INSTITUT PASTEUR — UNIVERSITE PIERRE & MARIE CURIE

The 2nd PSU International Teaching Platform on Tumour Immunology and Immunotherapy

Jointly organized by
Prince of Songkla University, Université Pierre et Marie Curie (Paris 6) and Institut Pasteur

December 15 – 20, 2003
At The Department of Biomedical Sciences
Faculty of Medicine, Prince of Songkla University,
Hat Yai, Songkhla, Thailand

Lecture 9:
Techniques for the detection of specific T-lymphocytes application to the immunomonitoring of cancer vaccine protocols
Prof. Eric Tartour

December 18, 2003

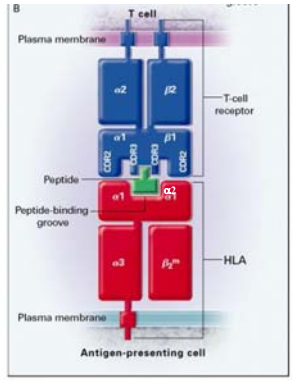
TECHNIQUES FOR THE DETECTION OF SPECIFIC T-LYMPHOCYTES APPLICATION TO THE IMMUNOMONITORING OF CANCER VACCINE PROTOCOLS

2nd PSU Workshop on Tumour Immunology and Immunotherapy, December 15-20, 2003. Prince of Songkla University, Université Pierre et Marie Curie and Institut Pasteur

PEPTIDE MHC TETRAMER STAINING

2nd PSU Workshop on Tumour Immunology and Immunotherapy, December 15-20, 2003. Prince of Songkla University, Université Pierre et Marie Curie and Institut Pasteur

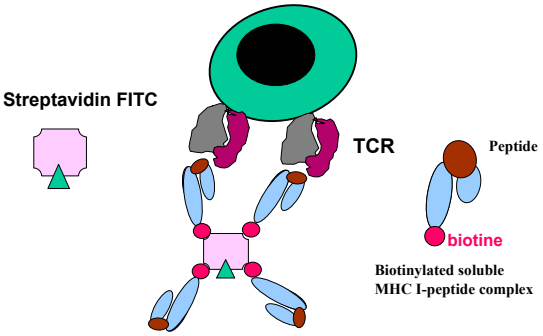
INTERACTION BETWEEN TCR AND MHC-PEPTIDE COMPLEXES



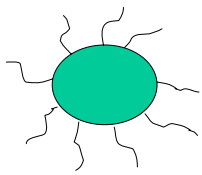
Klein et Sato NEJMed 2000

2nd PSU Workshop on Tumour Immunology and Immunotherapy, December 15-20, 2003. Prince of Songkla University, Université Pierre et Marie Curie and Institut Pasteur

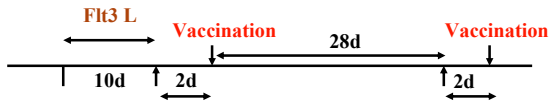
MHC PEPTIDE TETRAMERIC COMPLEX STAINING



2nd PSU Workshop on Tumour Immunology and Immunotherapy, December 15-20, 2003. Prince of Songkla University, Université Pierre et Marie Curie and Institut Pasteur



Pulse DC with modified peptide
CEA 605-613$^{Asp\text{-}Asn\ (610)}$

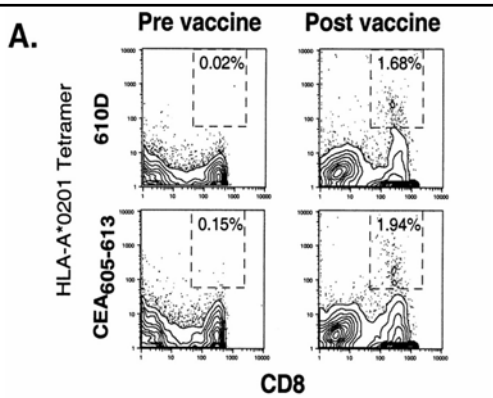


- Cytapheresis
- DC purification
(Density gradient)

- Cytapheresis
- DC purification
(Density gradient)

