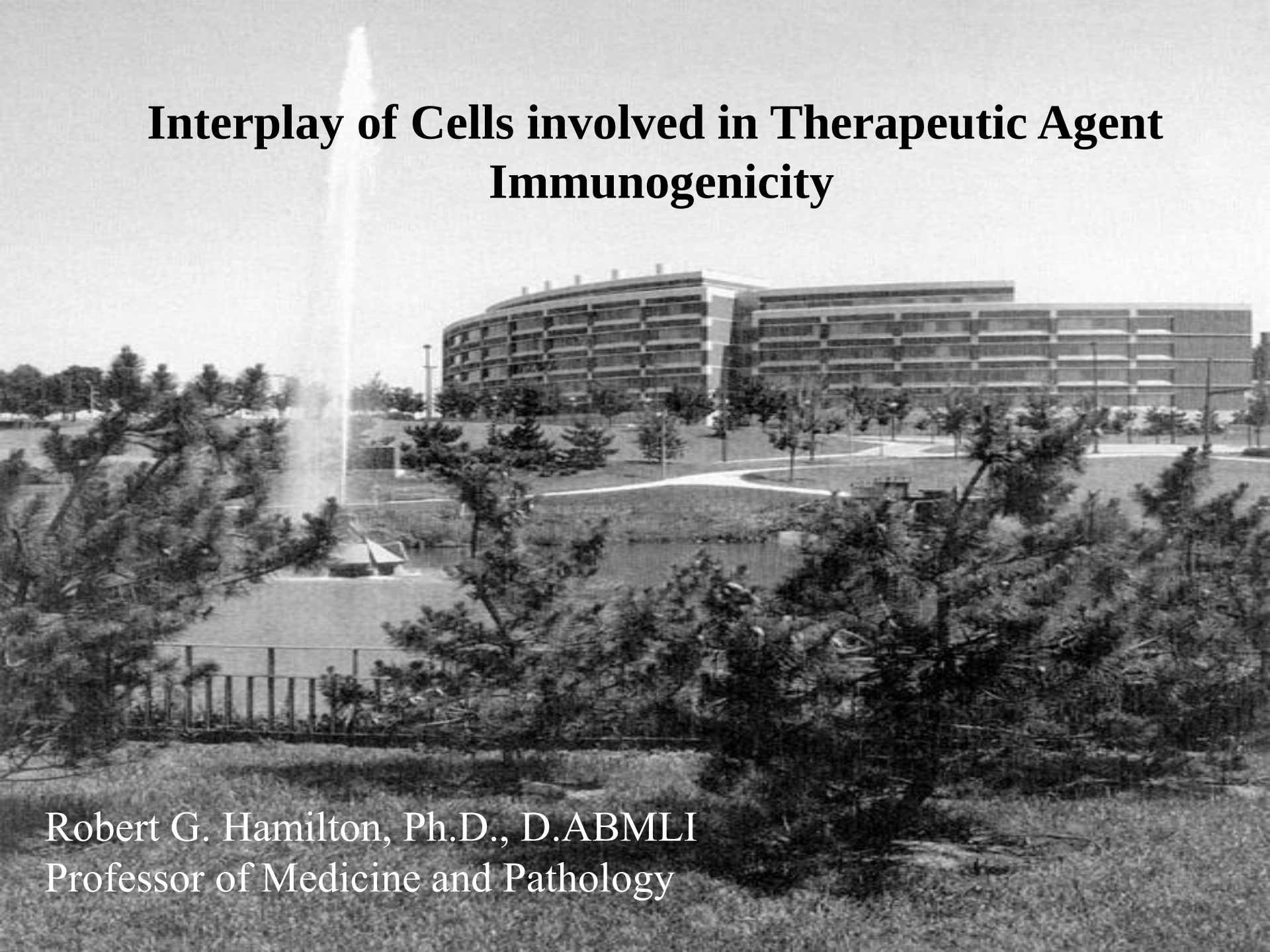
Interplay of Cells involved in Therapeutic Agent Immunogenicity



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Professor of Medicine and Pathology

Disclosure

- The author works with Amicus on an immunogenicity project related to enzyme replacement therapy for Pompe DIsease

- Otherwise nothing to disclose relevant to this presentation

Immunogenicity: propensity of a therapeutic protein to generate an **immune response** to itself and related proteins or to induce immunologically related adverse clinical event

FDA CDER/CBER Guidance for Industry:
Immunogenicity assessment for protein products

Immune response: a bodily response to an **antigen** that occurs when lymphocytes identify the antigenic molecule as foreign and induce formation of **antibodies and lymphocytes** capable of binding to it and rendering it harmless

Objectives

- Examine rationale for early immunogenicity assessment of drugs in development
- Overview the biology of principal cellular players relevant to the immunogenicity of therapeutic proteins. (B cell, T cell, APC)
- Discuss variables that influence the immunogenicity of a drug
- Review basic strategies for pre and post clinical immunogenicity testing

Rationale for early immunogenicity assessment of drugs in development

IMMUNOGENICITY OF INSULIN

Berson SA, Yalow R: Insulin-I131 metabolism in human subjects: demonstration of insulin binding globulin in circulating of insulin treated subjects JCI 1956;32:170-190

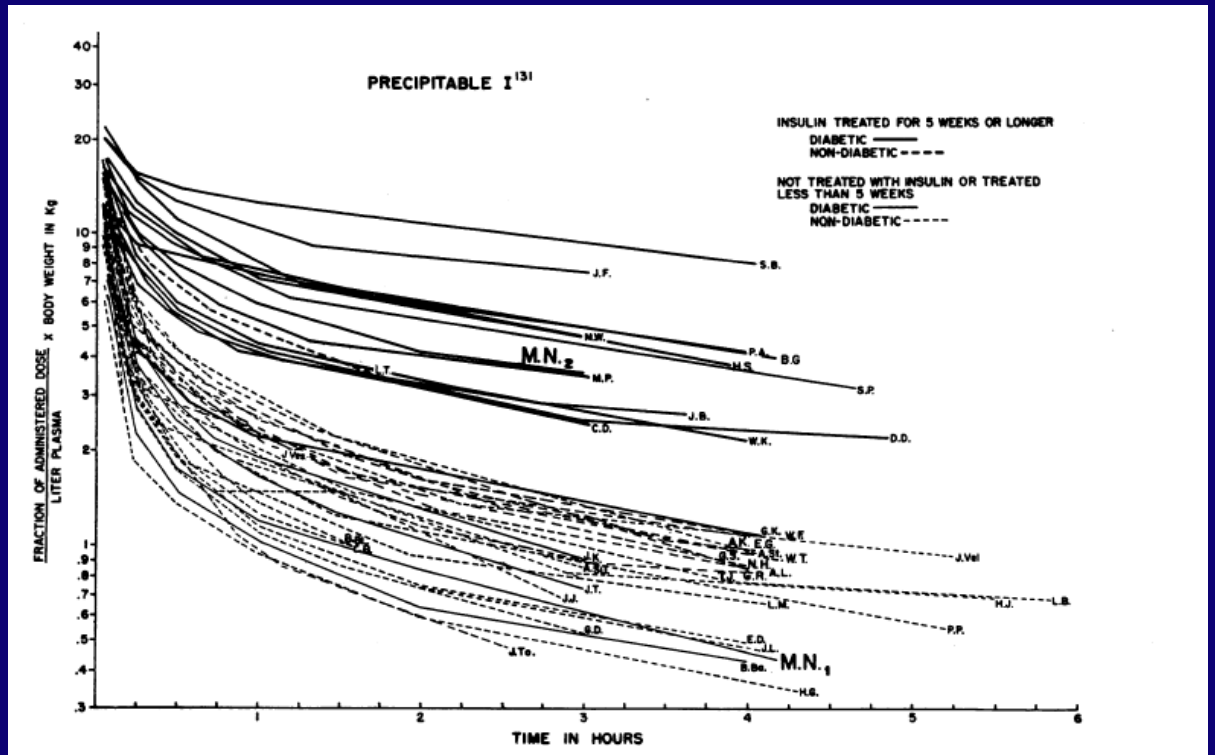


FIG. 2. TRICHLORACETIC ACID PRECIPITABLE RADIOACTIVITY IN PLASMA AS A FUNCTION OF TIME FOLLOWING INTRAVENOUS ADMINISTRATION OF INSULIN-I^{131}

Insulin-specific antibody was used to develop the first RIA for insulin

Nobel in Medicine 1964

Rationale for Immunogenicity Testing

- First recombinant therapeutic protein, human insulin (1982)
- Hamilton RG et al, Serological analysis of human IgG and IgE anti-insulin antibodies by solid-phase RIAs. J Lab Clin Med. 1980 ;96: 1022-36.
- Fineberg SE et al. Immunogenicity of recombinant DNA human insulin Diabetologia 1983;25:465-9.

