

# MHC Class II Tetramers Quantitatively Detect DP4-Restricted, Antigen Specific CD4+ T Lymphocytes

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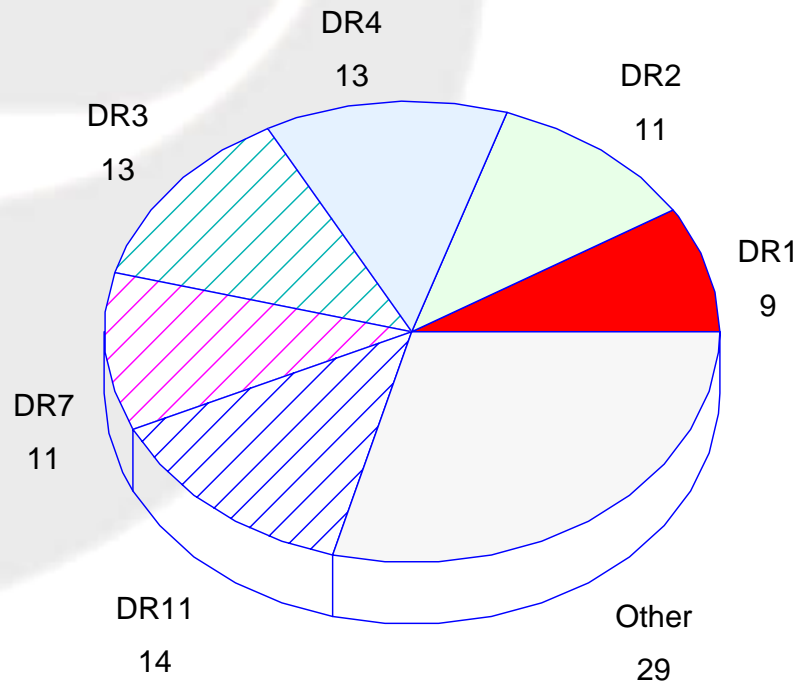
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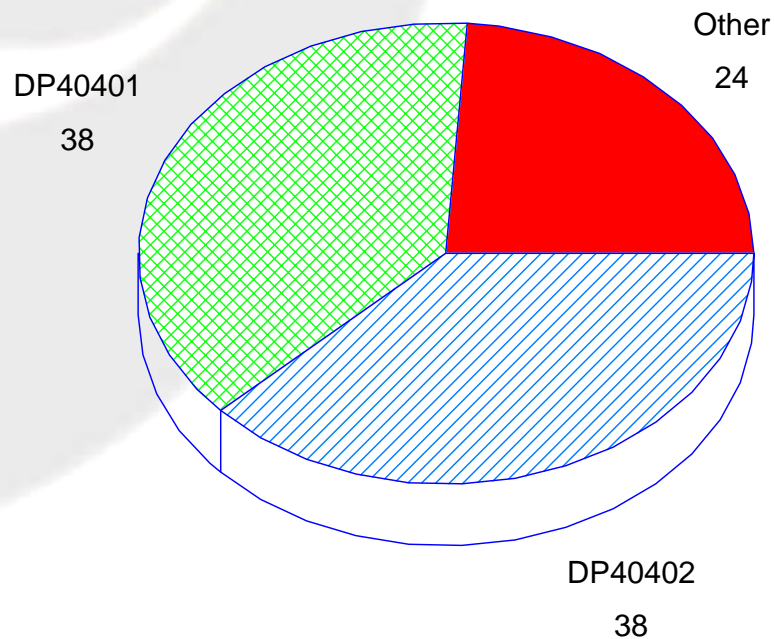
# Outline

- Class II MHC population frequencies and epitopes
- MHC Class II tetramer staining protocol
- T cell expansion protocol
- Measurement of DP401 specific T cells ex vivo and in expanded T cell cultures.
- Summary
- Challenges

