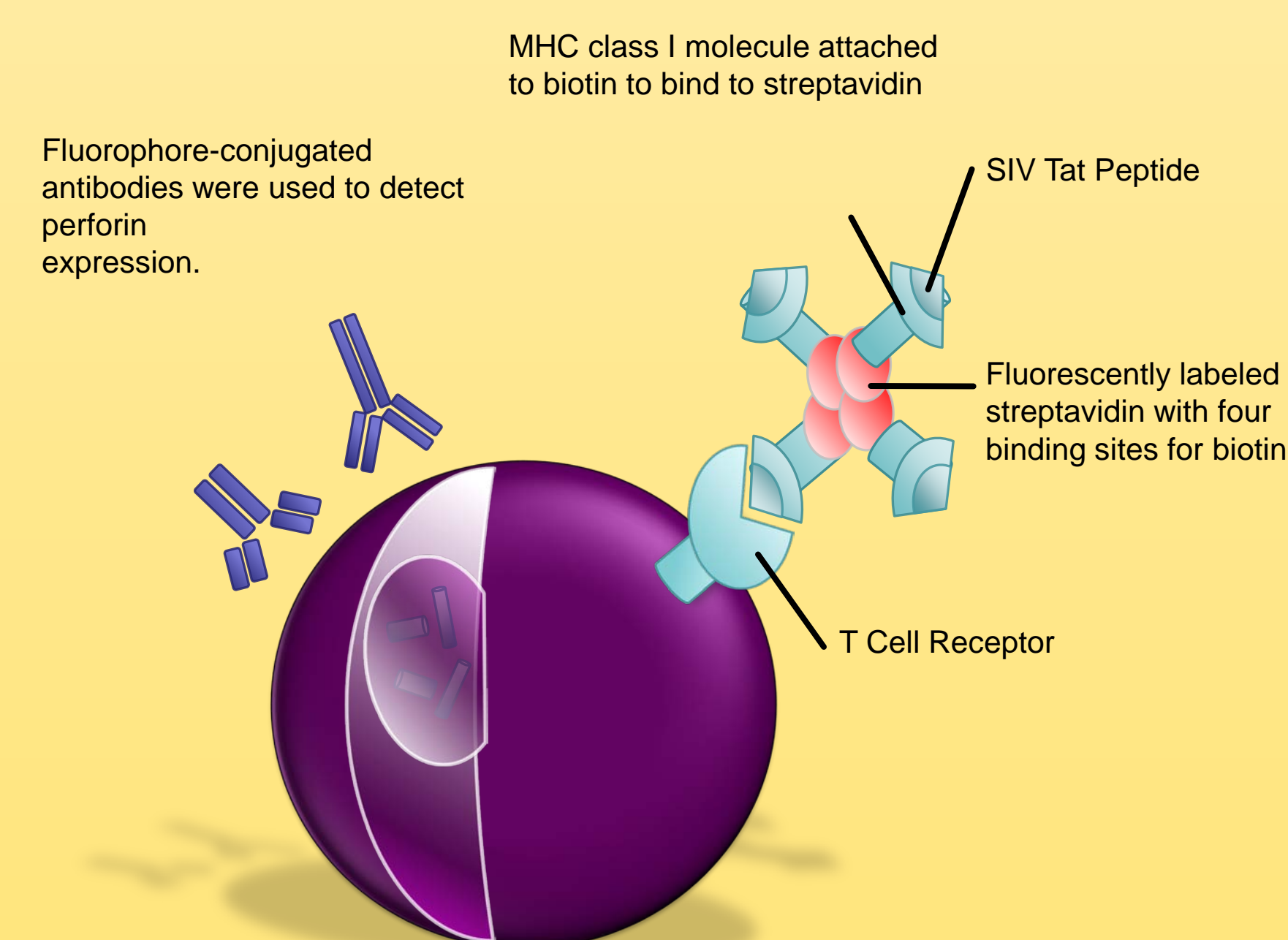
- Void of cytotoxic granules filled with perforin
- Phenotype of central memory CD8⁺ T cells (T_{CM} cells)

