Determinants of Immunogenicity and Tolerance

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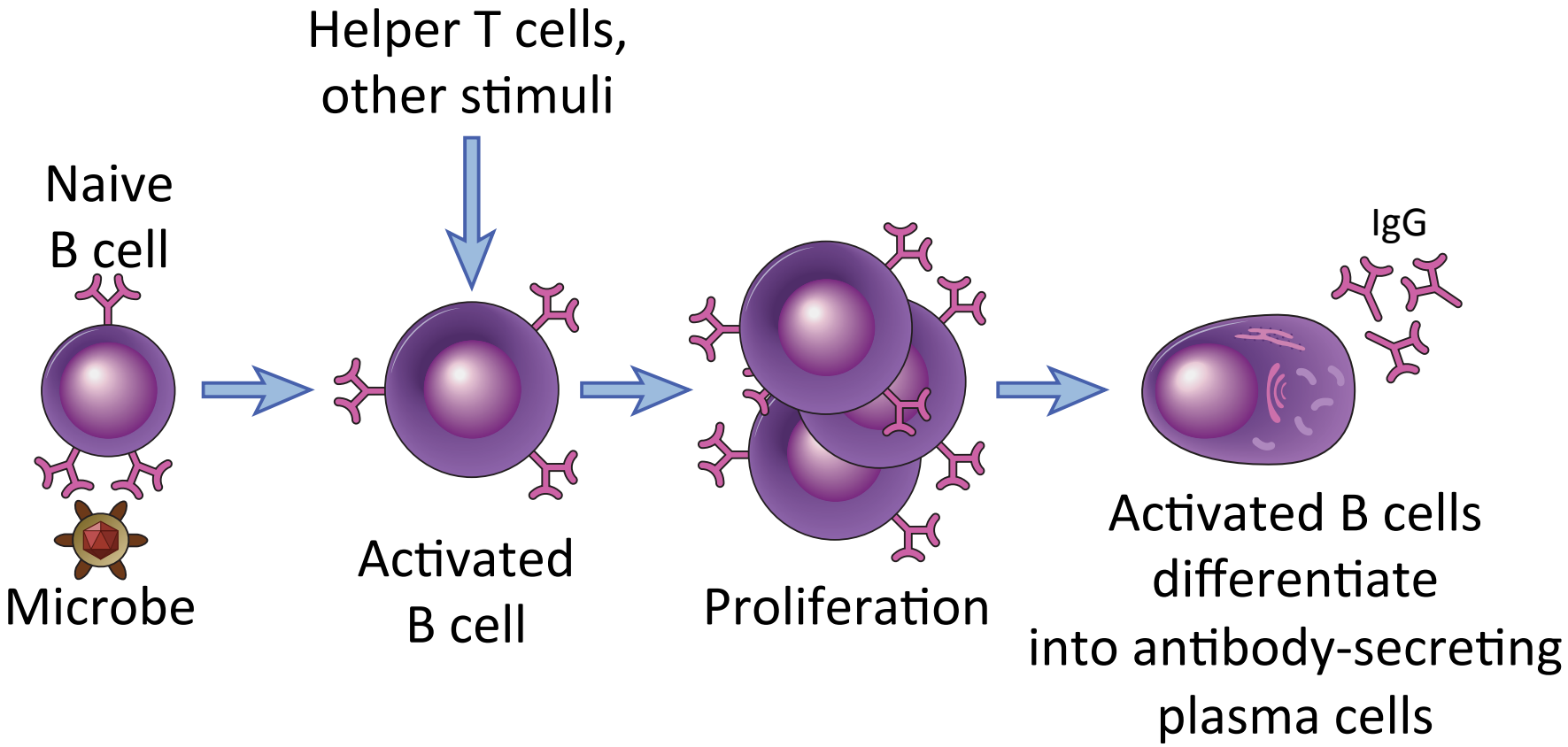
Why do some people respond to therapeutic proteins?