# DR Frequencies in the Caucasian Population

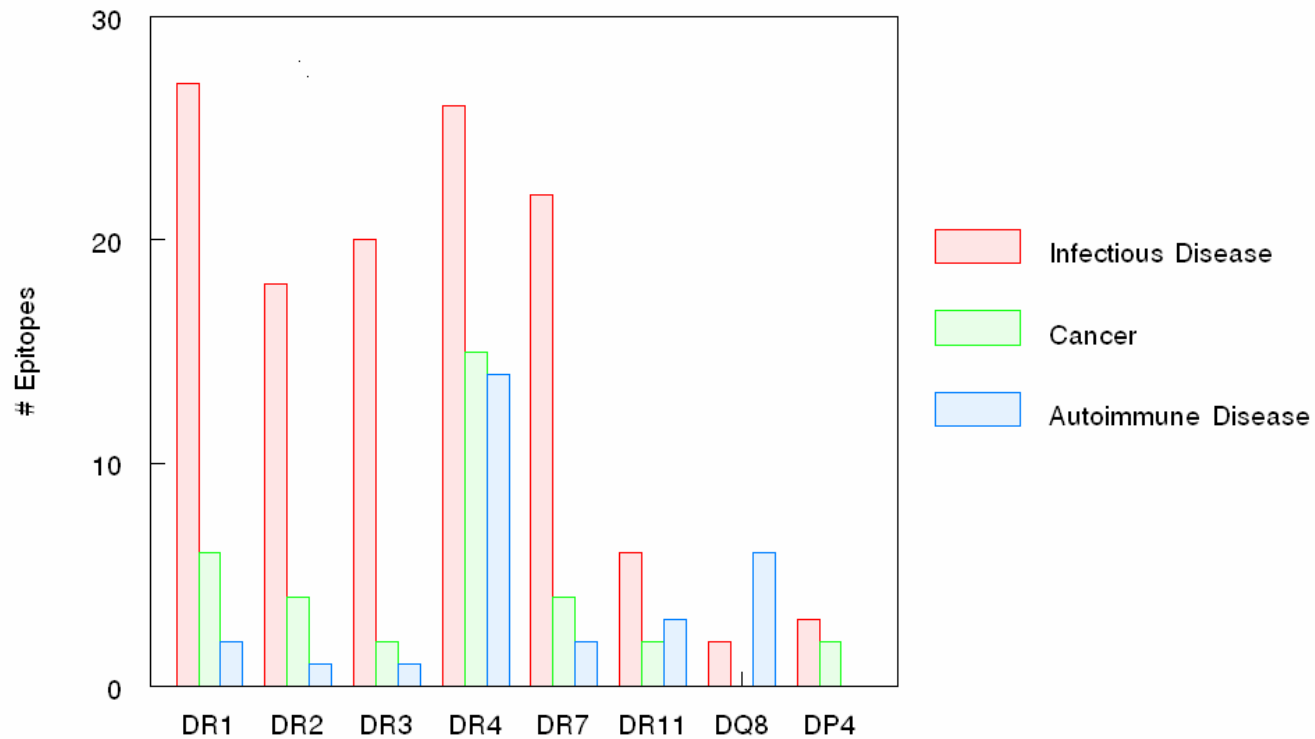


## DP4 Frequencies in the Caucasian Population



# T Cell Epitopes

## Associated with Different MHC Class II Alleles



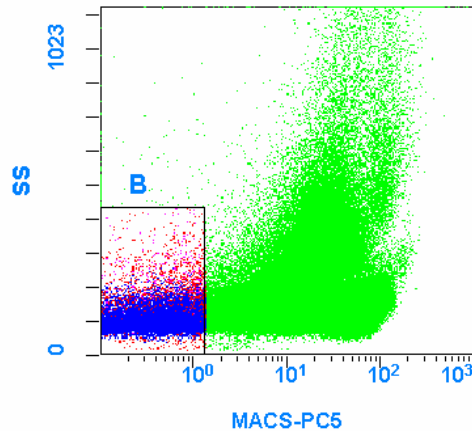
Sources: The HLA Fact Book, Institute Tumori, CANVAC, BCI Sales, BCI R+D