Phase I : 12 patients
(Colon adenocarcinomas and non small lung carcinomas)

2nd PSU Workshop on Tumour Immunology and Immunotherapy, December 15-20, 2003. Prince of Songkla University, Université Pierre et Marie Curie and Institut Pasteur



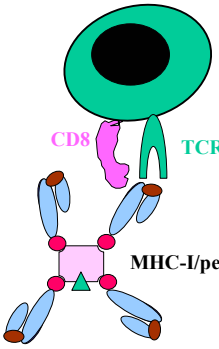
Identification and characterization of antigen-specific CD8 T cells with MHC/peptide tetramers

Correlation between immune and clinical responses*

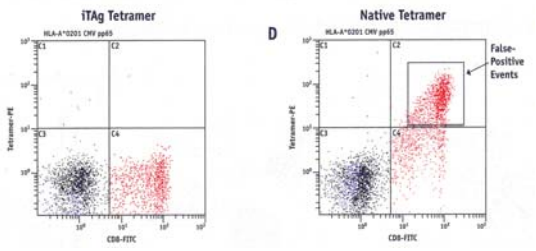
Patient	Clinical Response	Tetramer+ Prevaccine%	Tetramer+ Postvaccine%
1	PD	0.08	0.25
2	PD	0.03	0.08
3	SD	0.15	1.11
4	PD	0.18	0.04
5	CR-10Mo	0.4	1.03
6	PD	0.10	0.31
7	PD	0.26	0.49
8	SD	0.43	1.05
9	PD	0.16	0.07
10	PD	0.24	0.5
11	CR-10Mo	0.28	1.03
12	MR	0.12	1.68

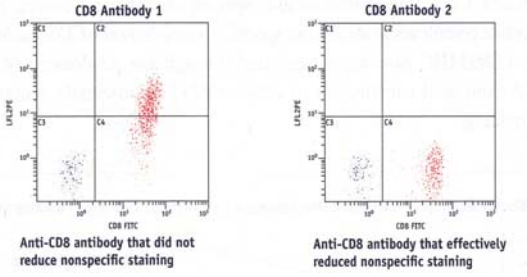
* P = 0.002

Non antigen-dependent interaction between CD8 and non polymorphic region of MHC-I



Addition of mutations in amino-acids of HLA molecules involved in the interaction between monomorphic region of HLA and CD8 molecules permits the reduction of non specific binding of tetramers

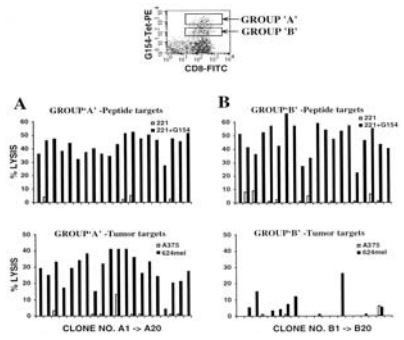




Sensitivity of this technique:

Threshold of detection 0.1-0.01% of specific T cells : $1/10^{-3}$ to $1/10^{-4}$

Although previous studies used the intensity of tetramer staining as a measure for recognition efficiency (or functional avidity) (Yee C et al J Immunol 1999)

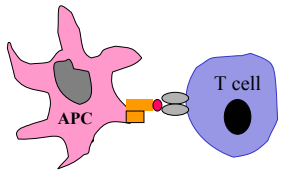


Isolation of high avidity, tumour-reactive T cell clones from a heterogeneous population following sorting of tetramer^{high} CD8^{+} T cells

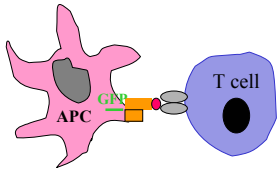
Yee C J Immunol 1999

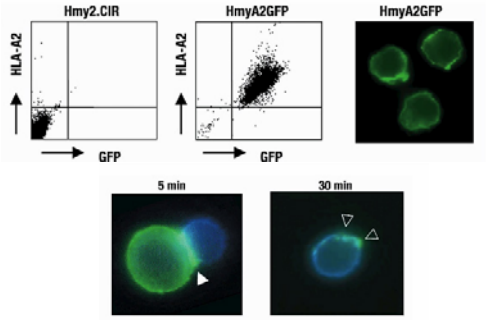
Although previous studies used the intensity of tetramer staining as a measure for recognition efficiency (or functional avidity) (Yee C et al J Immunol 1999)