Conclusions

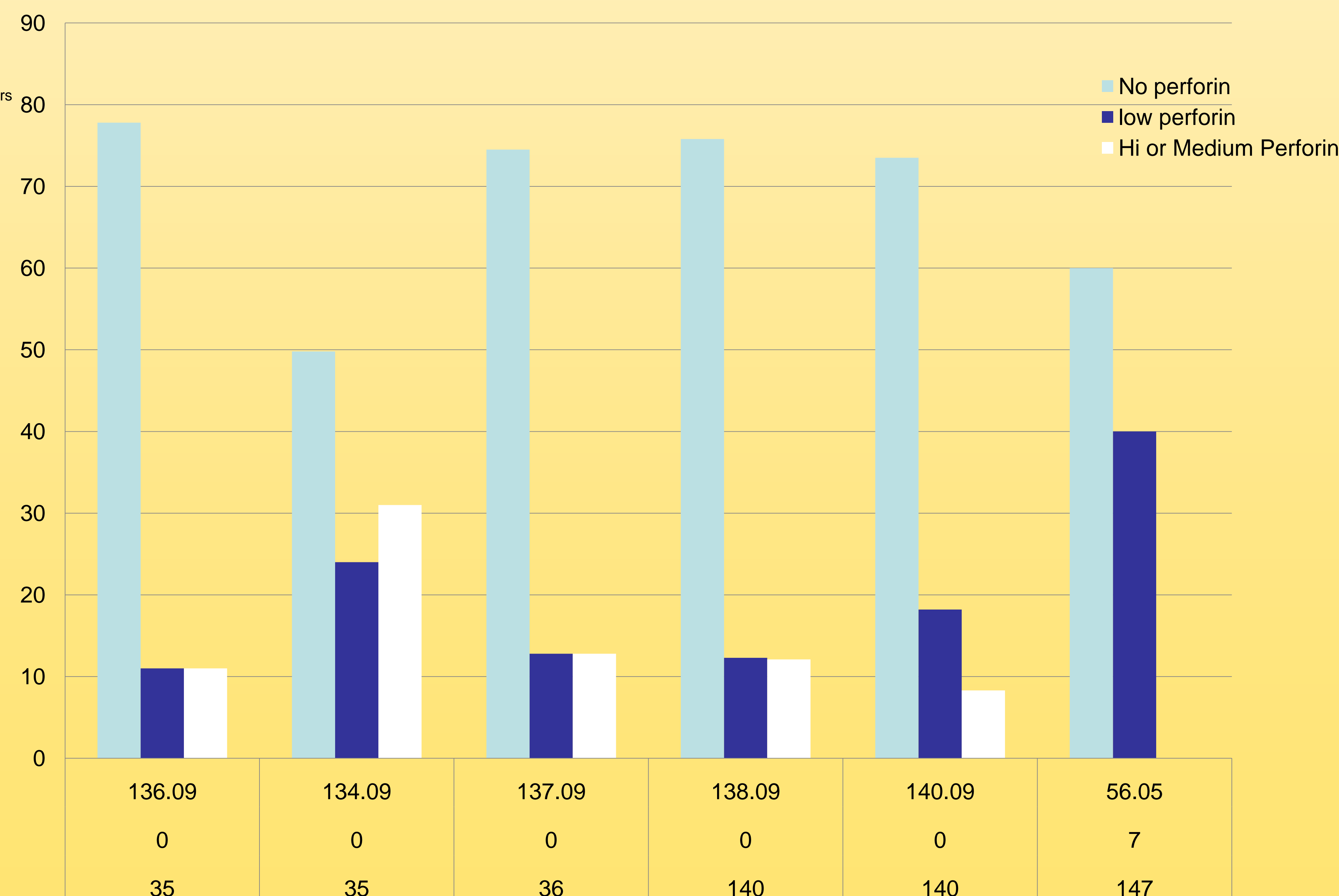
- SIV-specific CD8 T cells are present in the lymph nodes at time of challenge with SIVmac251
- Most of the SIV-specific CD8 T cells were perforin negative, consistent with being central memory cells
- A subset of SIV-specific CD8 T cells were perforin positive consistent with effector and effector memory cells
- A more complete quantification of levels of perforin post challenge with pathogenic SIVmac251 is underway. These results will provide insights into the phenotype of SIV-specific CD8 T cells associated with protection provided by a live, attenuated vaccine.