- Human IgG and IgE anti-human insulin induced insulin resisteance and insulin allergy

Rationale for Immunogenicity Testing

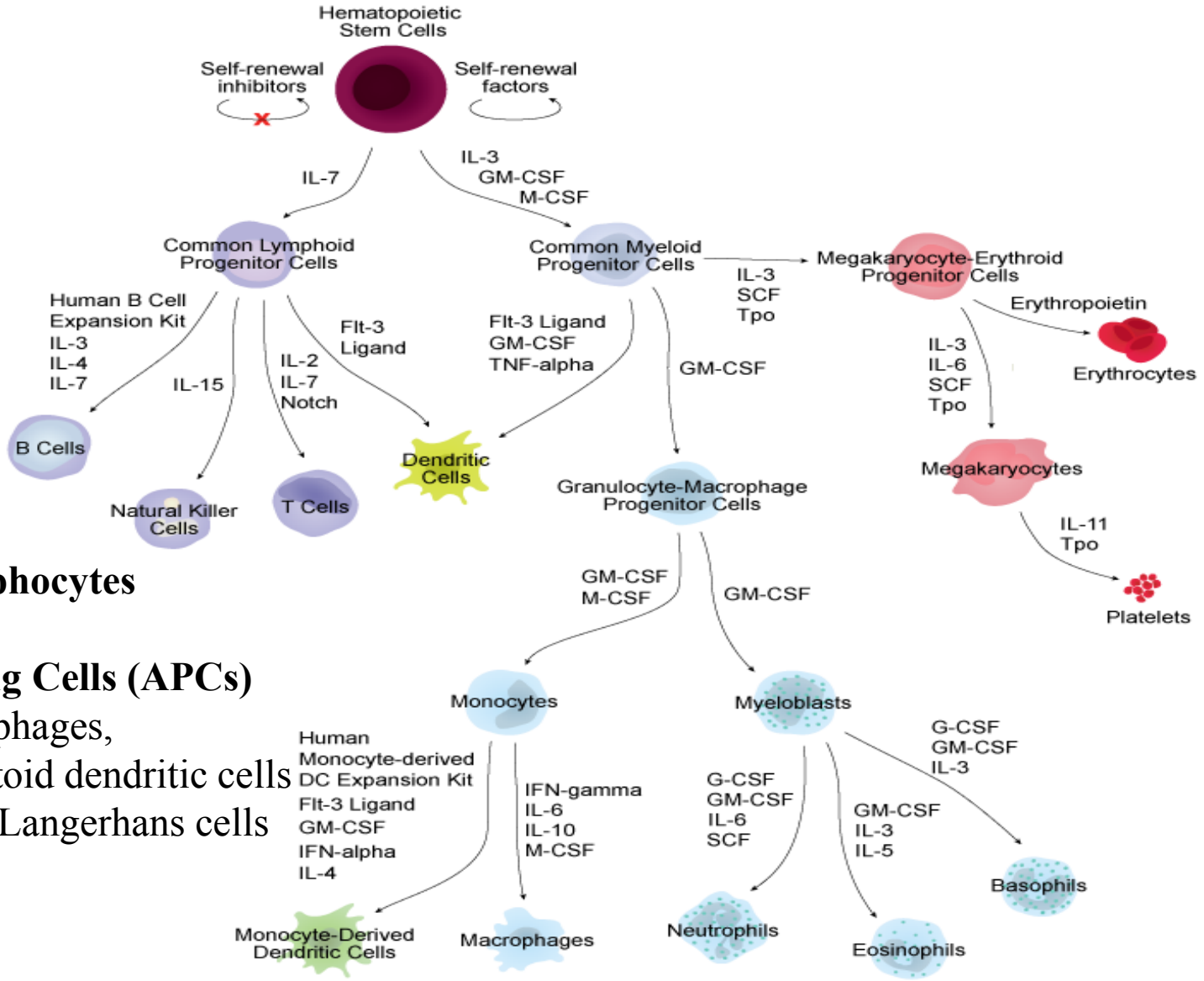
1. Anti-drug antibody block or neutralize the new drug's therapeutic effect and/or alter its pharmacokinetics
2. Anti-drug antibodies cross-react with autologous endogenous protein, blocking their effect
3. IgE anti-drug antibody arms mast cells and basophils for anaphylaxis potential
4. Give guidance to direct research and development strategies for drug redesign or deimmunization (modifications to decrease unwanted immunogenicity)

Overview of principal cellular players relevant to the immunogenicity of therapeutic proteins

not discuss T-cell independent immune responses

T-cell dependent immune responses:
more robust antibody response, isotype switching, memory B-cell generation

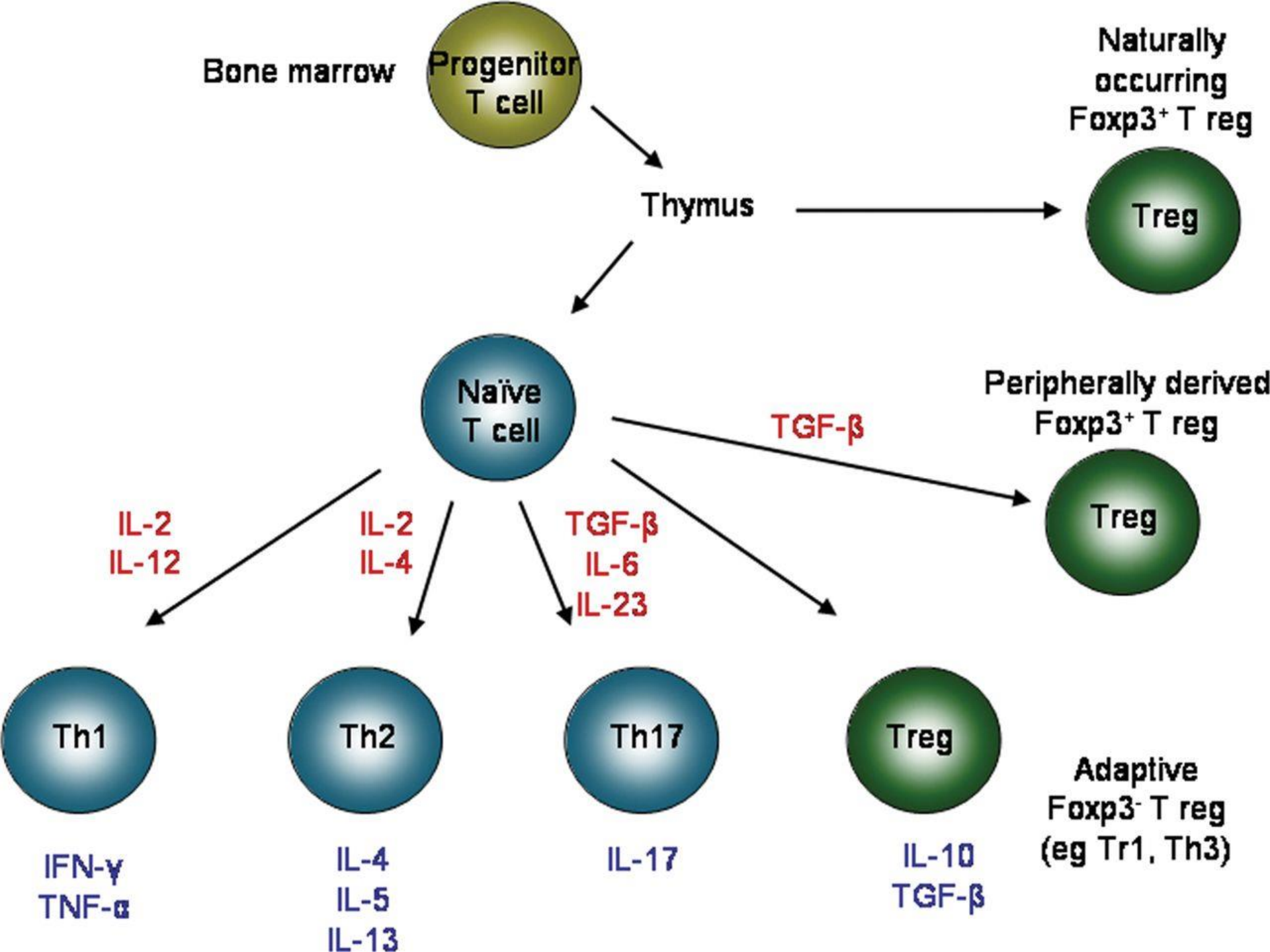
Cellular Players in the Immune Response to Therapeutic Drugs



B and T cell Lymphocytes

Antigen-presenting Cells (APCs)

Monocytes, macrophages,
Myeloid/plasmacytoid dendritic cells
B-cells, cutaneous Langerhans cells