Recent evidence suggests that tetramer staining does not directly correlate with recognition efficiency. (Echkakir H et al. PNAS 2002; Dutoit V. Eur J Immunol 2002)



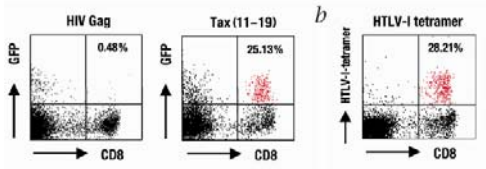
During peptide MHC-TCR specific recognition, peptide-MHC clusters are acquired by CD8^{+} T cells and internalized through the TCR (Stinchcombe JC Immunity 2001; Huang JF Science 1999)





Peptide–HLA-GFP complexes are acquired by HLA-A*201–restricted HTLV-I–specific CTL clone

Tomaru U. Nat Med 2003



Tax (11-19) specific HLA-GFP acquisition by CD8^{+} T cells from bulk PBMCs of HLA-A201 HTLV-1 infected patients.

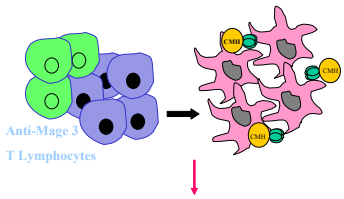
Tomaru U. Nat Med 2003

- MHC-peptide tetramer staining enumerates all specific T cells but does not discriminate functional or anergic specific T cells

- The avidity and the potential cytotoxic of these T cells are also not analyzed by these technique.

- Functional avidity or recognition efficiency of T cells is now emerging as a key factor in the effectiveness of an antigen specific T cell response.

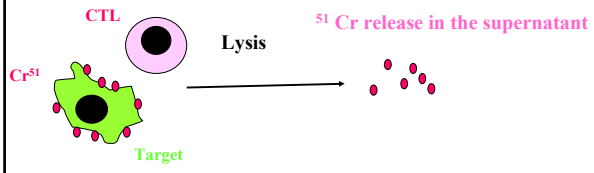
DETECTION OF FUNCTIONAL T LYMPHOCYTES



Functional T lymphocytes may be detected by:

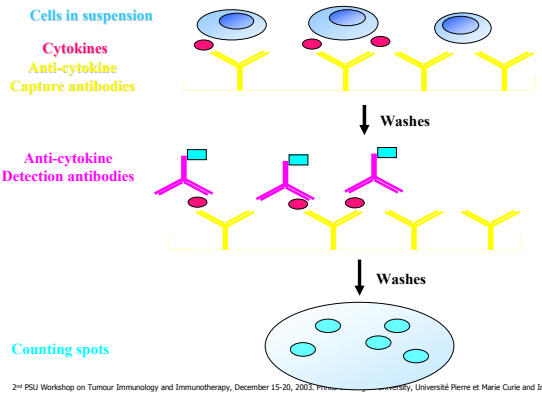
Lysis of APC	T cell activation measured	Phenotypic	Quantification of the
^{51}Cr cytotoxic assay	by cytokine production - Elispot - Intracellular cytokine detection	marker: CCR7, CD45RA CD27,CD28 Perforin	surface mobilization of CD107a

51Cr cytotoxicity assay



- Radioactive technique
- Low sensitivity → Threshold of detection: 0.1% of specific T cells ($1/10^{-3}$)
- Measure the functionality and avidity of T lymphocytes.

ELISPOT



ELISPOT

- Recognition of MHC-peptide complex by TCR will lead to activation of T cells and production of cytokines detected by Elispot.

- One of the most sensitive technique to measure cytokine

- Secreted cytokines are directly captured by antibodies coated on the Elispot plates which will avoid diffusion and dilution of the cytokine in the supernatant, degradation by proteases or binding to soluble cytokine receptors possibly present in the supernatant.

