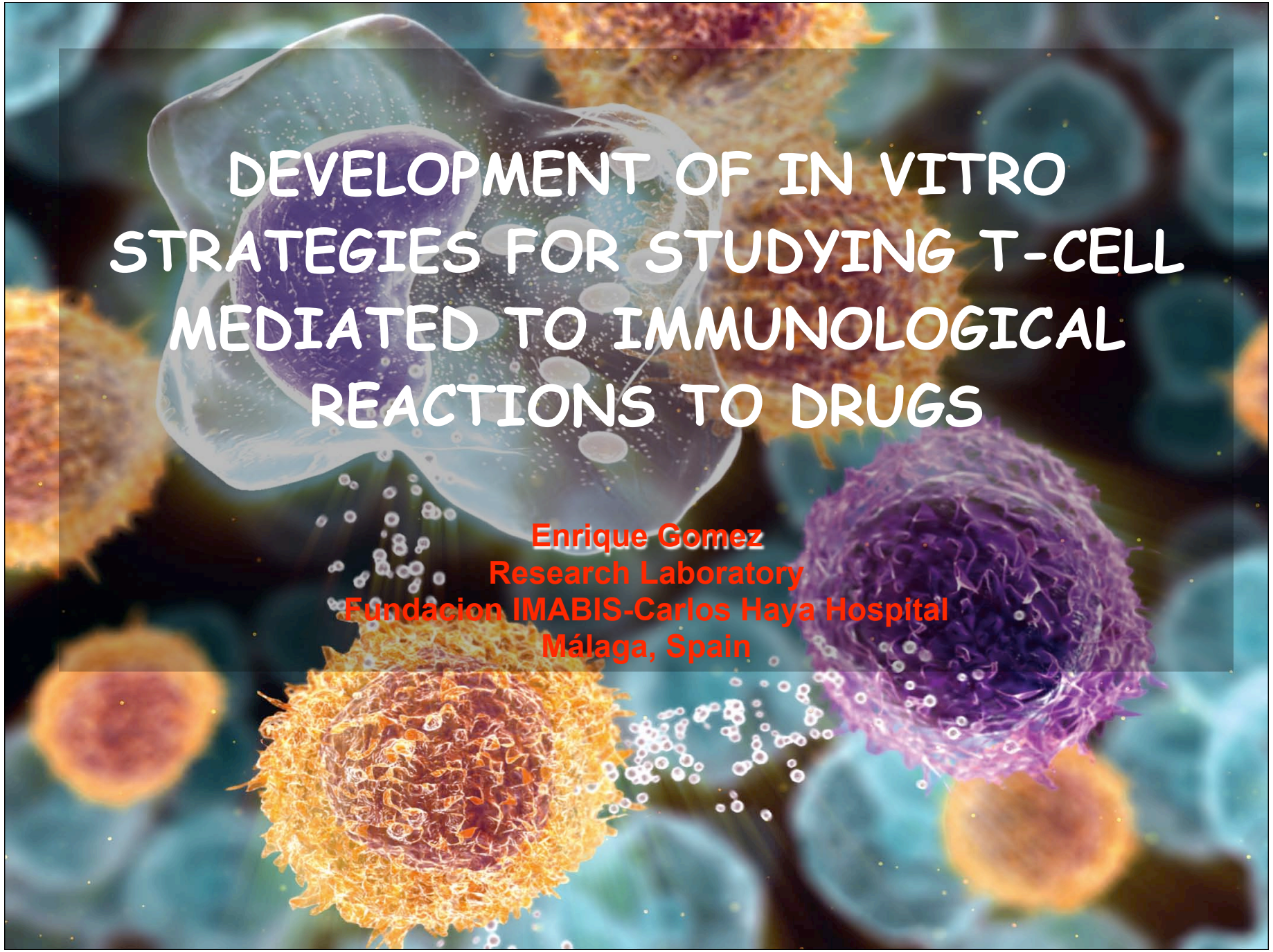
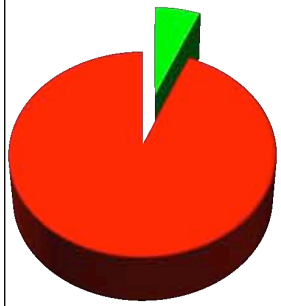

DEVELOPMENT OF IN VITRO STRATEGIES FOR STUDYING T-CELL MEDIATED TO IMMUNOLOGICAL REACTIONS TO DRUGS
Enrique Gomez
Research Laboratory
Fundacion IMABIS-Carlos Haya Hospital
Málaga, Spain

Adverse Drug Reactions (ADR): a response to a drug that is noxious and unintended and occurs at doses normally used in man for the prophylaxis, diagnosis or therapy of disease, or modification of physiological function. (*World Health Organization, 1972).*

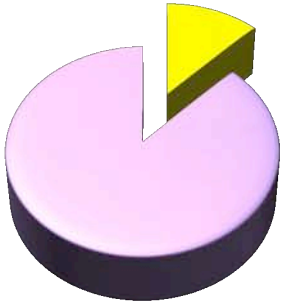
A percentage of all ADR are produced by an altered immunologic response to the drug and are named **Allergic drug reactions with an immunological basis or ADRIB** *(Gruchalla R., J Allergy Clin Immunol. Allergy 2003).*