Bone marrow
Progenitor T cell
Thymus
Naturally occurring Foxp3+ T reg
Treg
Naïve T cell
TGF-β
Peripherally derived Foxp3+ T reg
Treg
IL-2
IL-12
IL-2
IL-4
TGF-β
IL-6
IL-23
Th1
Th2
Th17
Treg
Adaptive Foxp3- T reg (eg Tr1, Th3)
IFN-γ
TNF-α
IL-4
IL-5
IL-13
IL-17
IL-10
TGF-β

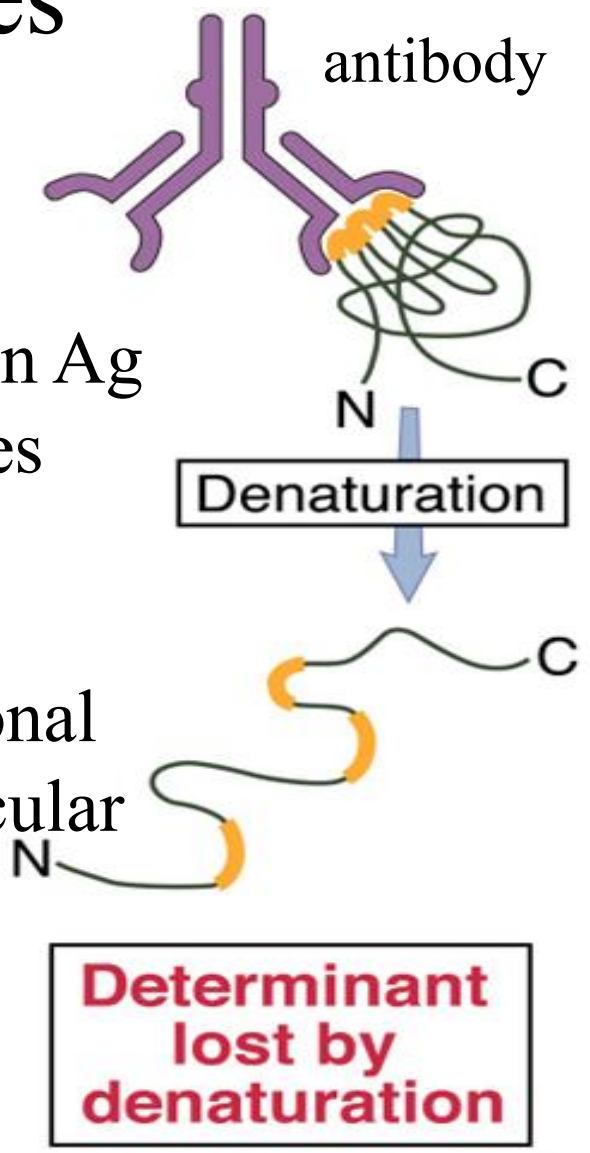
Lymphocytes

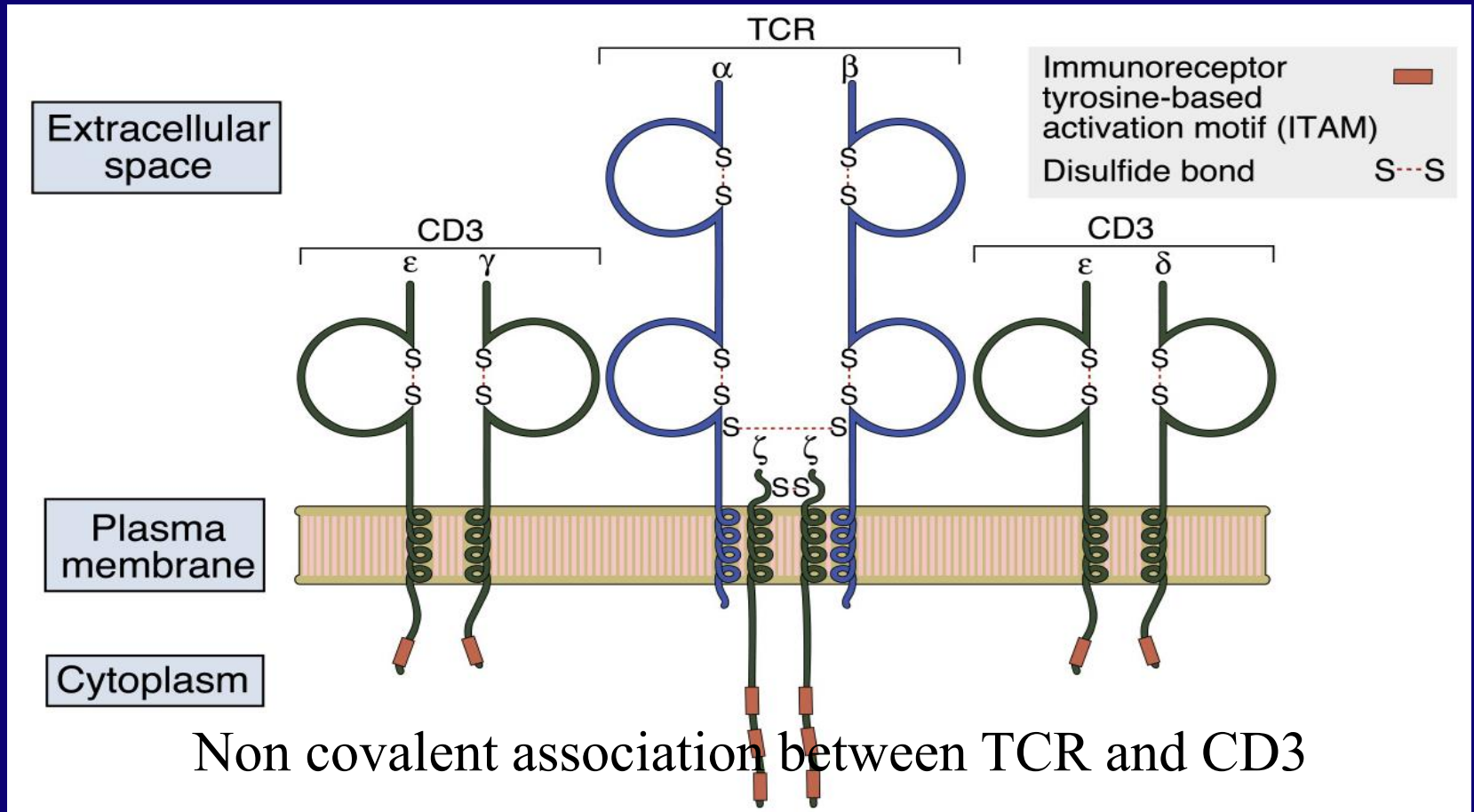
- **B cells** –membrane immunoglobulin receptors formed in bone marrow
- **T-cells**: maturation in thymus for rearrange-ment of receptors (self/non-self)
- Both have clonally-variable specific cell surface receptors for antigen based on gene rearrangement (**TCR; mIg** on B cells)
- T-cell recognition of peptide epitopes derived from antigen is key to T-dependent antibody generation

B and T cell differences

Benacerraf/Gell showed (1969)
T cells recognize denatured protein Ag
as linear 9-10 amino acid sequences
in MHC restriction

Antibodies recognize conformational
determinants from most any molecular
determinant