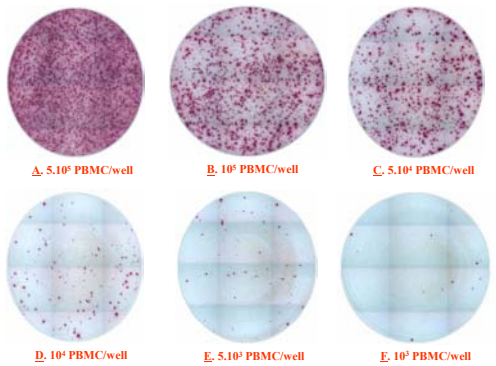


Illustration of an Elispot assay to detect IFNγ

SENSITIVITY OF ELISPOT

- The plate of Elispot is saturated for concentrations of PBMC above: 10^5

→ Theoretically: Threshold of detection: $1/10^5$ (0.001%)

- In routine analysis:
the test is considered positive if > 5-10 spots per 10^5 cells.

Sensitivity: $1/10^4$ et $1/10^5$

ADVANTAGES OF THE ELISPOT TECHNIQUE

- Detect functional T lymphocytes

- May be used even without the knowledge of MHC-peptide complex recognized by T cells (APC pulsed with pool of peptides or vectorized antigens, tumour cells as target).

- High sensitivity

- Possible automation

LIMITS OF THE ELISPOT TECHNIQUE

- DETECTS ONLY NON ANERGIC T CELLS WITH THE ABILITY TO PRODUCE THE CYTOKINE TESTED.

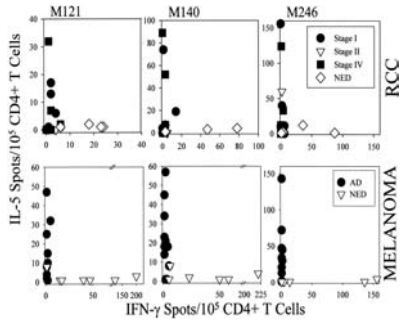
- DOES NOT ALLOW THE PHENOTYPING OF T CELLS PRODUCING THE CYTOKINE

- DOES NOT ALLOW SORTING OR PURIFICATION OF SPECIFIC T LYMPHOCYTES PRODUCING THE CYTOKINES.

INDICATIONS OF THE ELISPOT TECHNIQUE

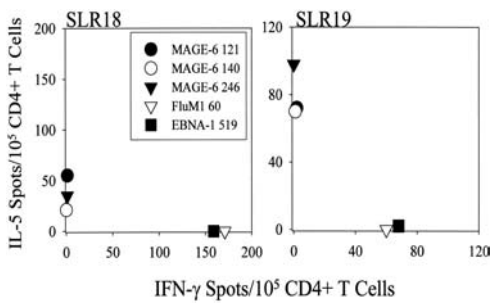
- Analysis of the frequency of T cell precursors producing cytokines (TH1, TH2, TH3…). Analysis of the polarisation of T lymphocytes.

- Identification of specific T lymphocytes with characterization of their polarisation.



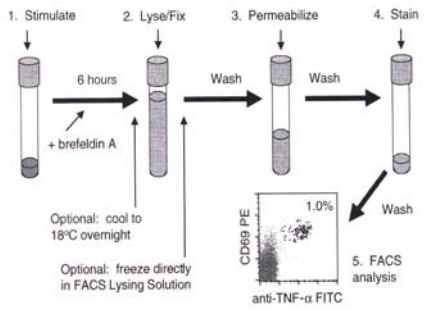
Analysis of the TH1-type vs TH2 type $CD4^+$ T cell response to Mage-6 peptides in HLA-DRβ10401 in RCC or melanoma patients

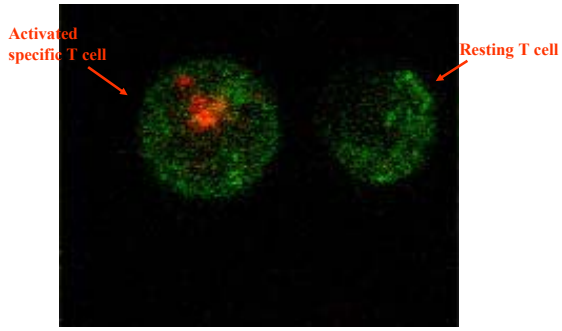
Tasumi et al J. Exp Med 2002



Peripheral blood from cancer patients display TH2-type reactivity to Mage-6 epitope but TH1 reactivity to viral epitopes.