HOSPITAL ADMISSIONS



ADR
6%

HOSPITALIZED PATIENTS



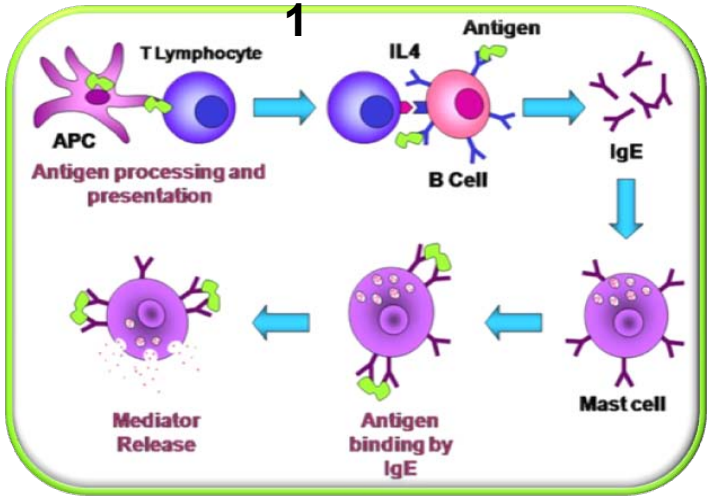
ALLERGIC REACTION
10-15%

Bigby M, et al. JAMA. 1986

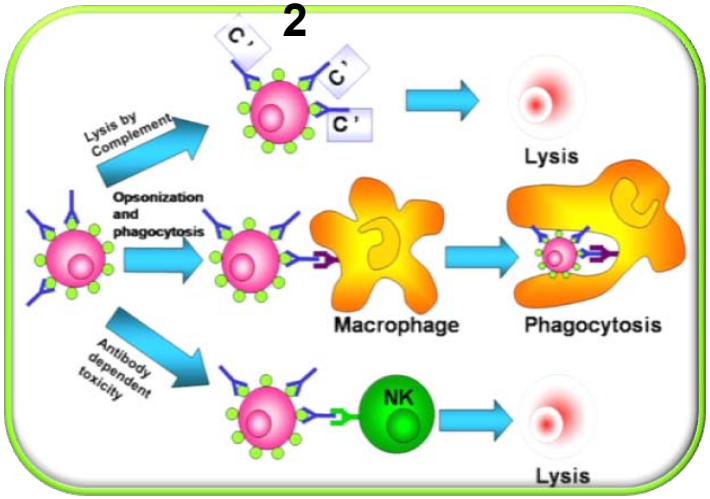
ALLERGIC REACTIONS CLASSIFICATION

MECHANISM INVOLVED

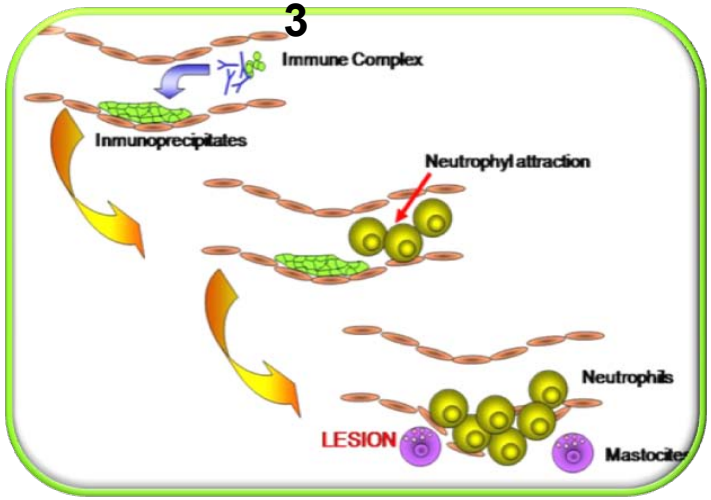
Type 1



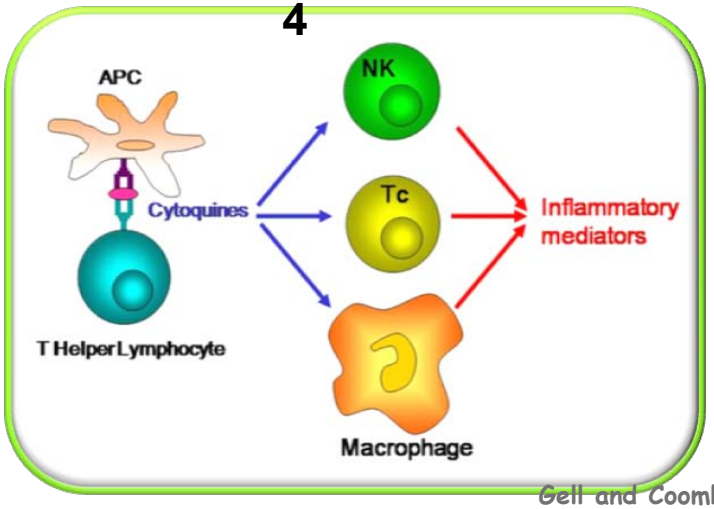
Type 2



Type 3



Type 4



Gell and Coombs 1963

CLASSIFICATION OF ALLERGIC REACTIONS

TIME INTERVAL

IMMEDIATE:

IgE mediated

Th2 (IL4, IL5, IL10, IL13)

NON-IMMEDIATE:

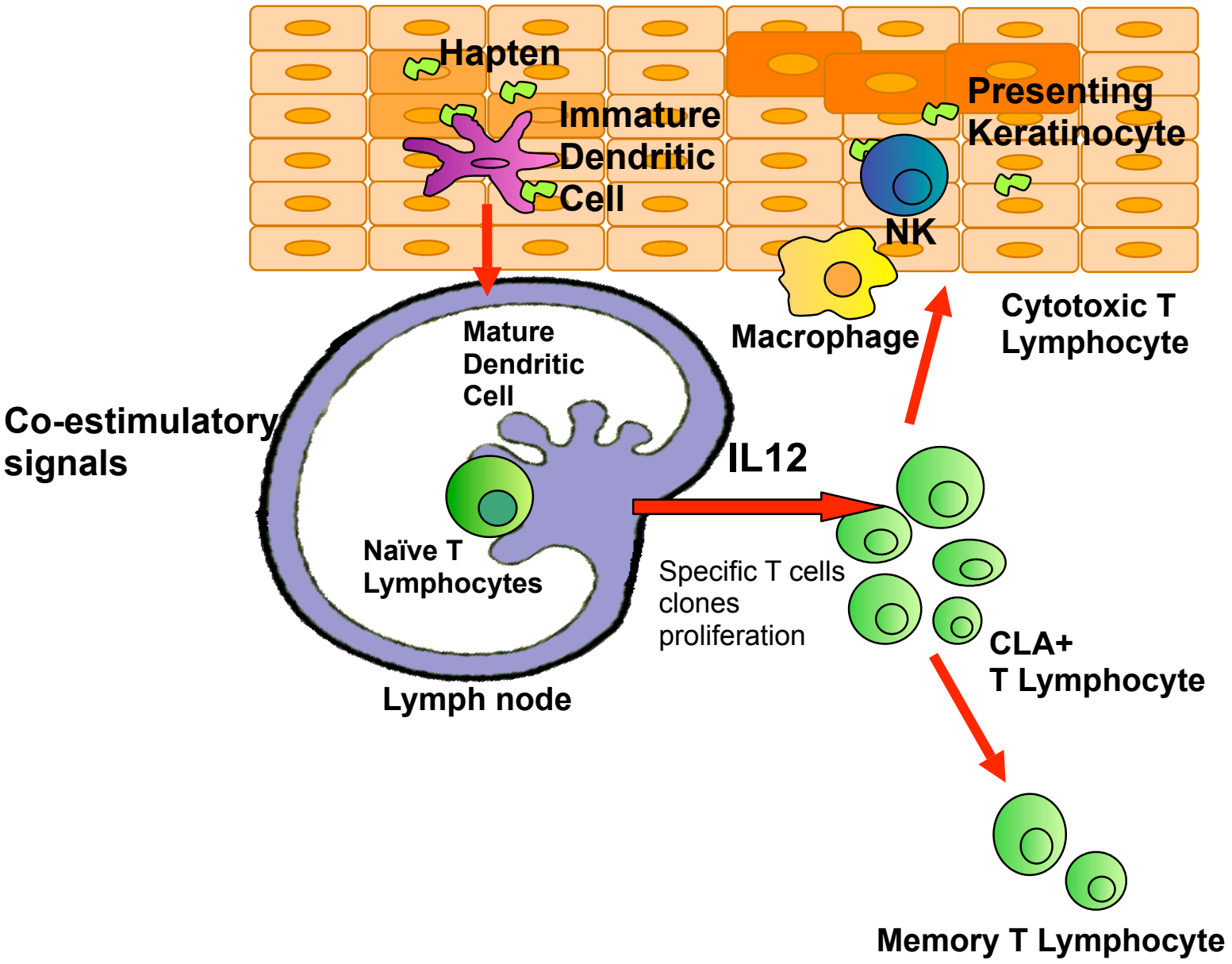
T-cell mediated

Th1 (IFNγ, TNFα, IL12)

