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

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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

- DR1: 9
- DR2: 11
- DR3: 13
- DR4: 13
- DR7: 11
- DR11: 14
- Other: 29
DP4 Frequencies in the Caucasian Population

- DP40401: 38
- DP40402: 38
- Other: 24
T Cell Epitopes
Associated with Different MHC Class II Alleles

Sources: The HLA Fact Book, Institute Tumori, CANNAC, BCI Sales, BCI R&D
Tetramer Staining Protocol

• Expanded or unexpanded PBMCs (2-3 x 10^6, fresh or freeze-thawed) were stained with 10 µg of DP401 tetramer at 37° C for 2 hours.
• Cells were cooled on ice for 10 min and 60 µl 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
**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 x 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% CO2 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\textsuperscript{st} Stimulation (9 days)

HBSAg\textsubscript{193-202/DP401} (Mismatch)

2\textsuperscript{nd} Stimulation (6 days)

TT \textsubscript{947-967/DP401}
Detection of RSV Specific CD4+ T Cells After *In Vitro* Expansion

**2nd Stimulation**

- 7 days
- 12 days

**TT947-967/DP401**
(Mismatch)

**RSVG162-173/DP401**
Detection of Hepatitis B Surface Antigen Specific CD4+ T Cells After *In Vitro* Expansion

2nd 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

Day 7

Day 12

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 \textit{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