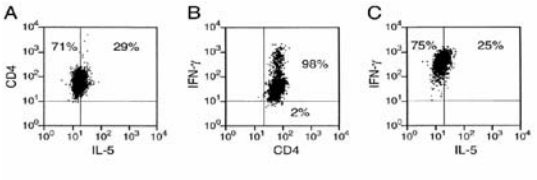
DETECTION OF SPECIFIC T LYMPHOCYTES BY MEASURING INTRACELLULAR CYTOKINE BY FACS ANALYSIS





Activated and resting T lymphocytes identified by the expression of CD3 (green) and production or not of IL-2 (red)

Single-cell analysis of IFNγ and IL-5 synthesis by an CD4+ anti-HA clone



Graham CM J Immunol 1998

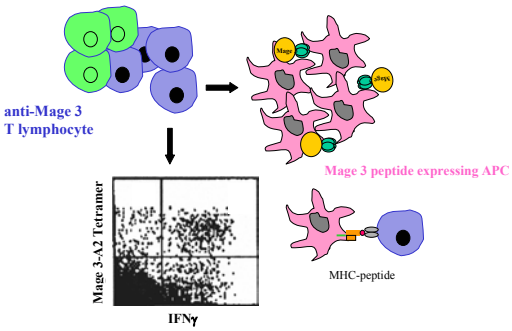
DETECTION OF SPECIFIC T LYMPHOCYTES BY INTRACELLULAR CYTOKINE ANALYSIS USING CYTOMETRY

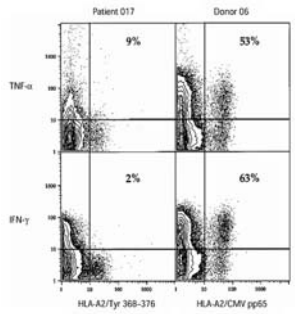
- **SENSITIVITY**: Ability to detect 0.1 to 1% specific T cells ($1/10^{-2}$-$1/10^{-3}$)

- Identification of functional specific T lymphocytes

- Possibility to phenotype but not to sort specific T lymphocytes producing cytokines.

COUPLING MHC-PEPTIDE TETRAMER ANALYSIS WITH DETECTION OF INTRACELLULAR CYTOKINES





Intracellular cytokine staining of antigen-specific T cells

Tyrosinase-specific $CD8^+$ T cells from patient 017 and CMV-specific $CD8^+$ T cells from donor 06 were stained for the expression of TNFα and IFNγ after stimulation with PMA and ionomycin for 6 h (last 3 h with Brefeldin A).

Lee et al Nat Med 1999

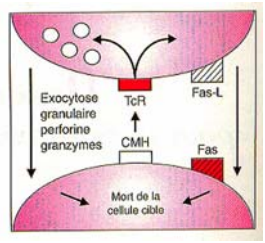
Comparative analysis of techniques to detect specific T lymphocytes

	ELISPOT	INTRACELLULAR CYTOKINES	MHC TETRAMER	CYTOTOXICITY ^{51}Cr
Sensitivity	10^4-10^5	10^3	10^3-10^4	10^3
T cell detection				
- functional	+	+	+	+
- anergic	-	-	+	-
Phenotypic analysis	-	+	+	-
Sort of cells	-	-	+	-
Automation	+	-	-	-