Non covalent association between TCR and CD3

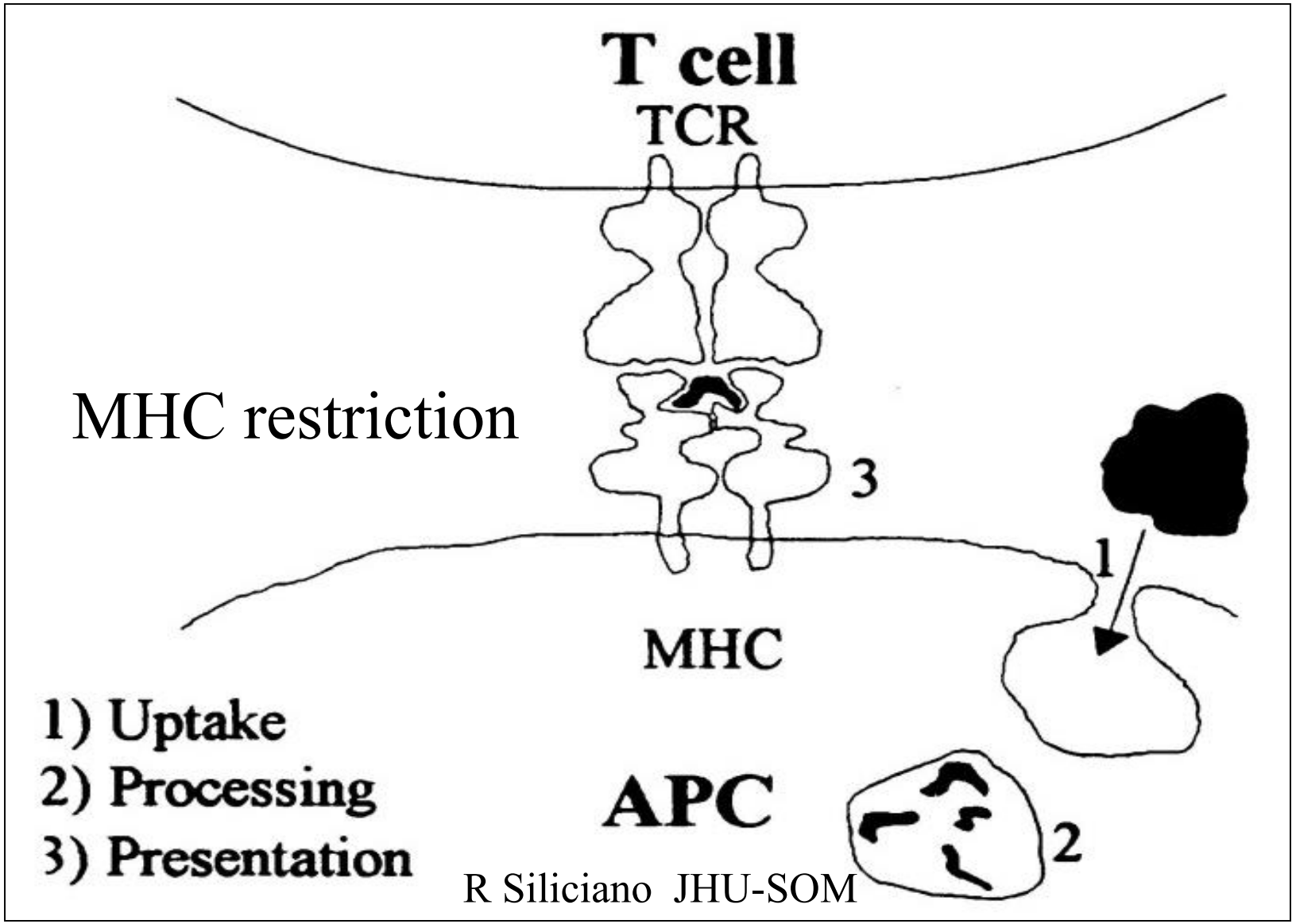
T-cell receptor- heterodimeric membrane molecule

Sees foreign Ag as processed foreign **peptide** associated with self protein encoded by the polymorphic **major histocompatibility complex (MHC) – up to 10^{18} different TCR structures possible**

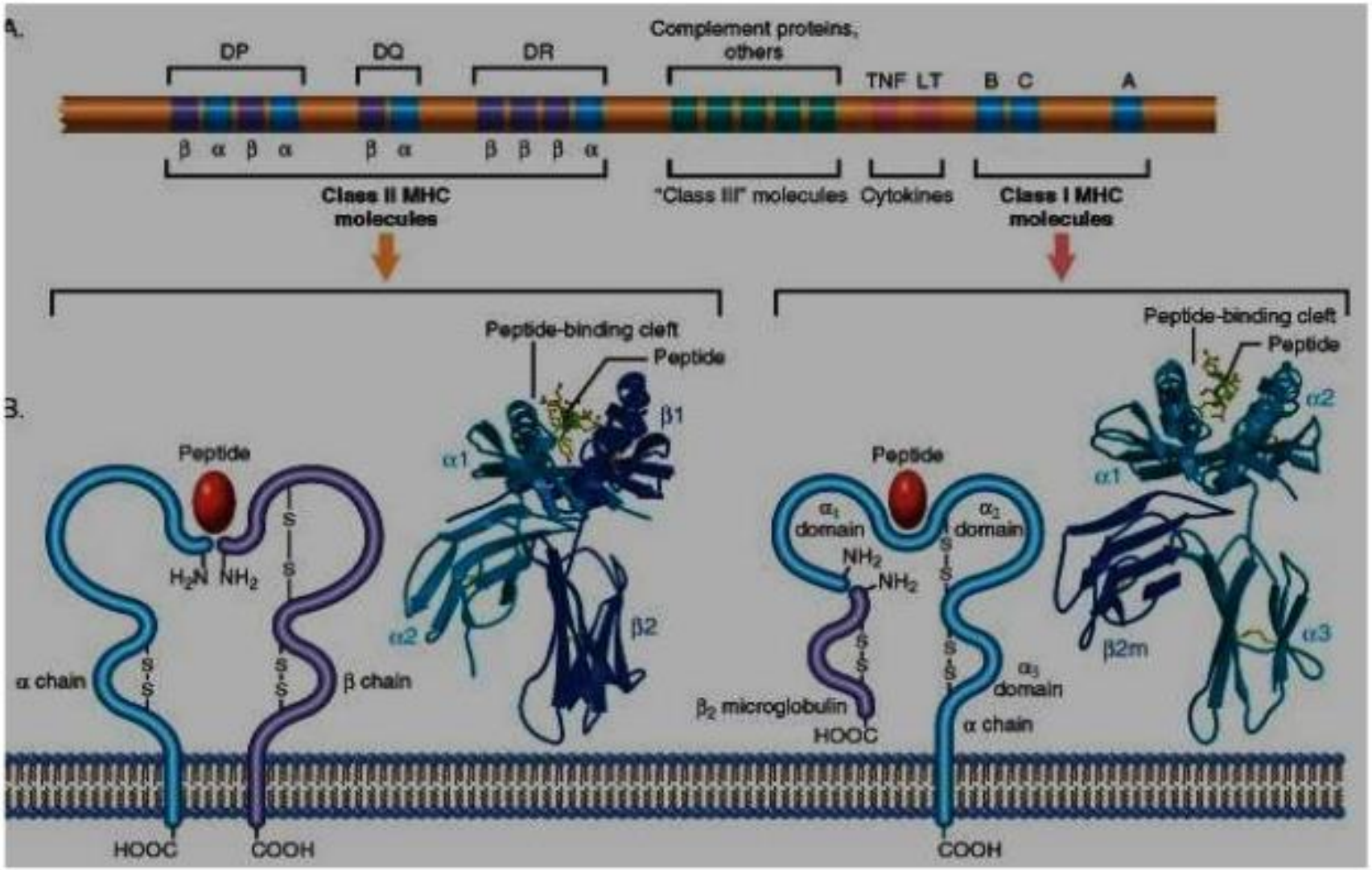
Antigen Presenting Cells (APCs)

- Monocytes, macrophages, dendritic cells, Langerhans cells, B-cells
- Functionally diverse cells specialized to present antigen peptides (8-10 AA) to T cell lymphocytes
- Features: expression of class I and II MHC molecules and accessory molecules for T-cell activation (B7, CD80)
- Upon activation: release cytokines