# Tetramer Staining Protocol

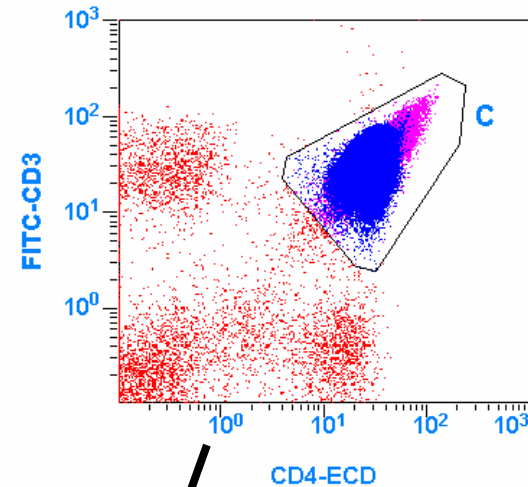
- Expanded or unexpanded PBMCs ( $2-3 \times 10^6$ , fresh or freeze-thawed) were stained with 10 ug of DP401 tetramer at 37° C for 2 hours.
- Cells were cooled on ice for 10 min and 60 ul of antibody cocktail (exclusion gate) was added (Anti-CD3-FITC, Anti-CD4-ECD, Anti-CD8-PC5, Anti-CD13-PC5, Anti-CD19-PC5, Anti-CD56-PC5).
- After 20 minutes of incubation at room temperature, cells were washed once with FACS buffer and resuspended for flow cytometric analysis.
- 100,000 to 200,000 CD4+ T cell events were acquired and analyzed using an EPICS XL flow cytometer (Beckman Coulter).

# Gating Strategy For DP401 Tetramer Flow Cytometric Analysis

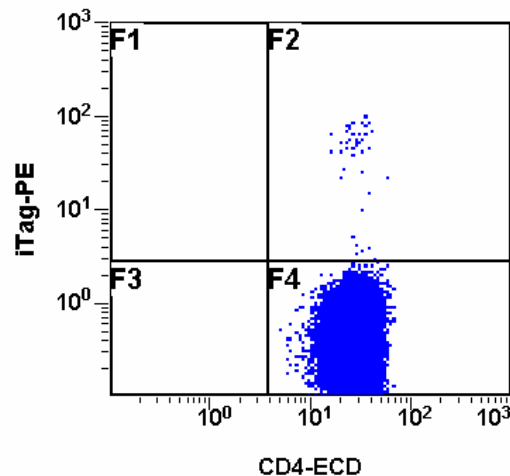
(F1)[A] G0016071.LMD : FL4 LOG/SS



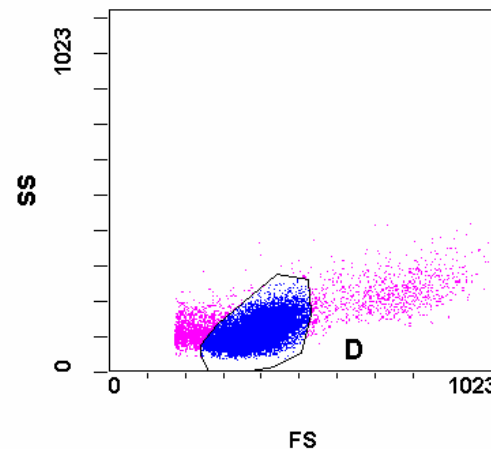
(F1)[A AND B] G0016071.LMD : FL3 LOG/FL1 LOG



AND B AND C AND D] G0016071.LMD : FL3 LOG/FL



(F1)[A AND B AND C] G0016071.LMD : FS/SS



# Cell Stimulation and Expansion

- Peripheral Blood Mononuclear Cells were isolated from heparinized whole blood using Histopaque 1077 (Sigma).  
CD8+ T cells were depleted using Dynal beads according to manufacturer's protocol.
- Cells were cultured in 24 well plates at  $5 \times 10^6$  cells/well/mL of RPMI (Gibco) complete media containing 10% Human AB serum and other supplements and 10 mg/mL of specific peptide
- Plates were placed at 37°C, 5% CO<sub>2</sub> incubator.  
On day 3, 1 mL of RPMI complete media supplemented with 20 IU IL-2 was added to cells (final concentration of IL2 = 10 IU/mL)
- Cells were re-stimulated with peptide between day 7 and day 14 depending on cell number.