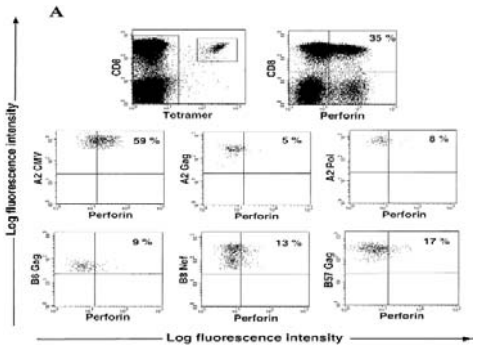
ANALYSIS OF CYTOLYTIC POTENTIAL OF CD8 T CELLS

- Labelling the target with: ^{51}Cr (standard cytotoxic ^{51}Cr assay)
  : fluorescent dyes (flow based killing assay)

- Assessment of perforin or granzyme cell content (Intracellular cytometry)
  release by CD8-T cells (Elispot)



Two main mechanisms responsible of the cytotoxicity mediated by CD8 T-cells



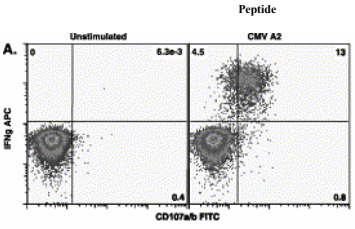
HIV specific $CD8^+$ T cells express low levels of perforin

Appay V J. Exp Med 2000

Lytic granules of CD8 T cells contain a dense core composed of various proteins including perforin and granzymes surrounded by a lipid bilayer containing lysosomal associated membrane glycoproteins (LAMPS) including CD107a (LAMP-1), CD107b (LAMP-2) and CD63 (LAMP-3).

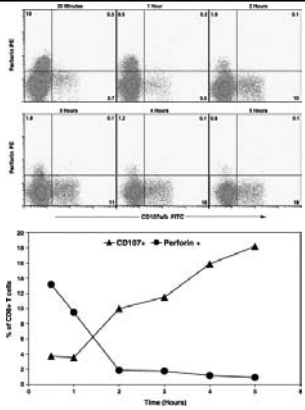
Cumulative exposure of granular membrane proteins (CD107a and b) on the cell surface of responding antigen-specific T cells provides a marker of degranulation.

Significant expression of cell surface CD107a and b can be observed as early as 30 min following stimulation of primary $CD8^+$ T cells, and reaches a maximum by 4h.



CD107a is expressed by *ex vivo* activated antigen-specific CD8⁺ T cells

Betts MR J Immunol Methods 2003



Betts MR J Immunol Methods 2003

Acquisition of cell surface CD107 is correlated with a loss of intracellular perforin

Cytolytic activity of tetramer-positive CD107a⁺ and CD107a⁻ clones directed against gp100

Rubio V Nature Med 2003

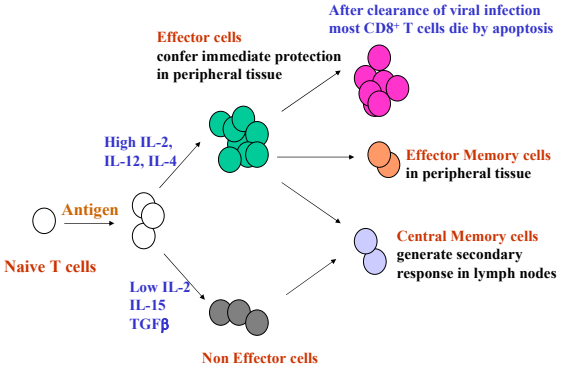
Sample 10545

Tetramer-positive CD107a⁺ clones				Tetramer-positive CD107a⁻ clones			
Malme-3M	mel526	A375	RE	Malme-3M	mel526	A375	RE
31	38	–1	10^{-12} M	0	0	0	10^{-8} M
20	22	–1	10^{-10} M	8	4	–1	10^{-9} M
20	22	–1	10^{-11} M	11	7	0	10^{-9} M
27	29	–1	10^{-11} M	24	20	1	10^{-10} M
20	15	–1	10^{-11} M	2	1	0	10^{-8} M
—	—	—	—	4	8	–1	10^{-9} M
—	—	—	—	1	5	–1	10^{-8} M
23.6	25.2	–1	Averages	7.1	6.4	–0.3	Averages