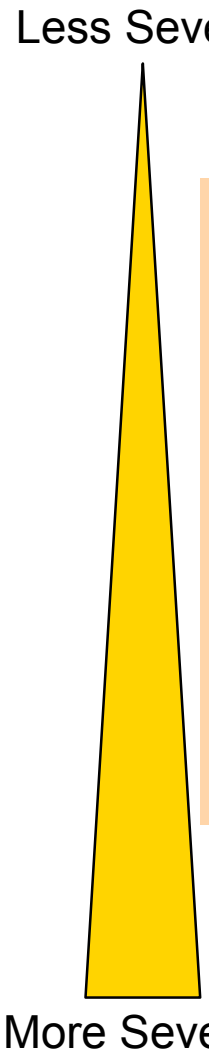
Levine BB. N Engl J Med 1966

T-CELL MEDIATED REACTIONS

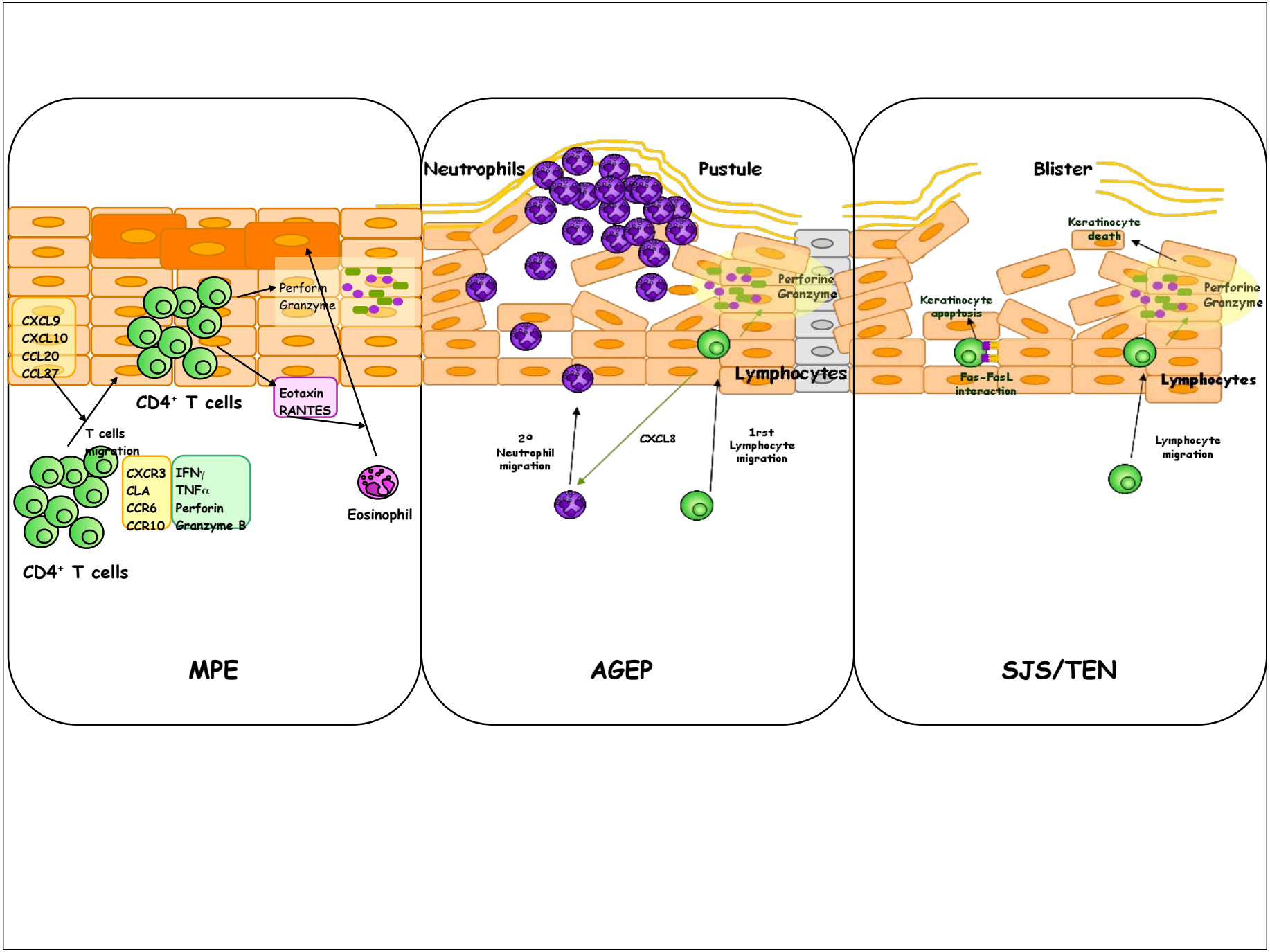
Cellular Response Th1



Pathologies



- Urticaria/angioedema
- Exanthema maculopapular
- Exanthema fixed
- Dermatitis
- Acute Generalised Exanthematic Pustulosis (AGEP)
- SJS
- Toxic Epidermal Necrolysis


CXCL9
CXCL10
CCL20
CCL27
Perforin
Granzyme
CD4+ T cells
Eotaxin
RANTES
T cells
migration
CXCR3
CLA
CCR6
CCR10
IFNγ
TNFα
Perforin
Granzyme B
Eosinophil
CD4+ T cells
MPE
Neutrophils
Pustule
Perforine
Granzyme
Lymphocytes
2°
Neutrophil
migration
CXCL8
1rst
Lymphocyte
migration
AGEP
Blister
Keratinocyte
death
Keratinocyte
apoptosis
Perforine
Granzyme
Fas-FasL
interaction
Lymphocytes
Lymphocyte
migration
SJS/TEN

DIAGNOSTIC APPROACH

Clinical history

In vivo

Skin test

Drug provocation test

In vitro tests

IN VITRO TESTS

- Advantages:
  - No risk.
  - Skin diseases or other treatments.
  - Less time consuming.
- Disadvantages:
  - Less sensitive.