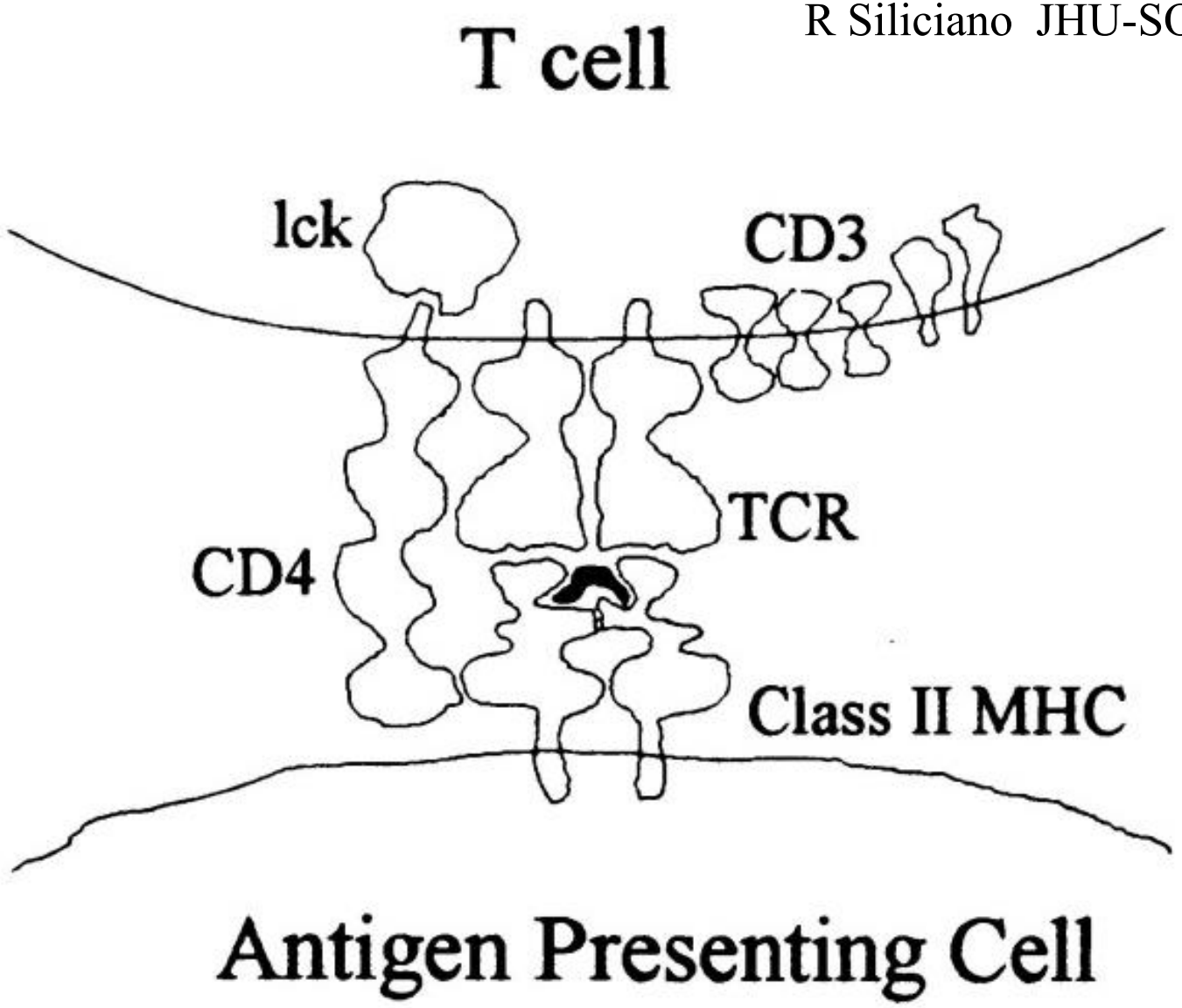
Antigen taken up by APCs (e.g, dendritic cell /macrophage);
Peptide epitopes bind to HLA MHC class II molecules



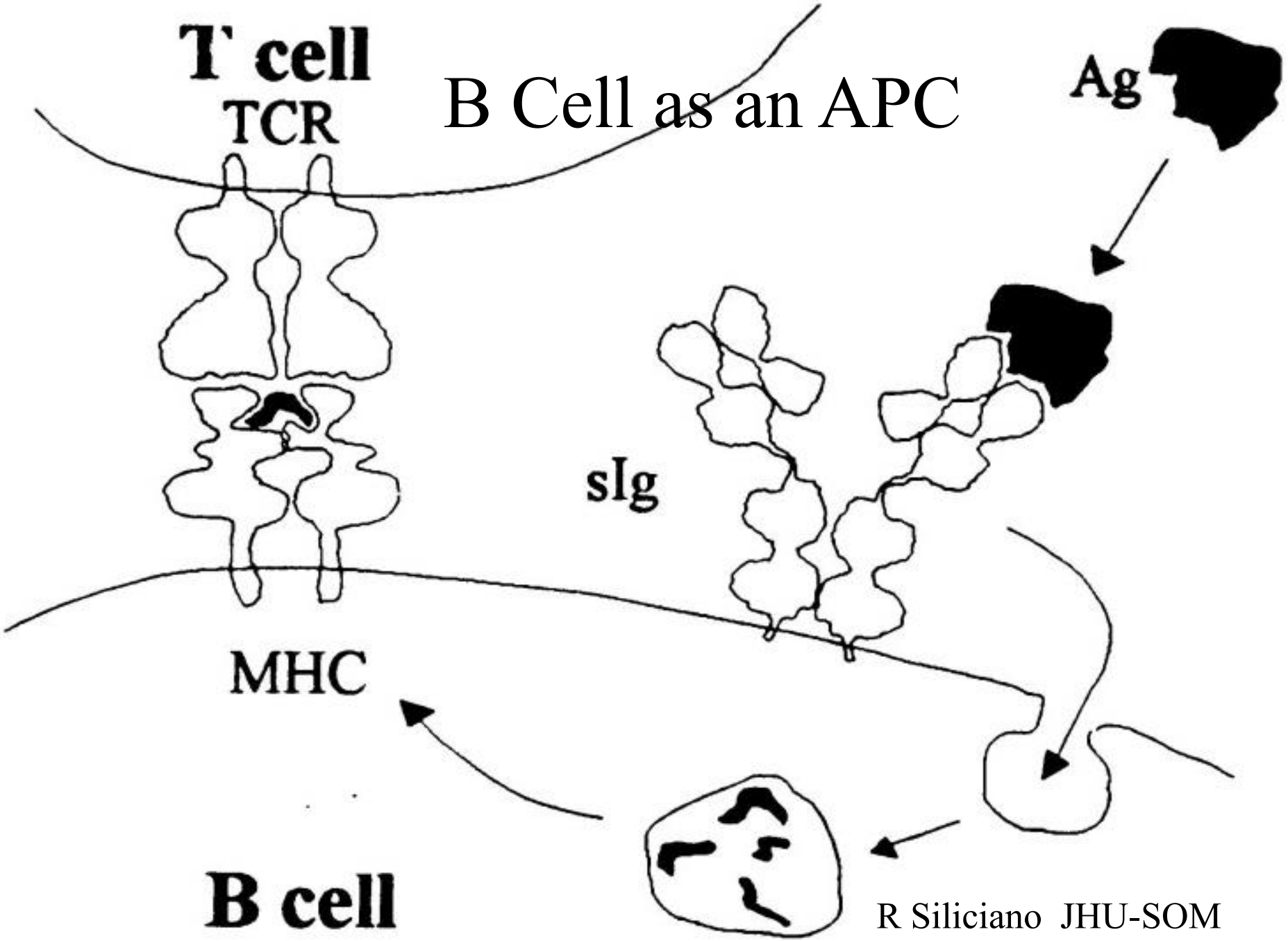
Major Histocompatibility molecules(HLA Complex)