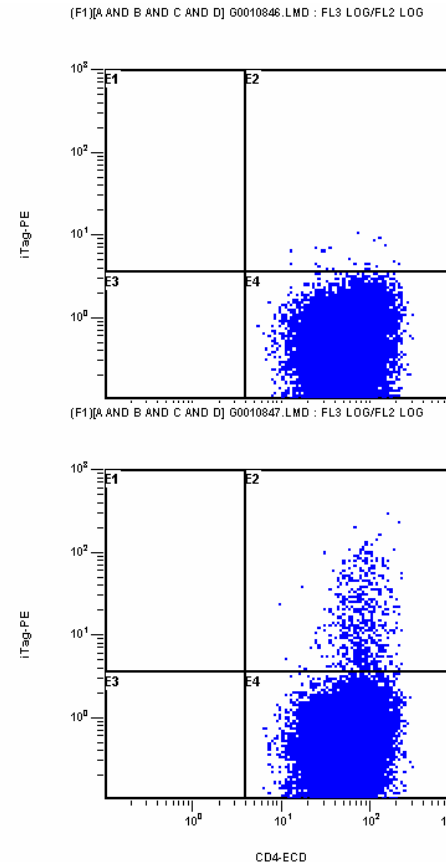
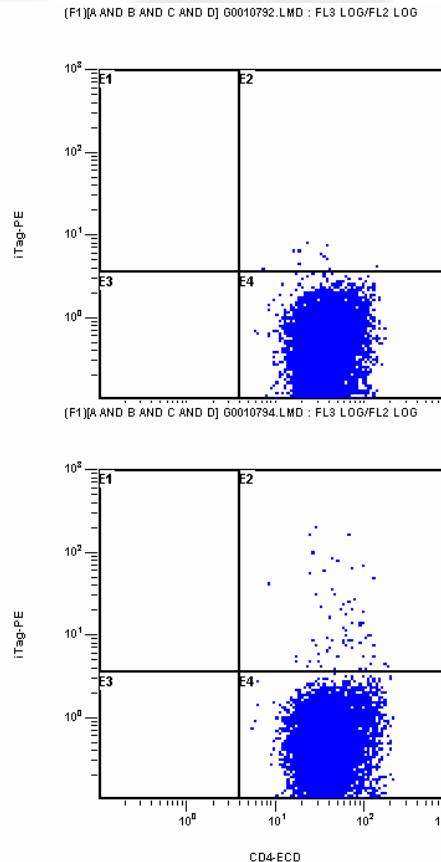
# Detection of Tetanus Specific CD4+ T Cells After *In Vitro* Expansion

1<sup>st</sup> Stimulation  
(9 days)

2<sup>nd</sup> Stimulation  
(6 days)

HBSAg193  
202/DP401  
(Mismatch)

TT 947-  
967/  
DP401



# Detection of RSV Specific CD4<sup>+</sup> T Cells After *In Vitro* Expansion

2<sup>nd</sup> Stimulation

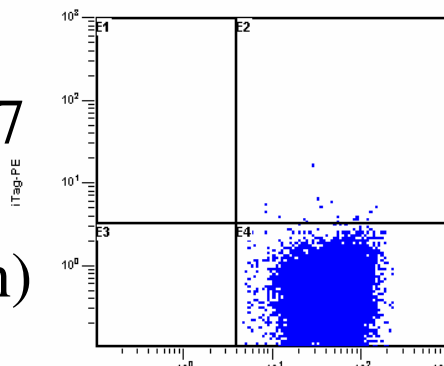
7 days

12 days

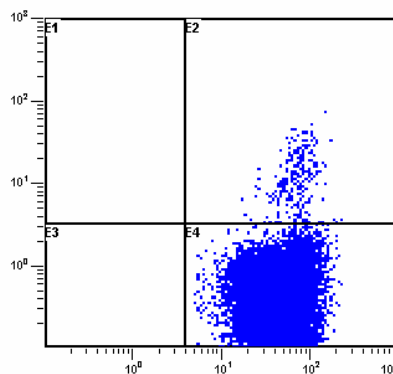
TT947-967  
/DP401  
(Mismatch)