NON IMMEDIATE REACTIONS

- Monitorization of the acute response
- Resolution phase study

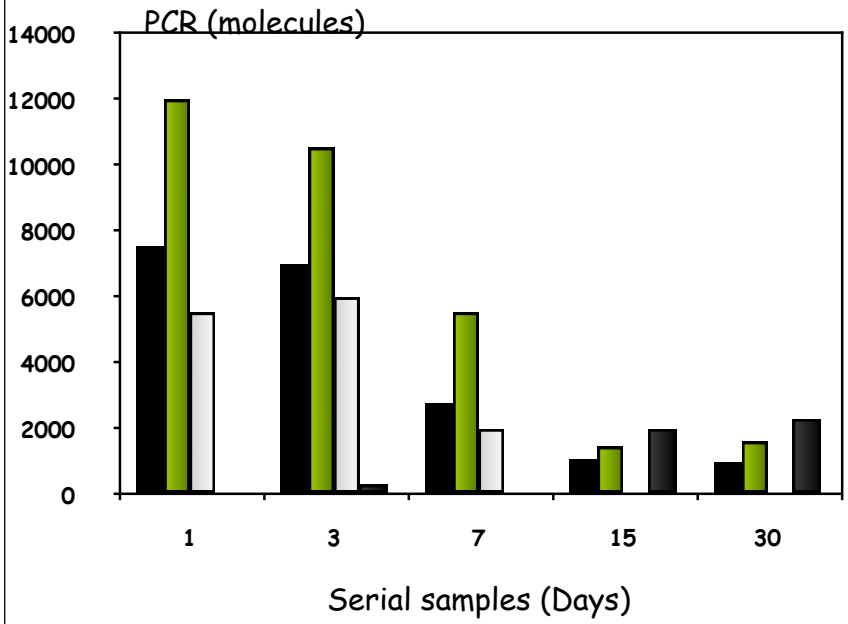
EVALUATION OF NON-IMMEDIATE REACTIONS

- IMMUNOHISTOCHEMISTRY
- REALTIME PCR
- FLOW CYTOMETRY
- CELL CULTURE

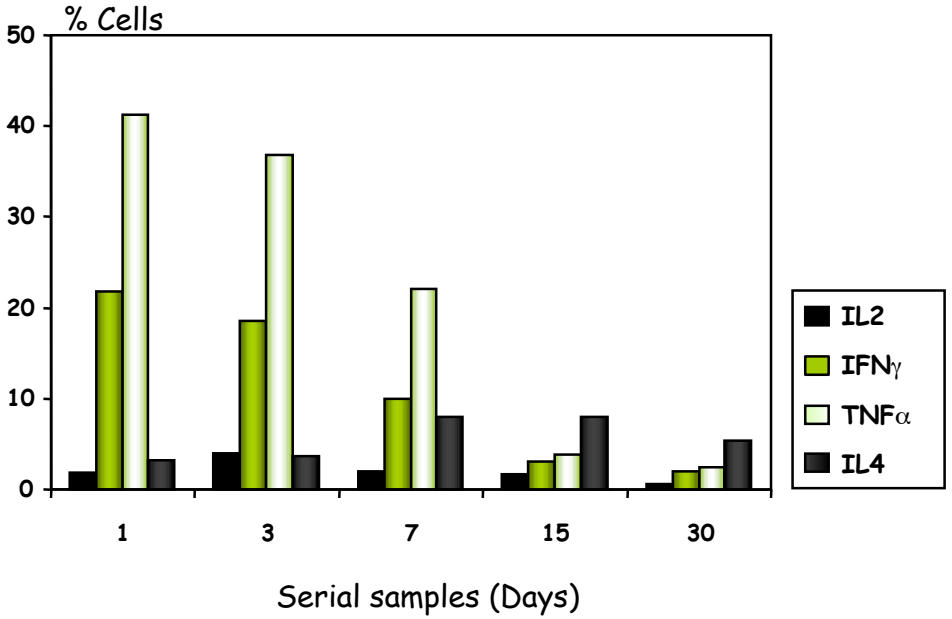
Monitorization of the acute response

Analysis with different methodology

RT-PCR

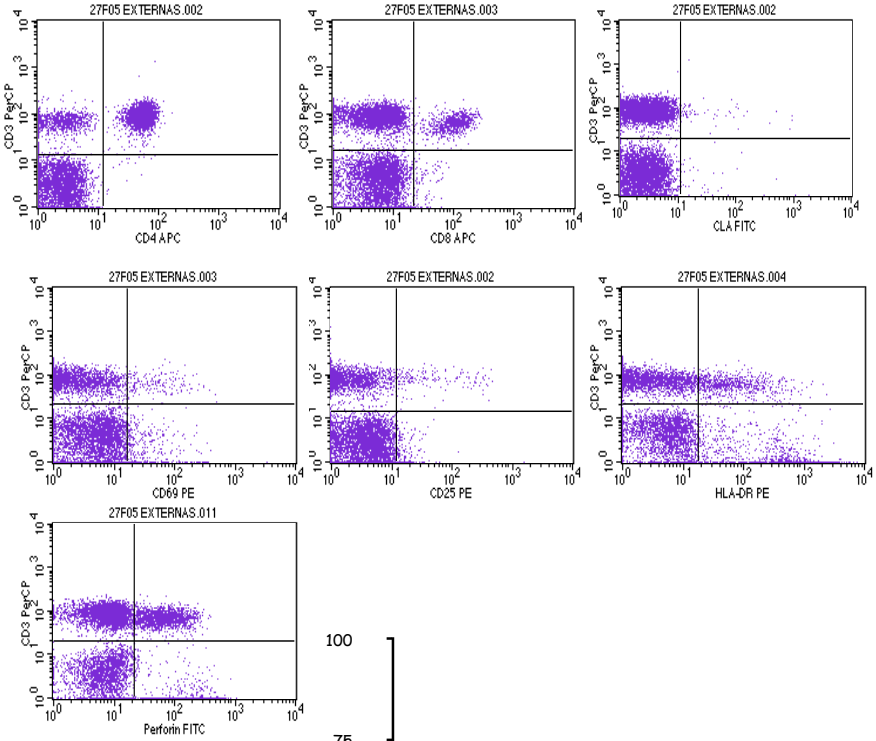


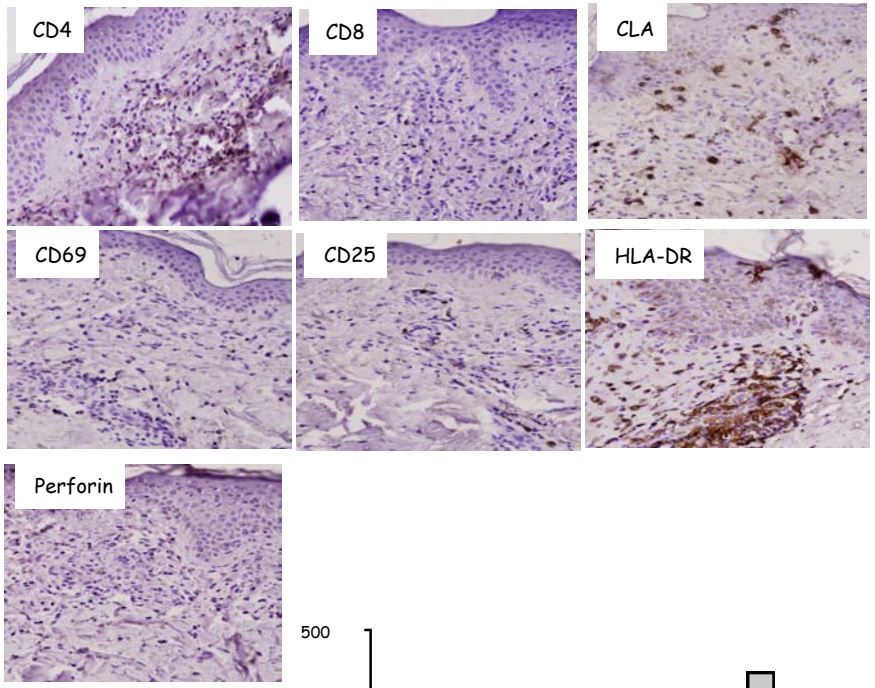
Flow cytometry

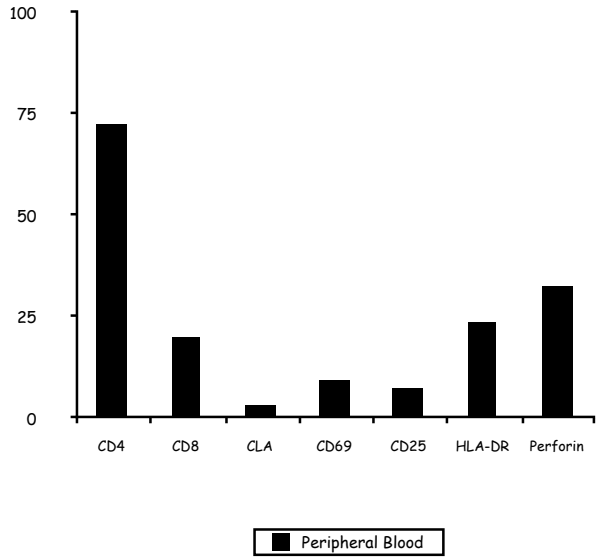