R Siliciano JHU-SOM



B Cell as an APC



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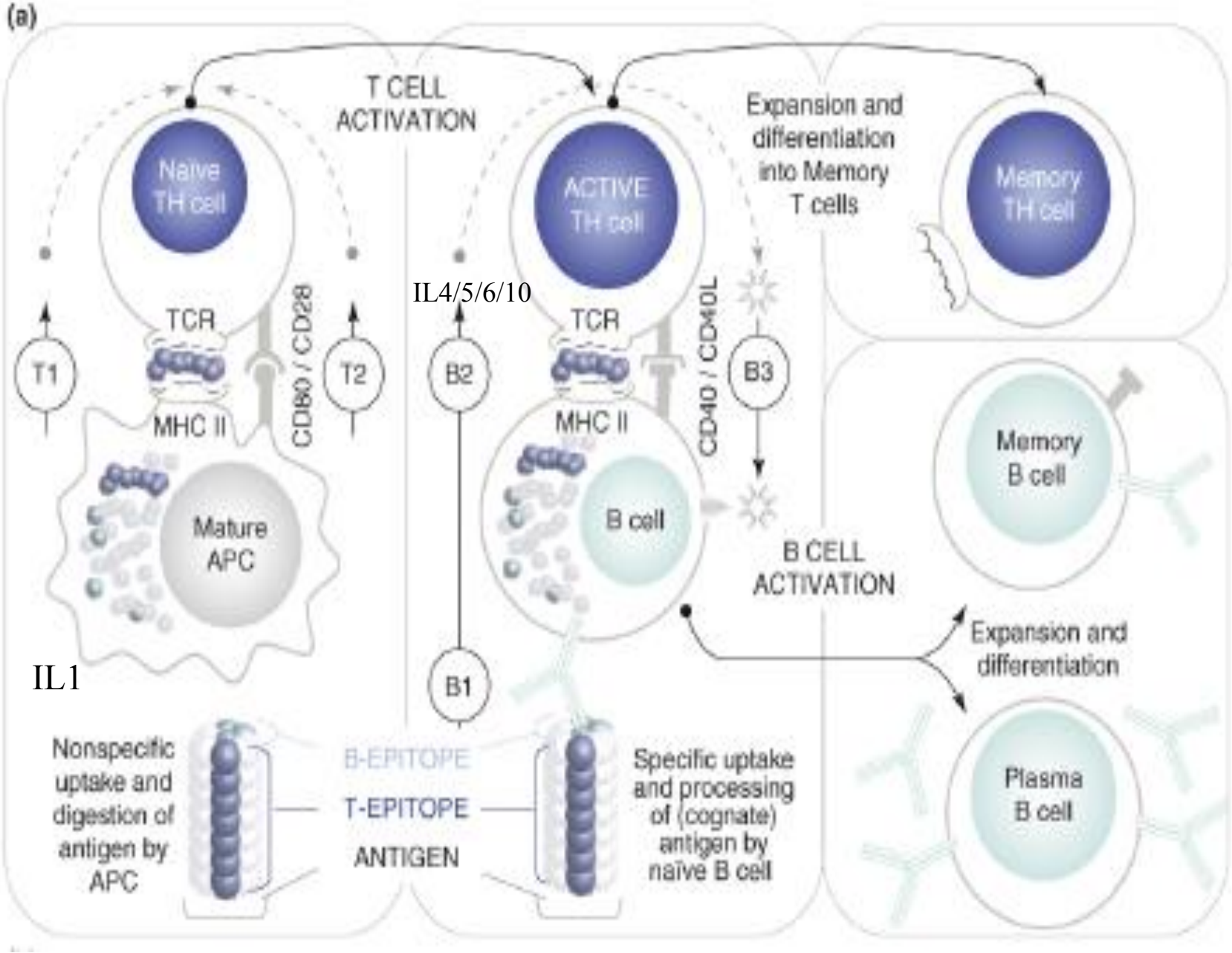
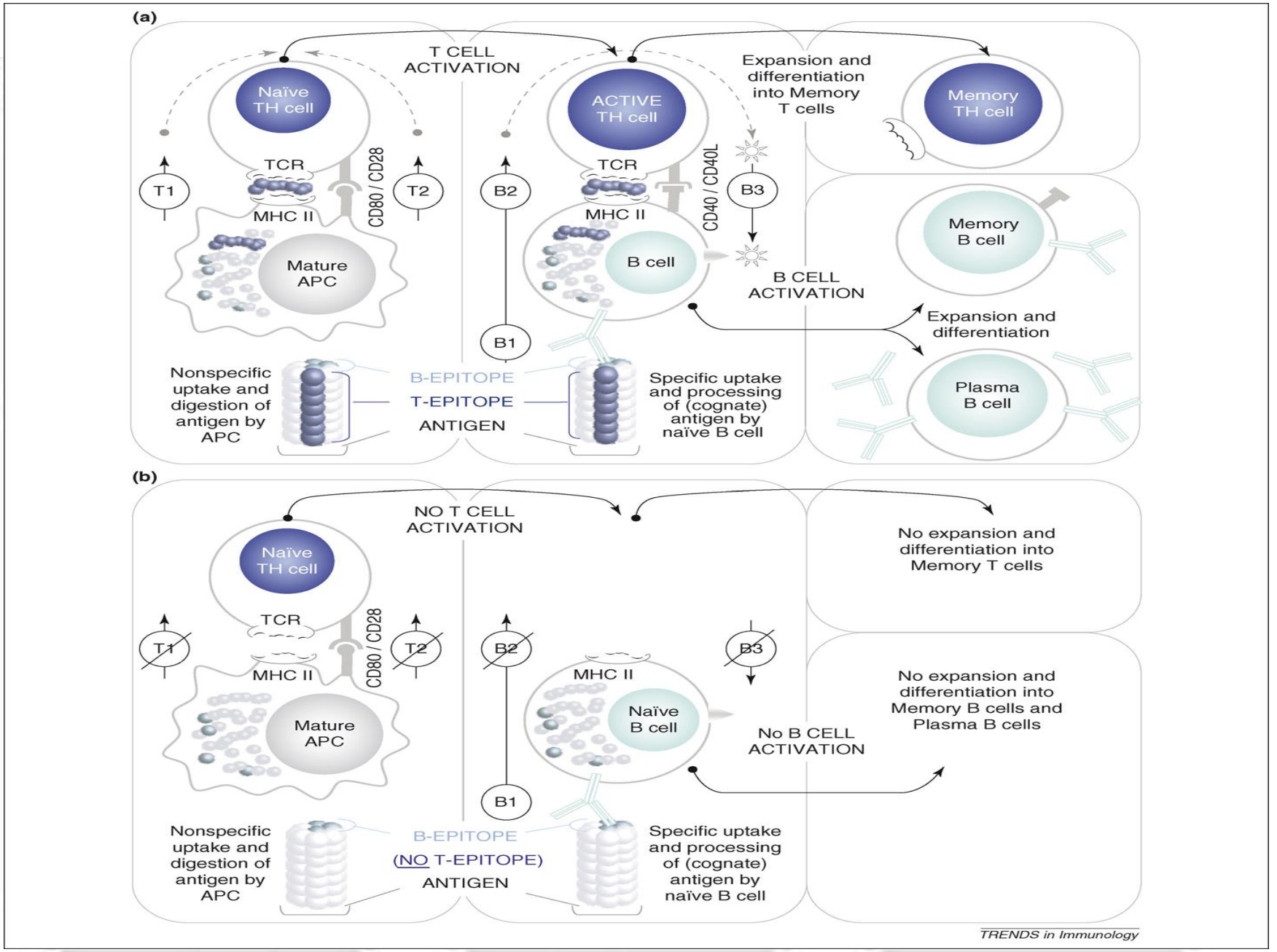
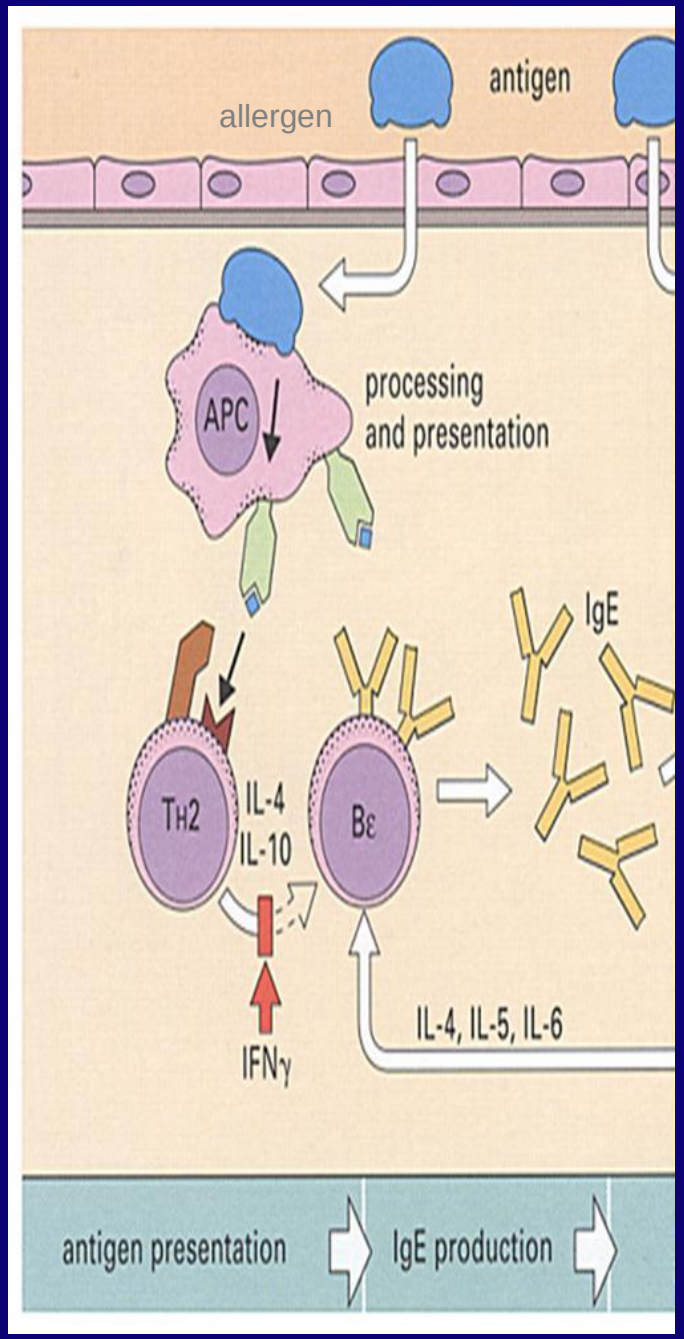


De Groot AS, Scott DW, Trends Immunol 28;482-90, 2007



De Groot AS, Scott DW, Trends Immunol 28;482-90, 2007

Variables that influence the immunogenicity of a therpeutic drug



Dynamic Factors That Alter the Immunogenicity

Patient Factors

age (child vs adult), gender, race , social economic status , immune status, **HLA background (MHC restriction)-allelic variation**

Therapeutic Drug Factors

antigen source (complexity-concentration), duration and route of exposure, doses, aggregates, adjuvants

↓ continued allergenic challenge

Antibody Isotype/Quantity/Quality Changes

Concentration (kUa/L)