- Characteristics of the protein
  - Conformation, post-translational modifications, etc
- Features of the host
  - Genes that influence immune activation vs tolerance: largely unknown
  - The immune "background" of the host
  - Defective control/tolerance mechanisms
- Environmental factors

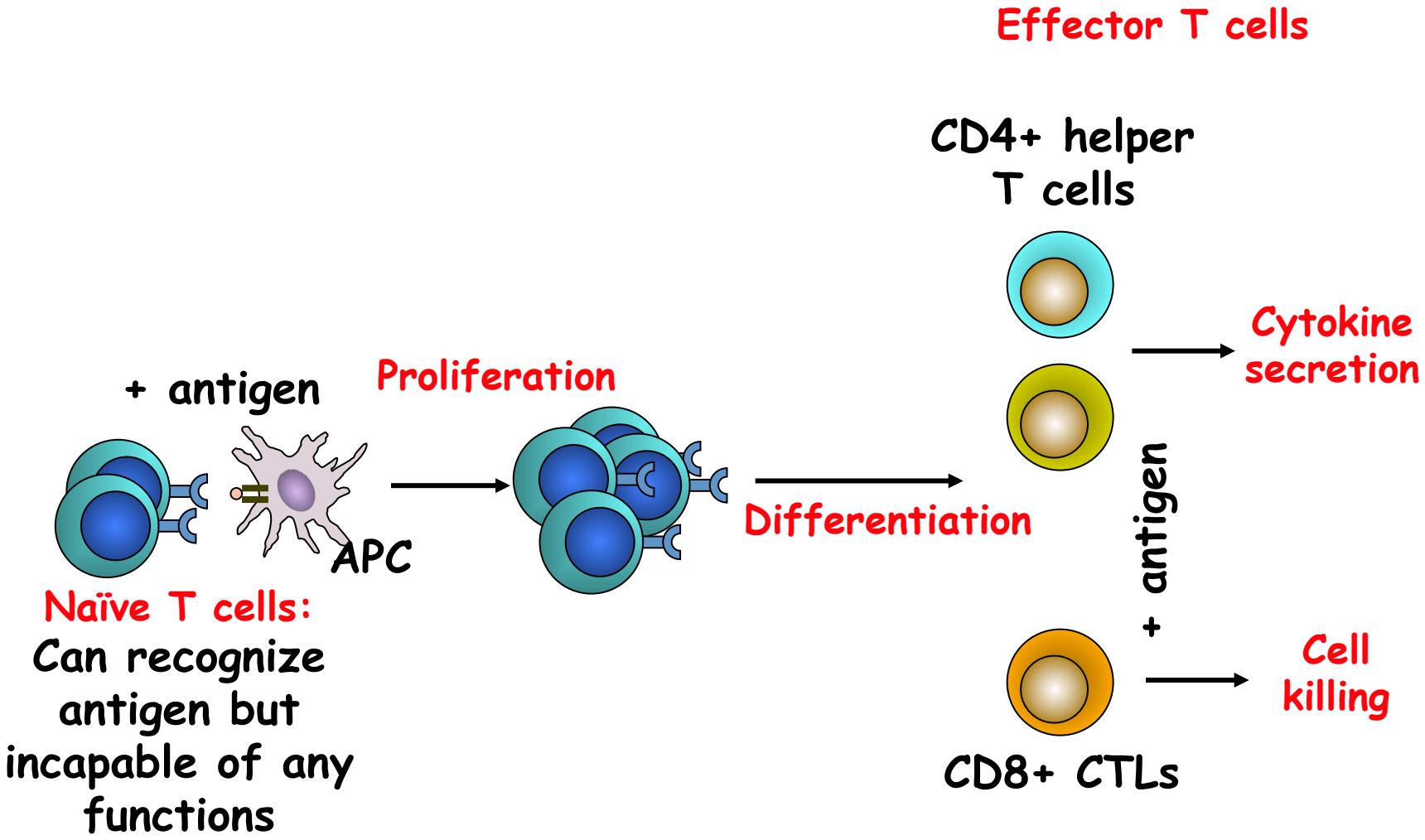
Critical questions

- Requirements for antibody response to a protein
- Why do some individuals produce antibodies when exposed to a "self" protein?
  - Why does tolerance to the protein fail?
- How can tolerance be restored?
- What assays need to be developed to better understand the loss and restoration of tolerance?