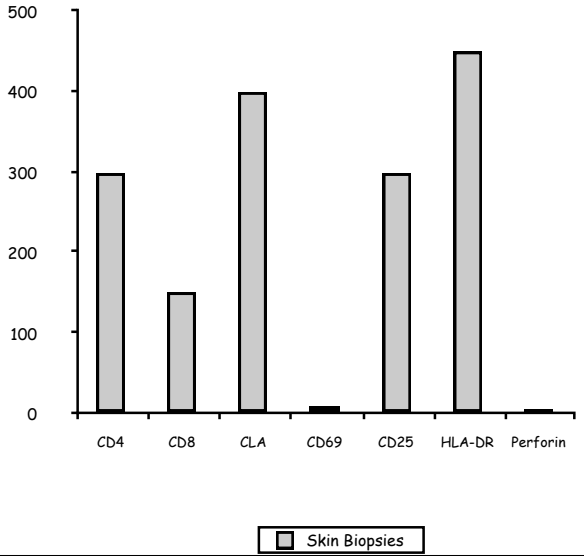
Monitorization of the acute response

Analysis in different compartments



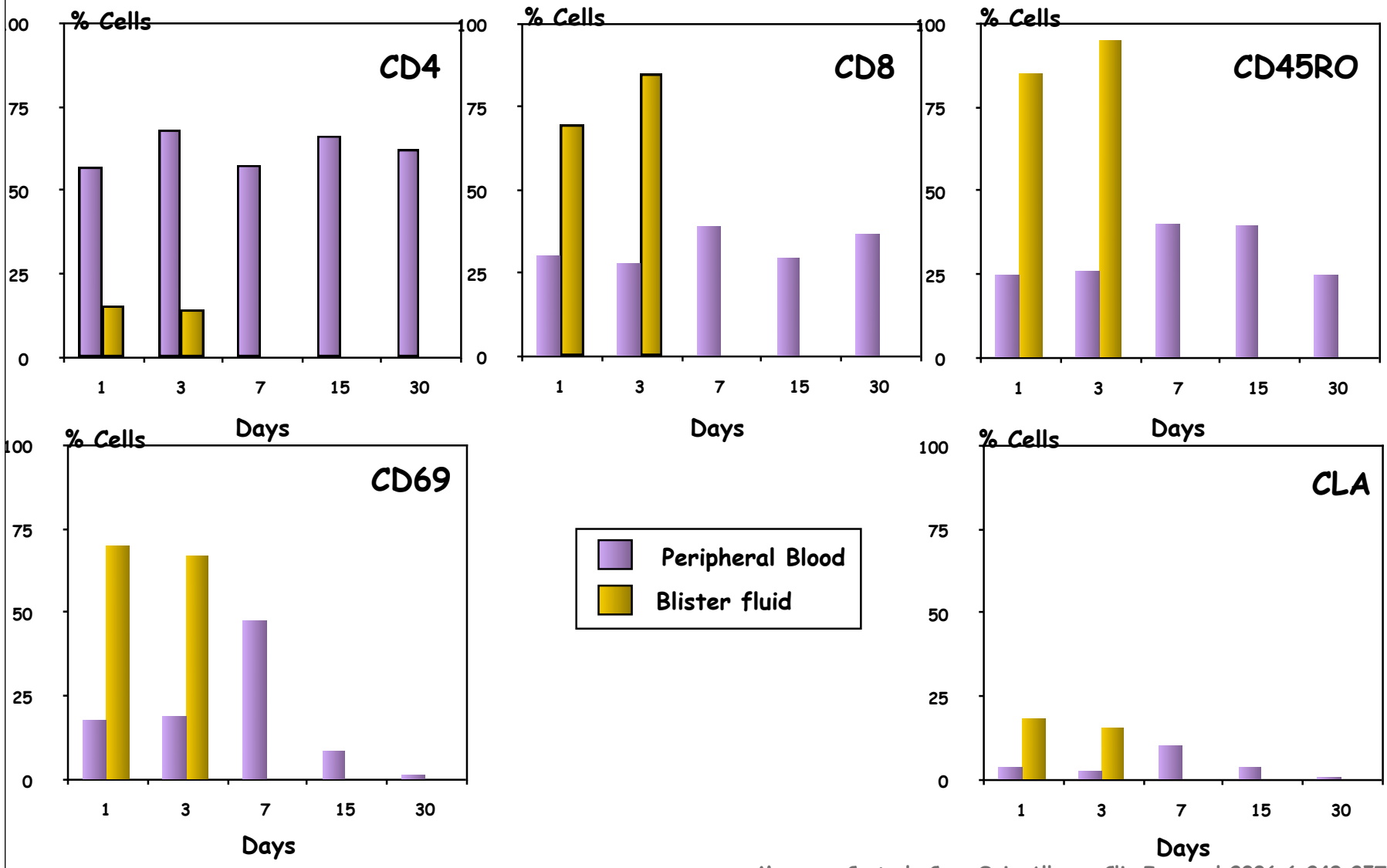






Monitorization of the acute response

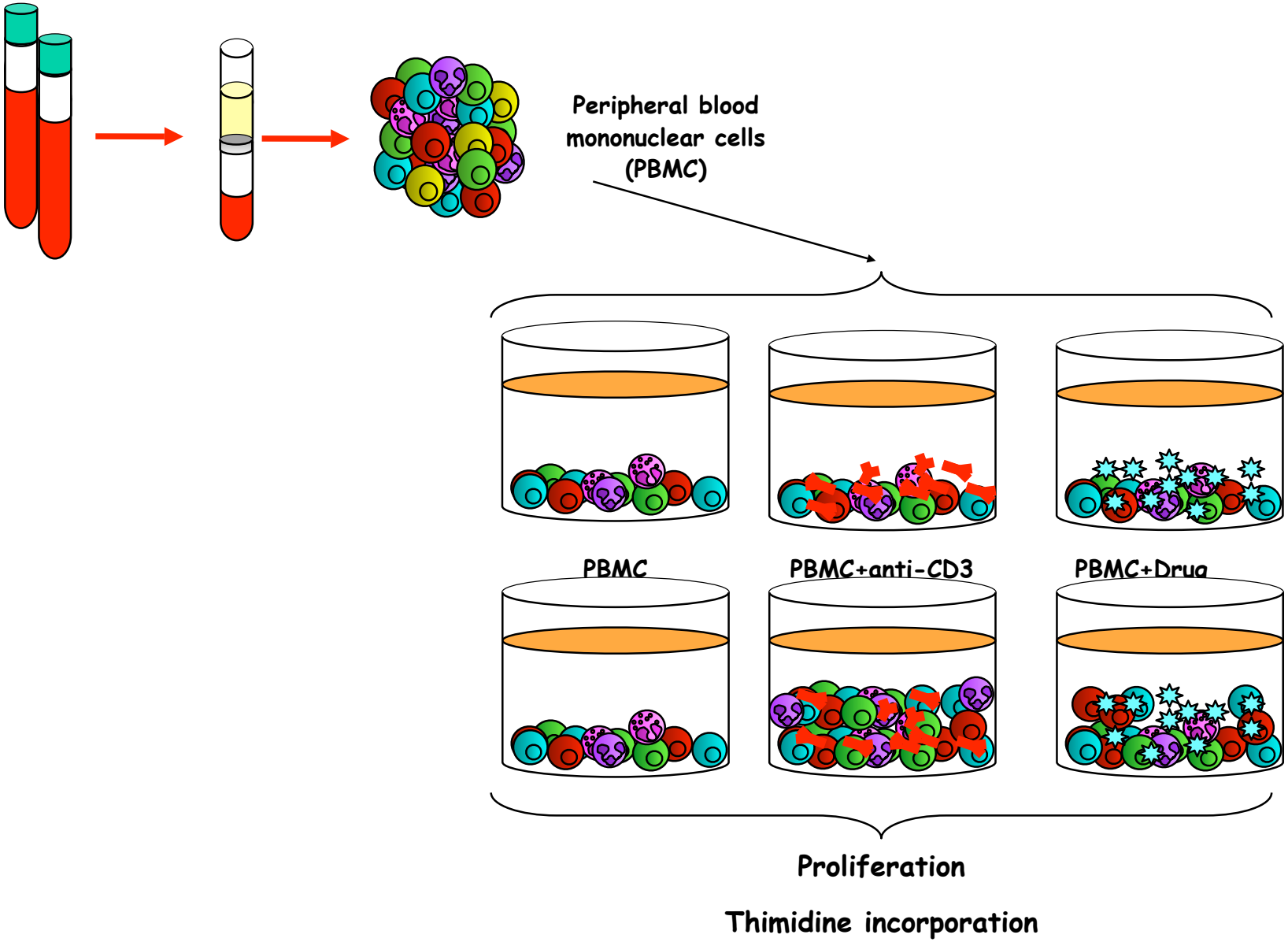
Analysis in different compartments



Mayorga C et al. Curr Opin Allergy Clin Immunol 2006;6:249–257.

LYMPHOCYTE TRANSFORMATION TEST (LTT)

LYMPHOCYTE TRANSFORMATION TEST (LTT)



LTT IN BETALACTAMS ALLERGY

SENSITIVITY	TOTAL	BP	AX	BP+AX
IMMEDIATE REACTIONS	64.5 %	19 %	26 %	19 %