RSVG162-  
173/DP401

(F1)[A AND B AND C AND D] G0009550.LMD : FL3 LOG/FL2 LOG

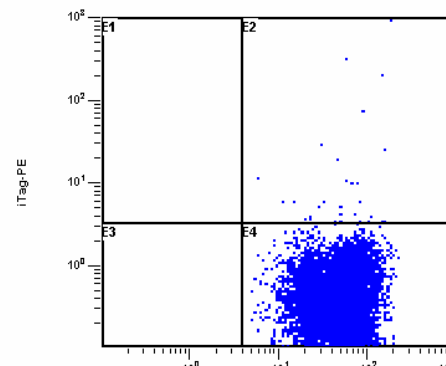


(F1)[A AND B AND C AND D] G0009549.LMD : FL3 LOG/FL2 LOG

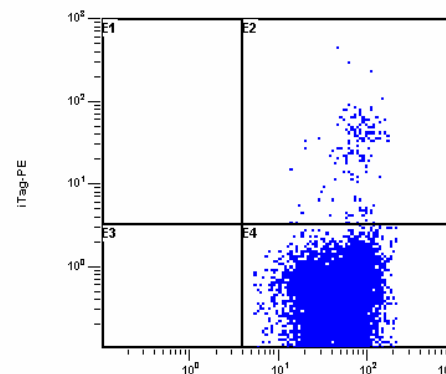


CD4-ECD

(F1)[A AND B AND C AND D] G0009587.LMD : FL3 LOG/FL2 LOG



(F1)[A AND B AND C AND D] G0009588.LMD : FL3 LOG/FL2 LOG



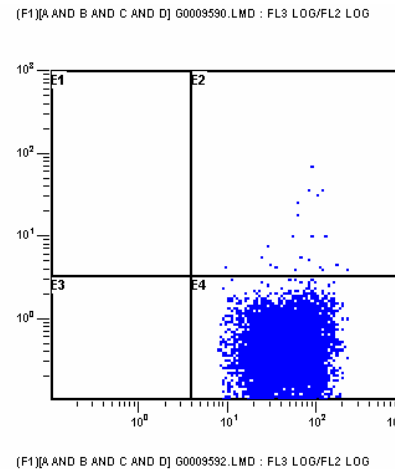
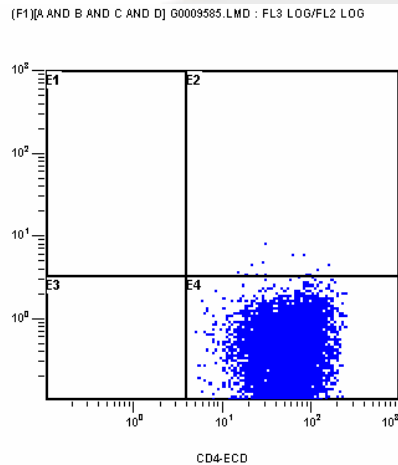
CD4-ECD

# Detection of Hepatitis B Surface Antigen Specific CD4+ T Cells After *In Vitro* Expansion

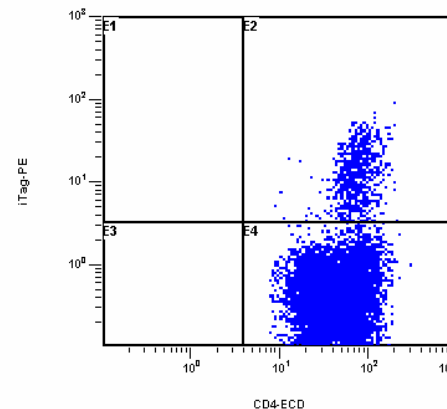
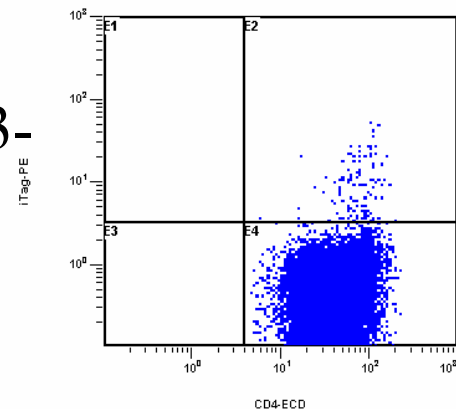
2<sup>nd</sup> Stimulation  
7 days