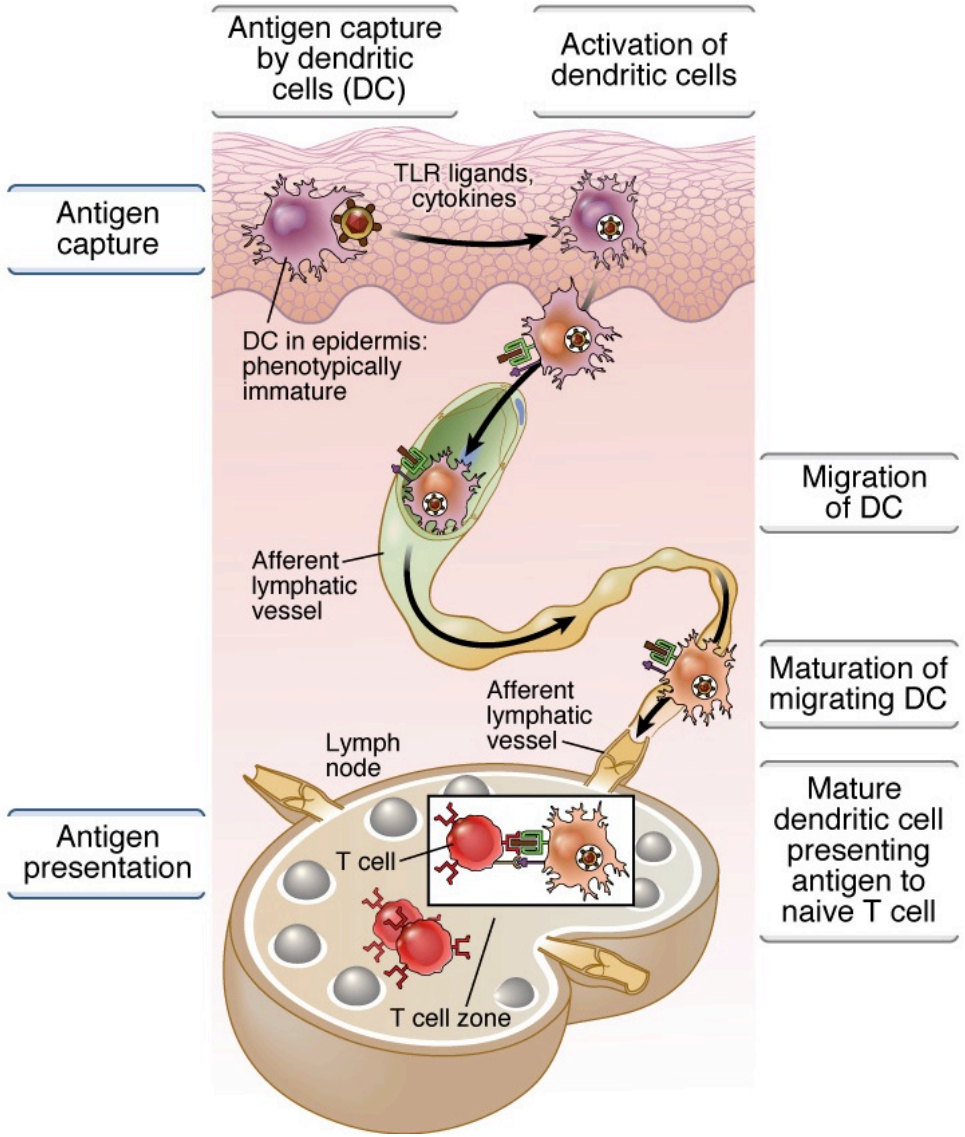
Antibody production: activation of B cells