In Situ Tetramer Staining and Immunofluorescence Staining

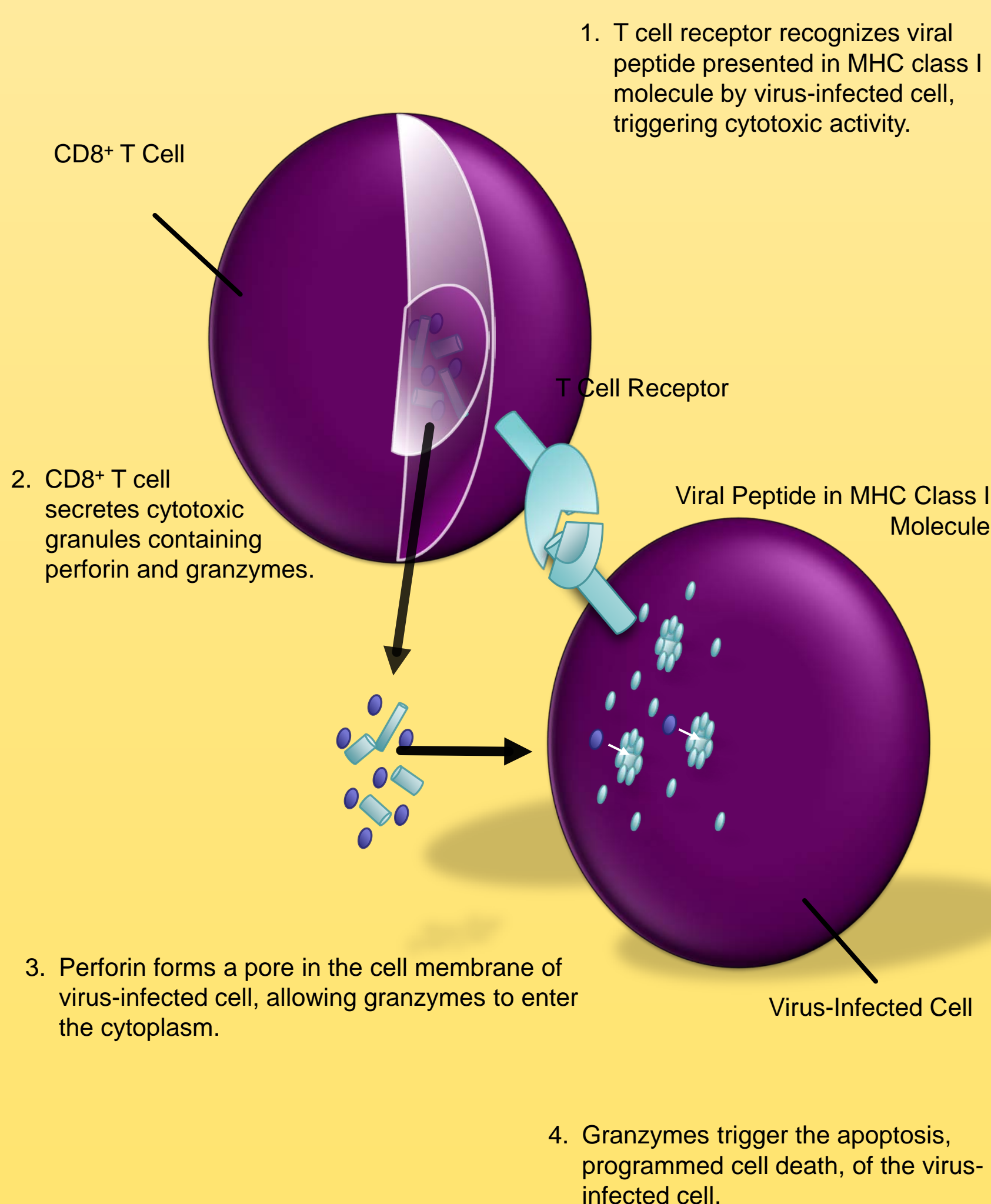
Fluorescently labeled MHC class I tetramers were used to identify SIV-specific CD8⁺ T cells.



Created by Kris Schwebler



The Role of Perforin in Protection Against Viral Infection



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