NON IMMEDIATE REACTIONS	57.91%	5 %	26 %	26 %
TOTAL	62%			

SPECIFICITY	92.8 %

Luque et al. Allergy 2001;56:611-618.

LTT IN DRUG HYPERSENSITIVITY

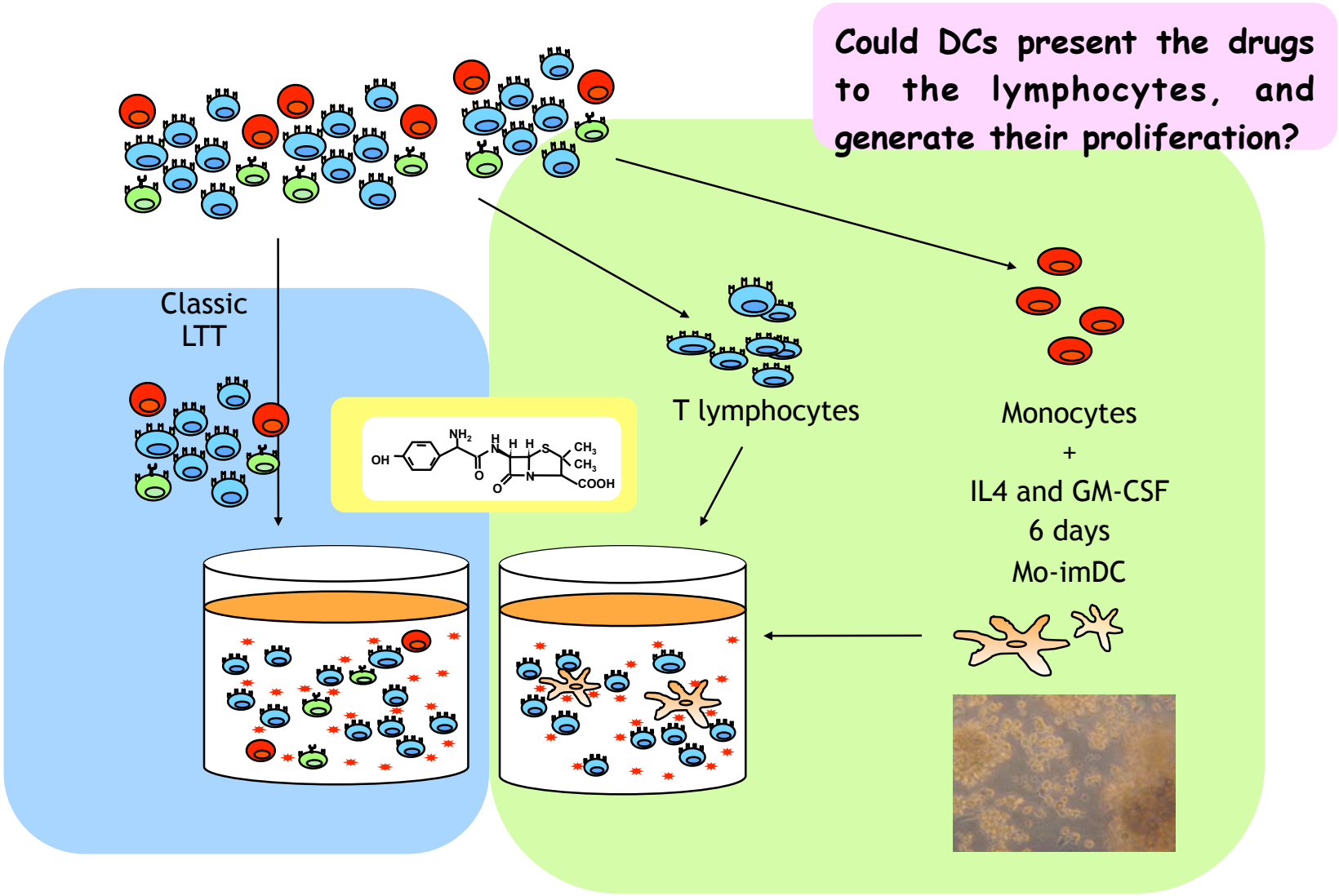
SENSITIVITY	SPECIFICITY
60-70%	85%

- Sensitivity depends on the drug responsible
- High values in LTT are associated with high precursor frequency of drug specific T cells.
- Drug-specific cells persist for as long as 12 years in peripheral blood even after strict avoidance of the drug

Pichler WJ et al. Allergy 2004;59:809-820.