12 days

TT947-967  
/DP401  
(Mismatch)



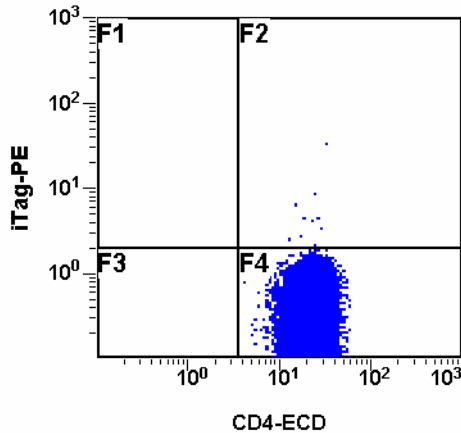
HBSAg193-  
202/DP401



# *Ex Vivo* Measurement of DP401/TT947-967 Specific CD4+ T Cells Following Vaccination with Tetanus Toxoid

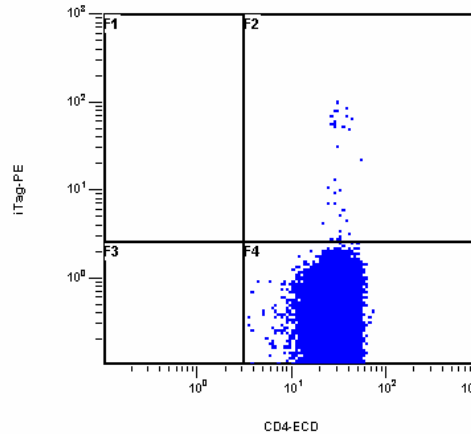
Day 0

AND B AND C AND D] G0016309.LMD : FL3 LOG/FL



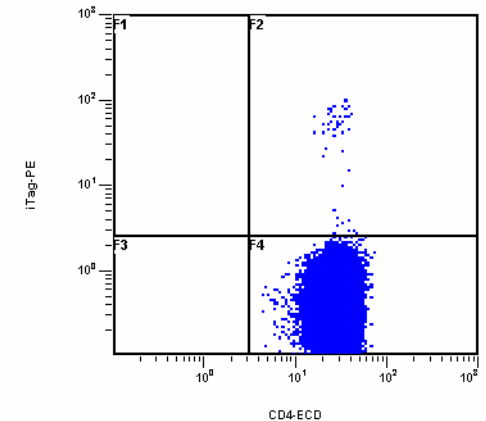
Day 7

(F1)[A AND B AND C AND D] G0016068.LMD : FL3 LOG/FL2 LOG

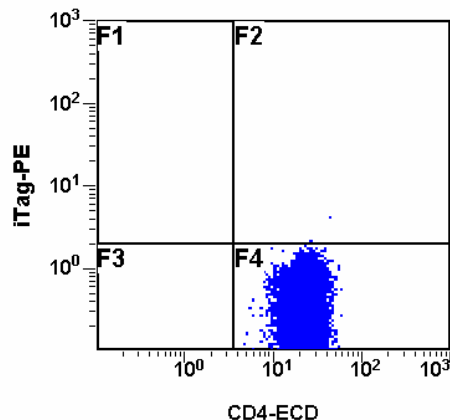


Day 12

(F1)[A AND B AND C AND D] G0016071.LMD : FL3 LOG/FL2 LOG



AND B AND C AND D] G0016308.LMD : FL3 LOG/FL



MAGE3:243-258  
/DP401  
(Negative Control)

# Summary

- DP401 tetramers quantitatively measured Tetanus Toxoid, RSV and hepatitis B surface antigen CD4+ T lymphocytes in PBMCs following *in vitro* expansion.
- DP401 tetramers are able to monitor CD4+ T lymphocyte responses following Tetanus Toxoid vaccination.
- DP401 tetramers may be used to measure Tetanus Toxoid related immune competence.

# Challenges to the Measurement of Antigen Specific, CD4+ T Cells

- Identification of biologically relevant T cell epitopes in cancer and autoimmune diseases.
- DP4 HLA typing is not routine, especially in the US.
- Rare event analysis
- Cell preservation and volume