T cell responses



Capture and presentation of antigens by dendritic cells



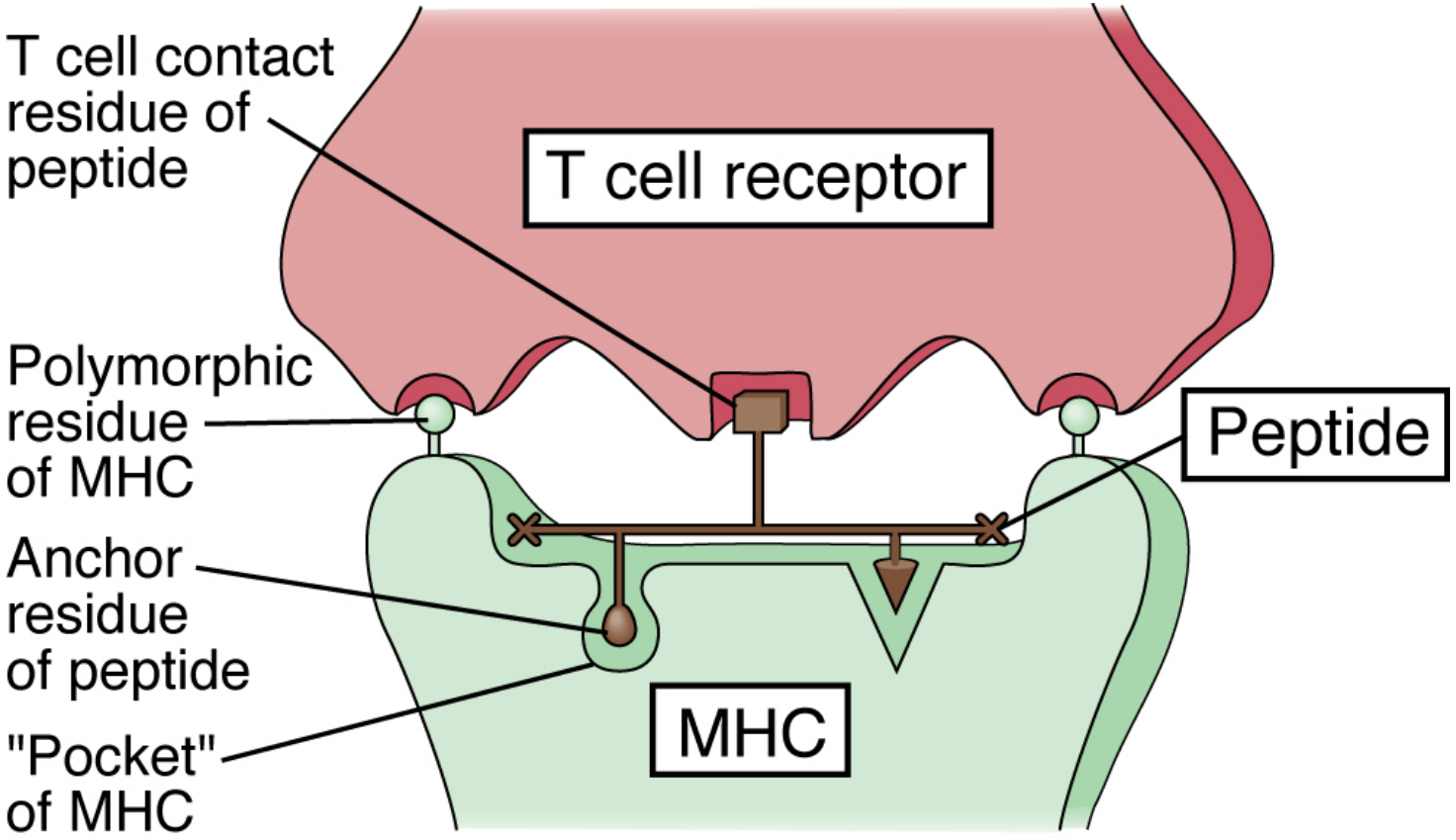
Abbas, Lichtman and Pillai. *Basic Immunology, 5th edition, 2016, Elsevier*

Sites of microbe entry: *skin, GI tract, airways (organs with continuous epithelia, populated with dendritic cells). Less often -- colonized tissues, blood*

Sites of lymphocyte activation: *peripheral lymphoid organs (lymph nodes, spleen), mucosal and cutaneous lymphoid tissues*

Antigens and T cells come together in the same organs

Schematic model of T cell recognition of antigen: what antigens do T cells see



MHC molecules are the peptide display molecules of the immune system

Human MHC: HLA (human leukocyte antigens)