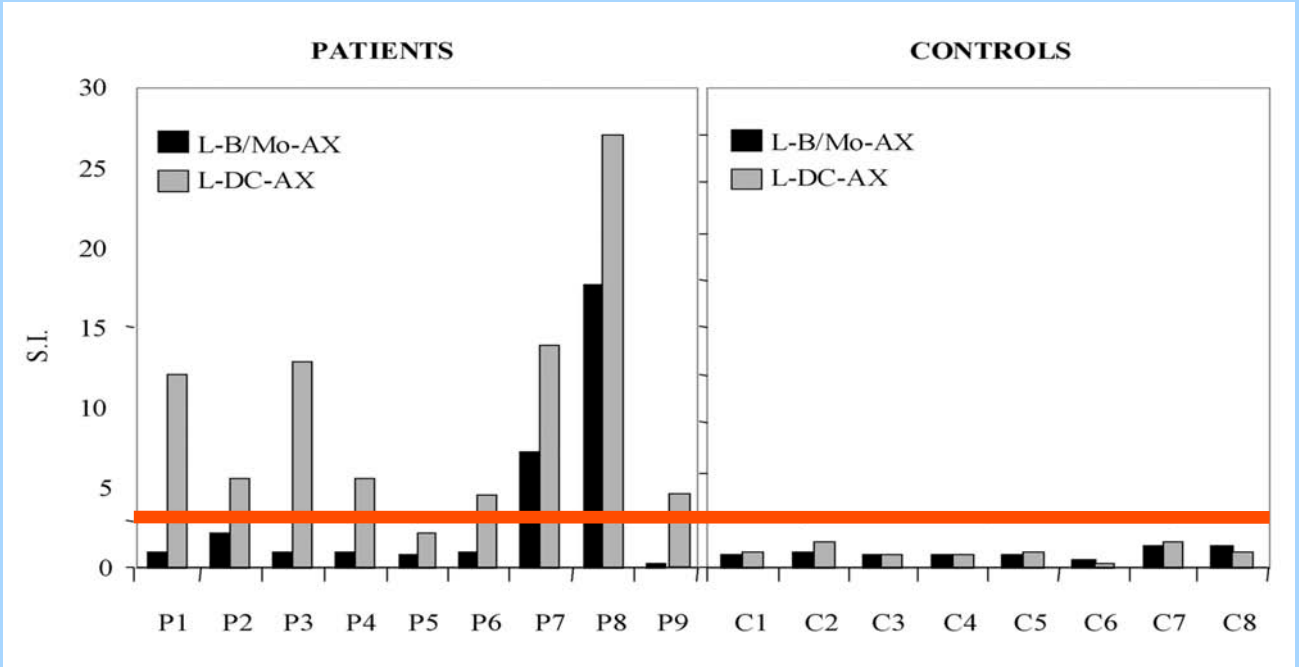
Beeler A et al. J Allergy Clin Immunol 2006;117:455-62.