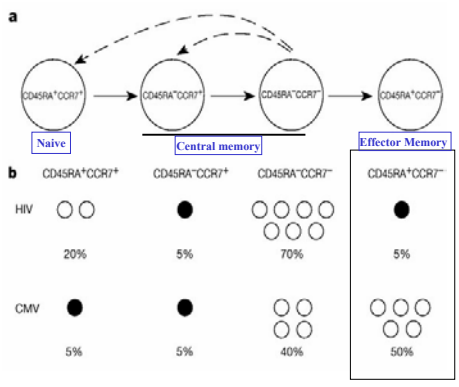
Sample 10356

Tetramer-positive CD107a⁺ clones				Tetramer-positive CD107a⁻ clones			
Malme-3M	mel526	A375	RE	Malme-3M	mel526	A375	RE
40	42	1	10^{-11} M	2	6	0	10^{-8} M
37	32	2	10^{-11} M	2	5	0	10^{-7} M
40	42	3	10^{-11} M	1	3	1	10^{-7} M
33	32	3	10^{-10} M	42	47	0	10^{-11} M
32	34	2	10^{-10} M	2	5	1	10^{-9} M
39	51	1	10^{-12} M	—	—	—	—
36.8	38.8	2	Averages	9.8	13.2	0.4	Averages

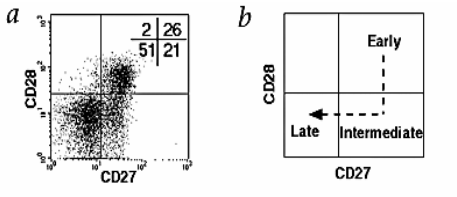
Phenotypic markers to identify functional (effector T cells)



Pathways for memory T cell generation

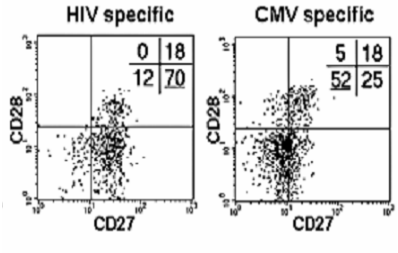


Differences in the composition of the HIV and CMV specific memory CD8⁺ T cell subsets



Model of antigen-specific CD8$^+$ T cell differentiation

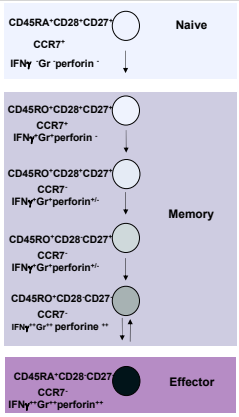
Appay V Nature Med 2002



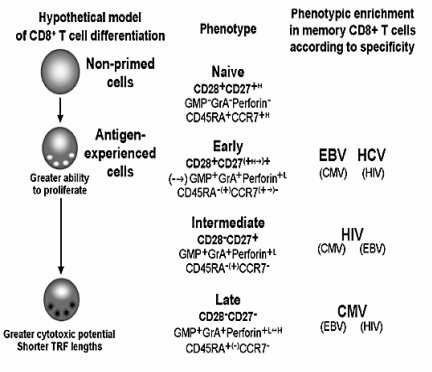
CD28/CD27 expression on virus-specific CD8$^+$ T cells during chronic viral infection

Appay V Nature Med 2002

Differentiation of CD8$^+$ T cells



Van Baarle et al.
Trends in Immunology 2002



	CCR7	CD62L	Perforin	CD45RA	CD44	CD122 β chain IL-2R/IL-15R
Central memory CD8 T cells	+	+	-	-	+	-
Effector Memory CD8 T cells	-	-	+	+	+++	+

Phenotypic markers of effector and central memory CD8$^+$ T cells in mice

CONCLUSION AND PERSPECTIVES

-Numerous techniques for the detection and characterization T-lymphocytes have been reported during these last years. They will allow to better discriminate functional activity and anergic T-cells.

-Surrogate T lymphocytes markers of the *in vivo* functional activity of these T cells have to be selected among different parameters.

- This monitoring will guide the improvement of strategies for the development of cancer vaccines.