Affinity (tightness of binding) Ka/Kd

Clonality (epitope specificity)-some neutralizing

Specific Activity (Ab/total Ig ratio)-isotype

Duration of Immune response

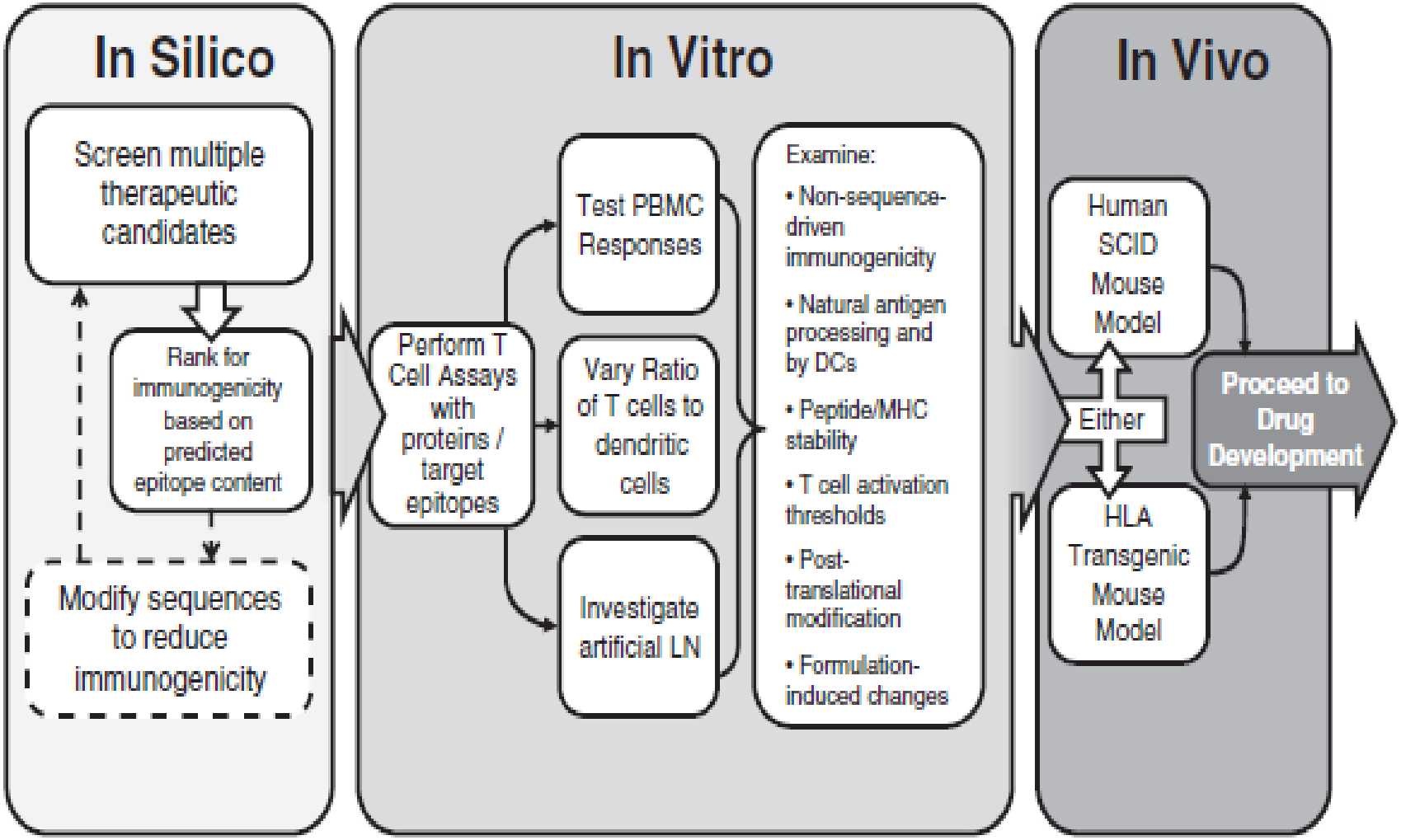
Overview basic strategies for early immunogenicity testing

Pre-Clinical Immunogenicity Prediction based on Proliferation Assays for Antigen-specific T cells

- **Premise**: Antigen-specific (CD4+) T helper cells responding to protein epitopes are critical for robust anti-drug antibody responses

- **Limitation**: T cell assay results do not directly correlate with prediction of anti-drug antibody responses that ultimately elicit clinical outcomes (neutralization/anaphylaxis)

Pre-clinical T-cell Dependent Immunogenicity Testing



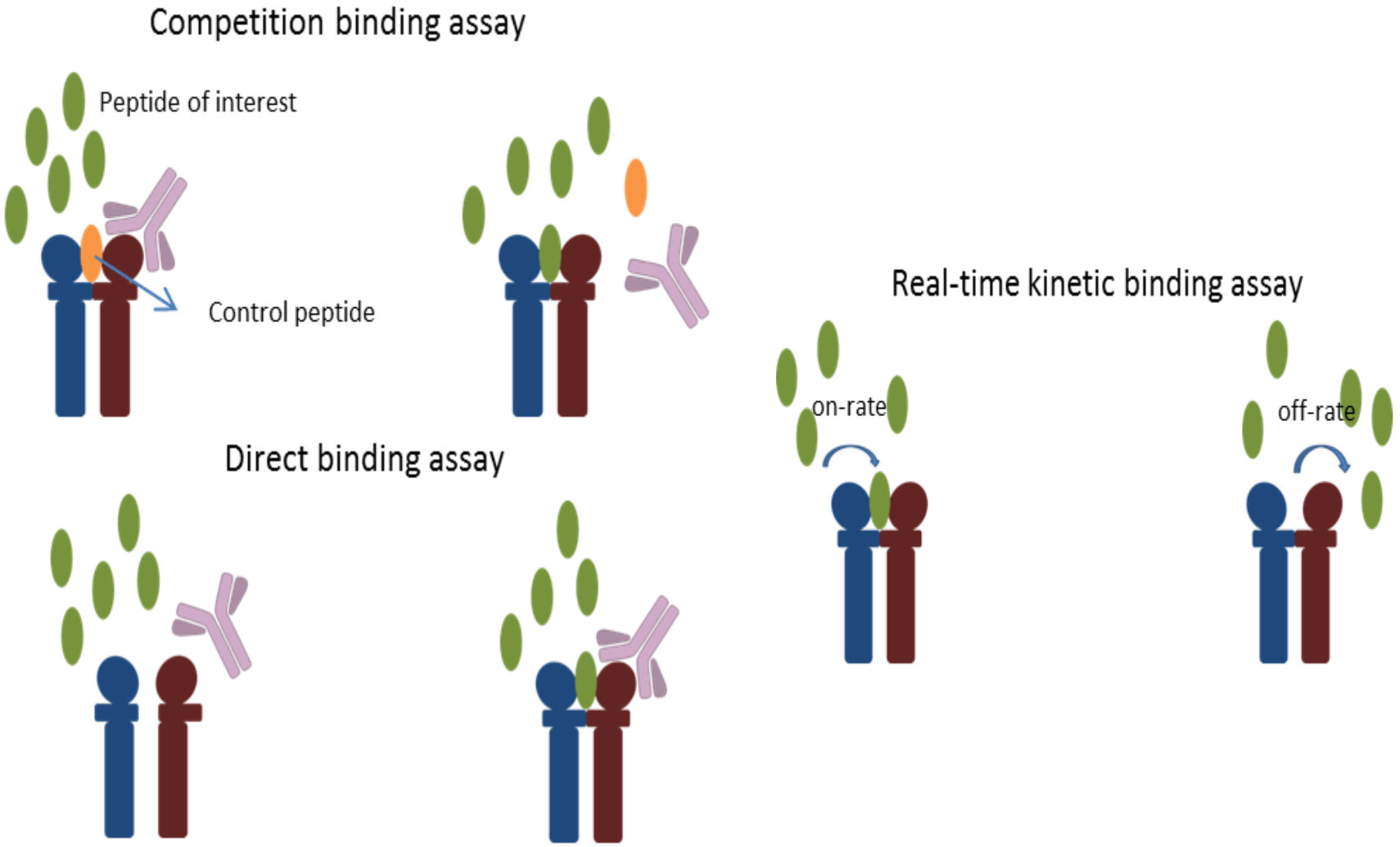
Jawa V …De Groot AS. T-cell dependent immunogenicity of protein therapeutics Clin Immunol 149:534-55, 2013

Current Pre-Clinical Methods Used to Identify Immunogenic Peptides in Therapeutic Proteins

Class	Method	Immune Response Probed	Rationale
In Silico epitope mapping Computational	Common HLA-II binding haplotype Algorithms	Peptide Antigen Presentation	Screen linear 9-mer sequences of candidate drugs to identify T cell epitopes and clusters against 3-D structure database models
In vitro	Peptide/HLA Binding Assay	Peptide Antigen Presentation	Assess potential T-helper cell epitope binding affinity