Monocytes+B cells vs DC as APC in the LTT



LTT in NIR to AMOXICILLIN

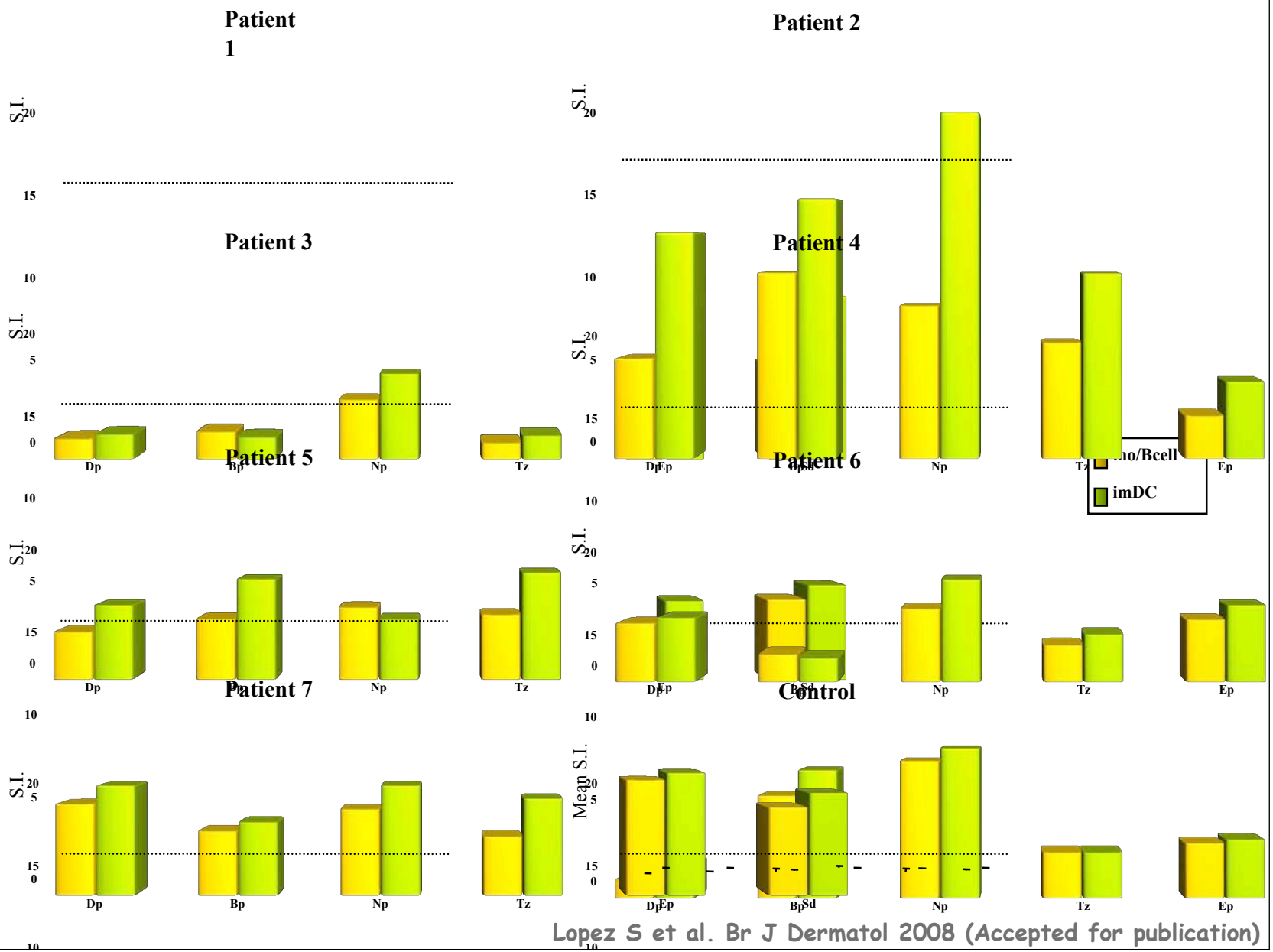
SI>3 Positive



Monocytes+B cells versus DC as APC in the LTT

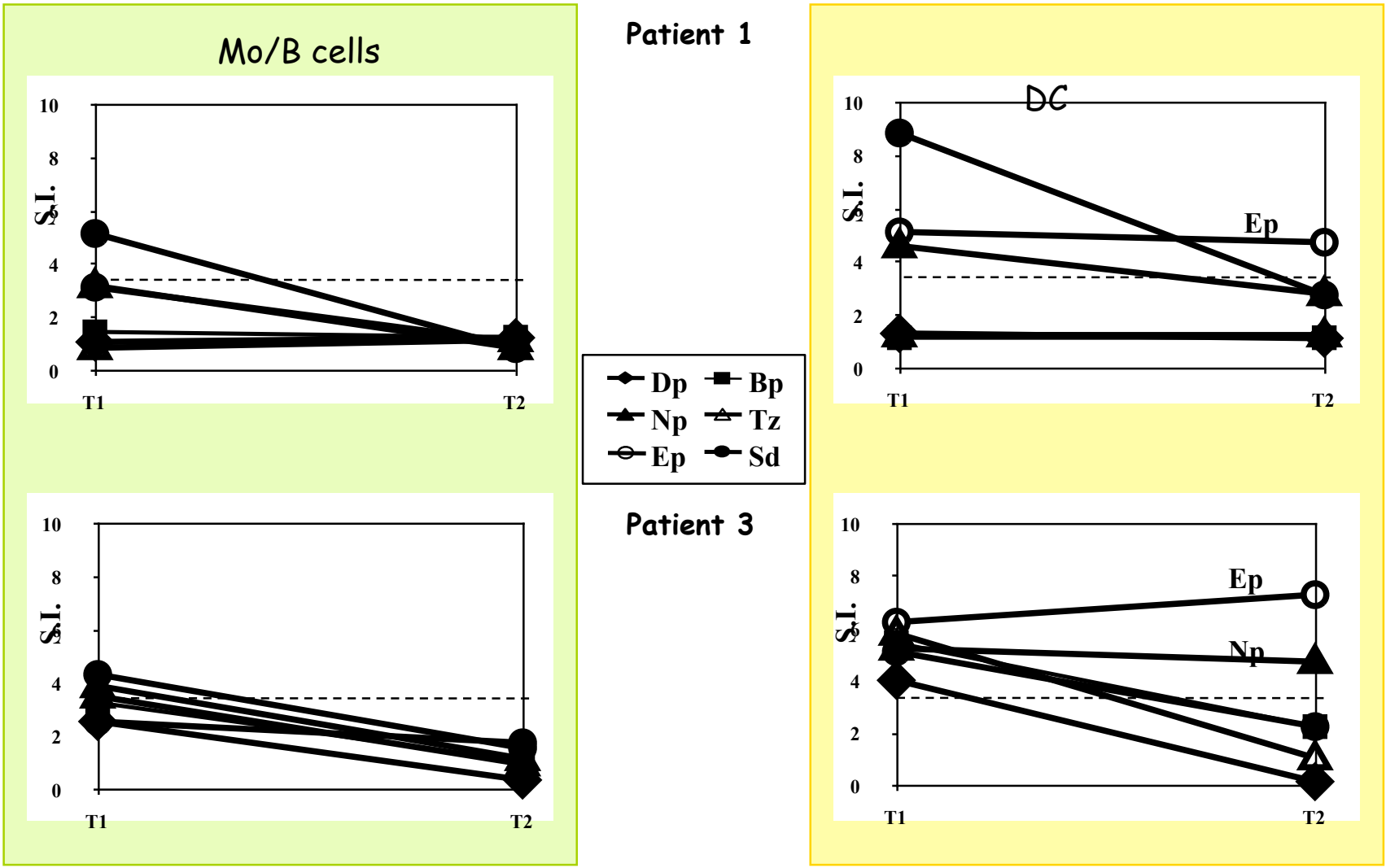
Rodríguez-Pena R et al, J Allergy Clin Immunol 2006;118:949-56.

LTT in NIR to HEPARINS



Lopez S et al. Br J Dermatol 2008 (Accepted for publication)

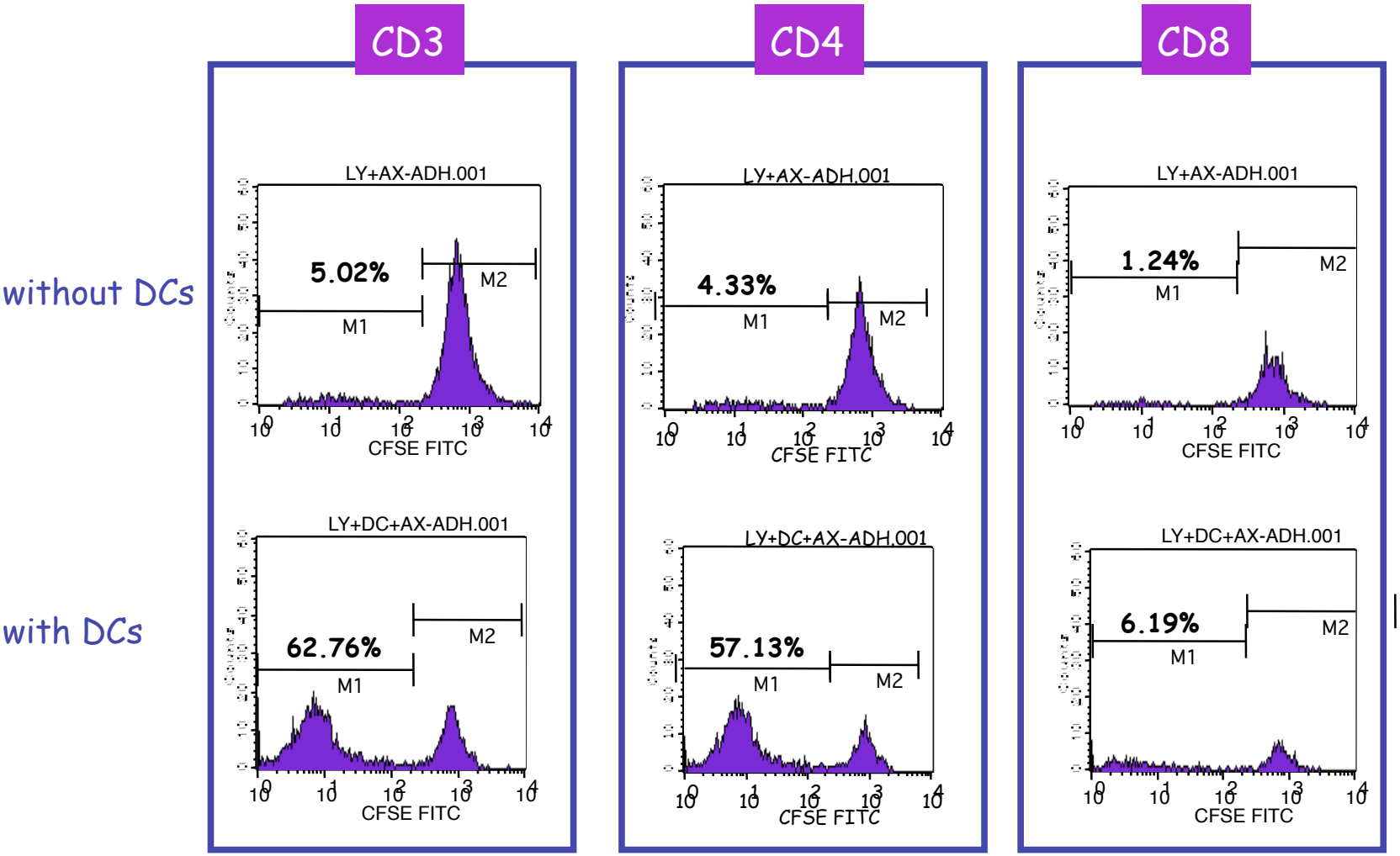
LTT in NIR to HEPARINS



Lopez S et al. Br J Dermatol 2008 (Accepted for publication)

LTT with CFSE

NIR to AMOXICILLIN



CONCLUSIONS

- ✓ The LTT has demonstrated to be useful in various diseases and with many different drugs.
- ✓ It can be improved with the use of dendritic cells as antigen presenting cells.
- ✓ The application of flow cytometric methods will help to identify the cell subpopulation involved in the drug recognition.
- ✓ More studies need to be carried out in different allergic drug reactions.

FUNDING SOURCES

Health Ministery (FIS): PI05290, PIO61503, PIO61561, PI071220
Science and Technology Ministery (MCYT):BQU2001-3624
Health Andalucia Ministery: PI-0199/07, PI-0243/07, PI-0201/07, PI-0200/07
Science, Innovation Andalucia Ministery: CTS 570
FIS-Thematic Networks and Co-operative Research Centres: RIRAAF (RD07/0064)