Sauna ZE et al, Trends in Biotechnology 36:1068-83, 2018

SIAT® *In Vitro* Class II HLA Binding Assay
Creative Biolabs



Up to 60 DR, DQ and DP alleles

Current Methods Used to Identify Immunogenic Peptides in Therapeutic Proteins

Class	Method	Immune Response Probed	Rationale
Ex vivo	LC/Mass Spec based MHC associated peptide proteomics (MAPPs) assay	Antigen processing and presentation	Identifies naturally processed peptide Ags (Th cell epitopes)
	MHC-II tetramer guided epitope mapping	Antigen recognition	Mapping HLA restricted epitopes
	Protein specific T cell amplification	Ag processing presentation recognition	Ag-specific T cell lines generated from naïve PBMC donors
	Human blood derived cell based assays	Released cytokines	DC-T cell activation measured by proliferation

Sauna ZE et al, Trends in Biotechnology 36:1068-83, 2018

Current Methods Used to Identify Immunogenic Peptides in Therapeutic Proteins

Class	Method	Immune Response Probed	Rationale
Animal model/ in vivo	HLA transgenic mice (humanized immune system	Antigen processing presentation recognition	Assessment of risk of anti-drug antibody development in context of human HLA

Sauna ZE et al, Trends in Biotechnology 36:1068-83, 2018

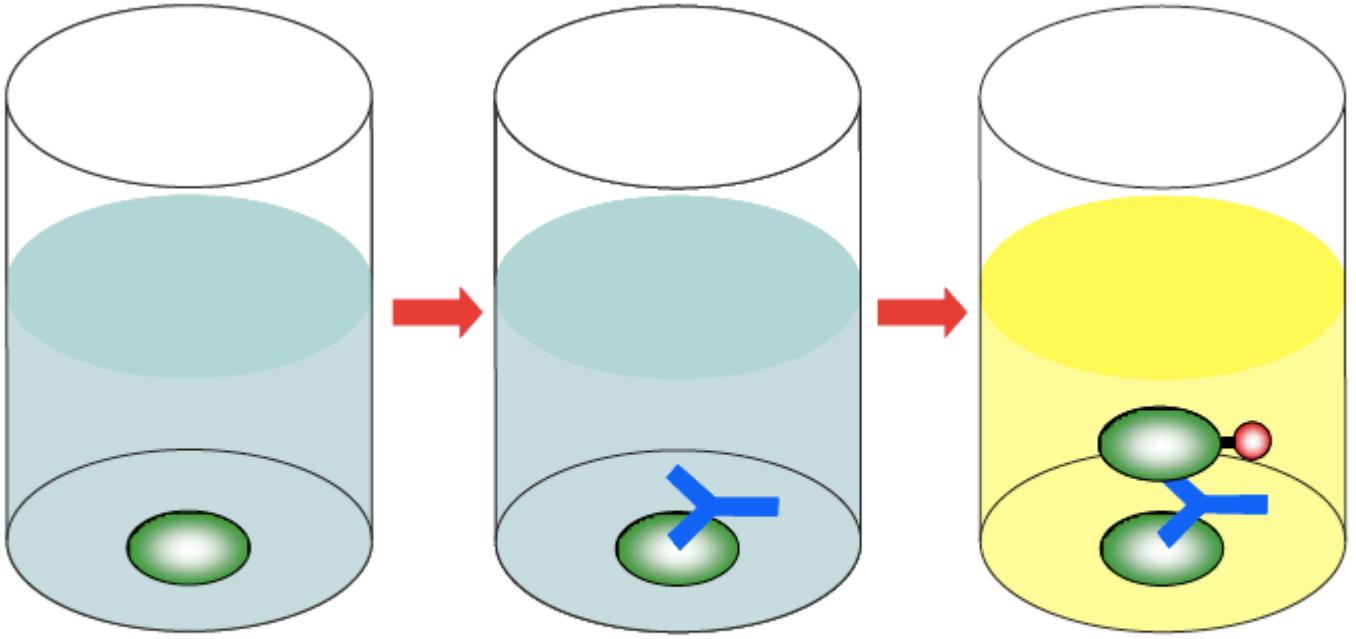
Assays For Monitoring Anti-Drug Antibody Development During Clinical Trials

- Screening Assay (presence)-Baseline/3-6-9-12 Mo
- Confirmatory Assay (specificity)
- Titering Assay (quantity)-no Ab assay standards
- Neutralization Assay (blocking)
- IgE Sensitization Assay (hypersensitivity)

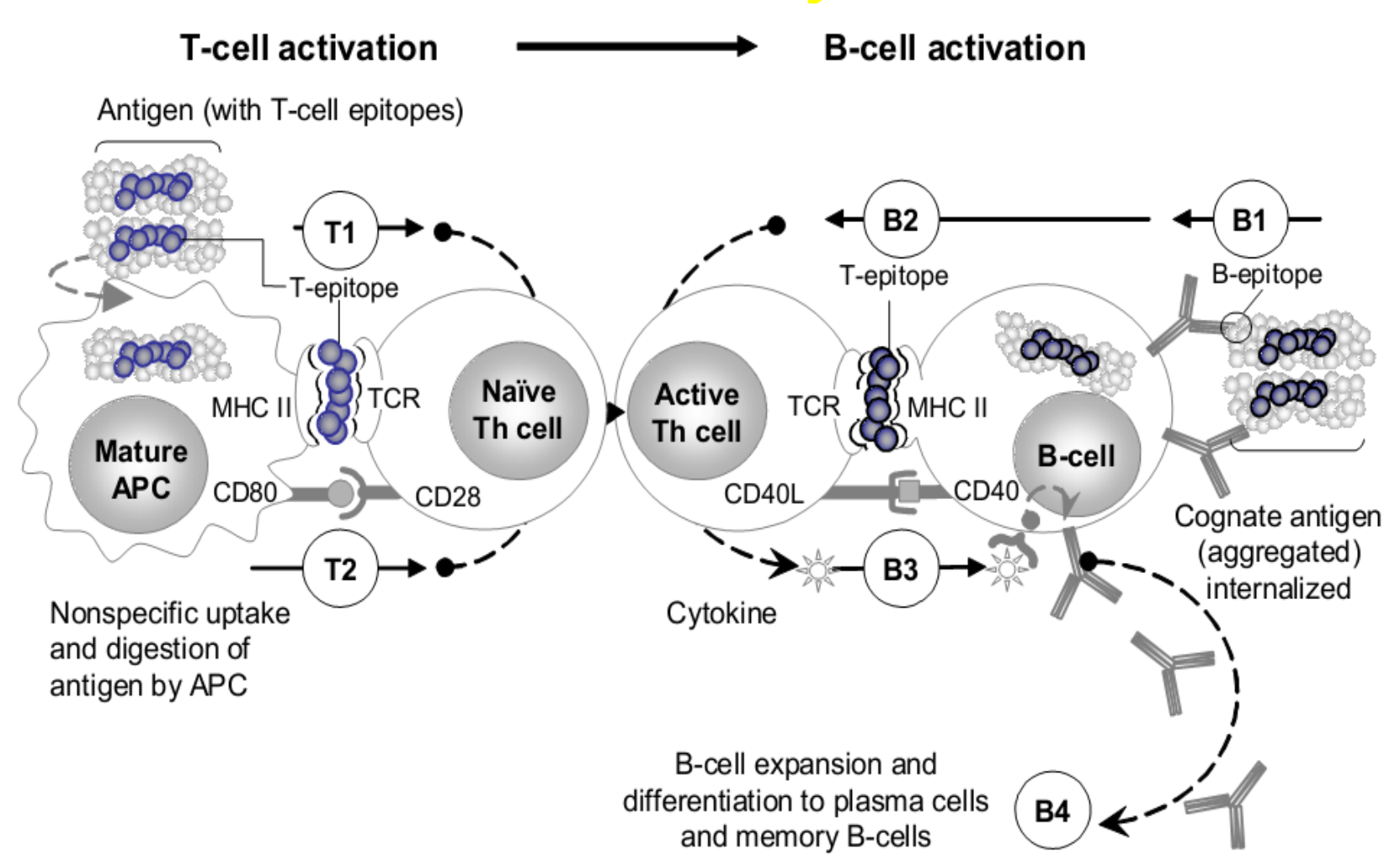
Validation (lack of standardization)
Analytically Sensitive
Drug Specific
B cell epitope mapping
Cross-reactivity

Mire-Sluis AR et al:. J Immunol Methods 289, 1-16, 2004

Anti-drug Antibody Bridging Assay
Creative Biolabs



Cellular Players in Immunogenicity Summary



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Big Picture References

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