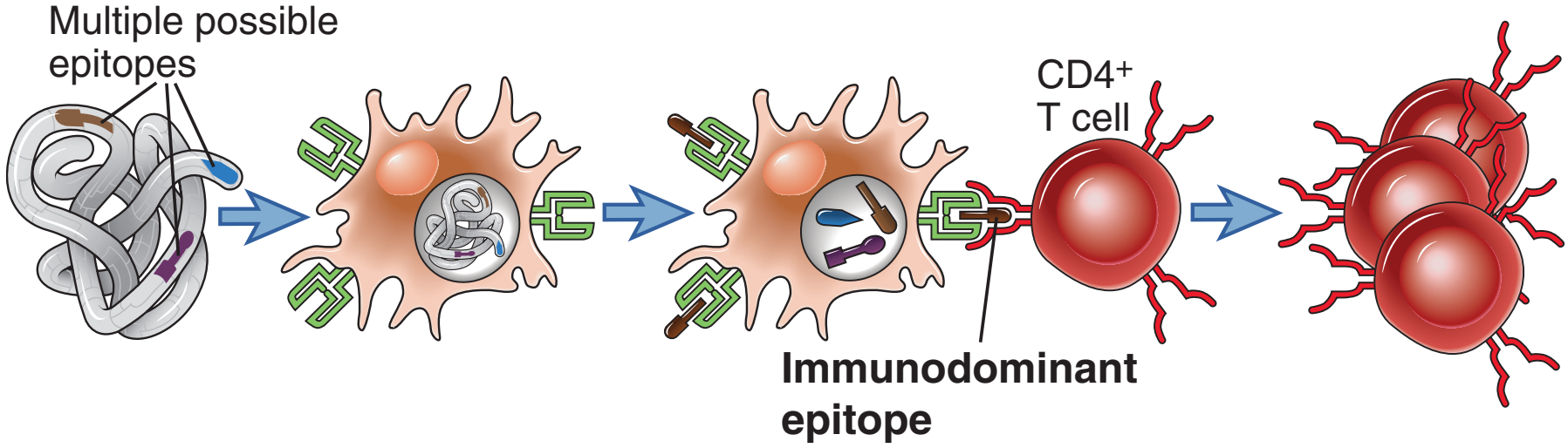
HLA molecules

- Most polymorphic genes in biology (many variants – alleles – in the population)
  - Each of us inherits different combinations of alleles
  - Each HLA variant can display many (but not all) peptides; hence, individuals may display different peptides and respond to different antigens

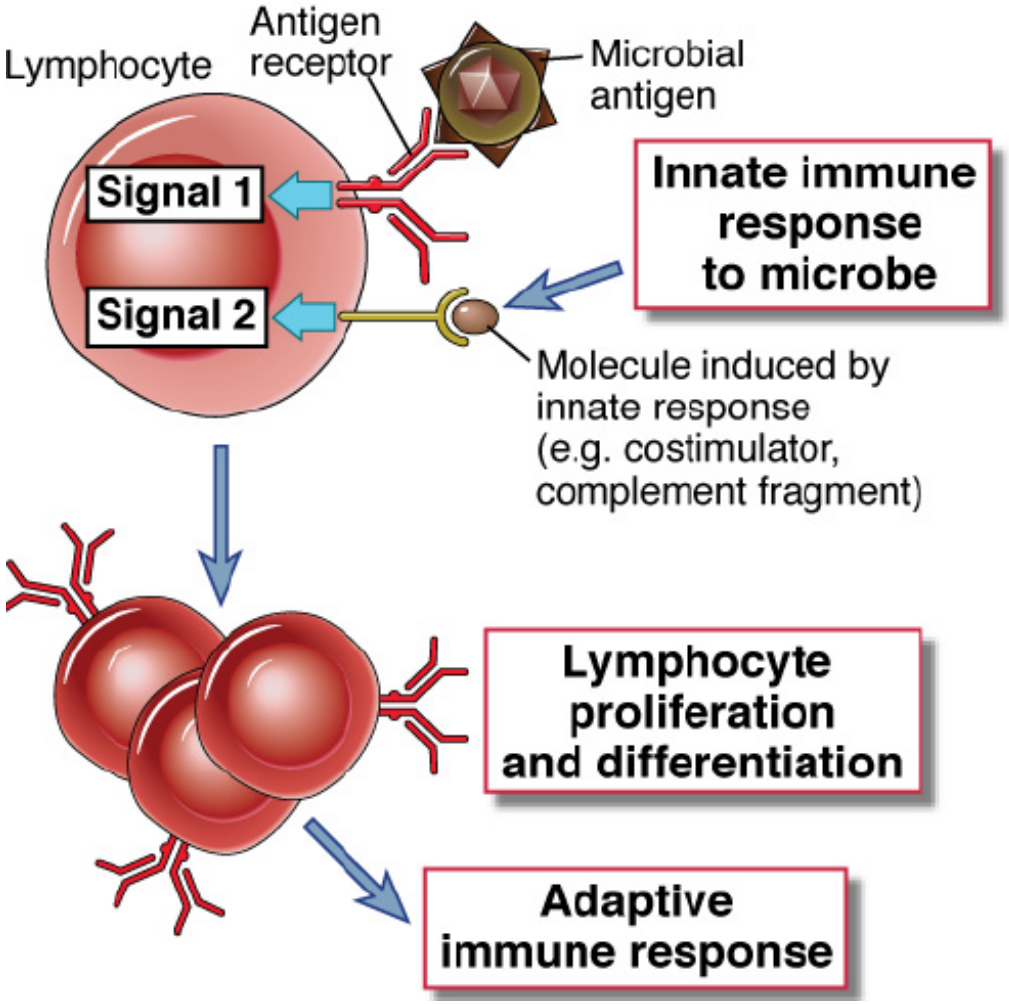
- Two major classes:
  - Class I MHC molecules bind to CD8
  - Class II MHC molecules bind to CD4

Each HLA molecule presents a limited number of peptides

Internalization of antigen into APC	Antigen processing	Processing generates multiple peptides, one of which can bind to class II allele	T cells respond to immunodominant peptide epitope



Costimulation: the second signal for T lymphocyte activation

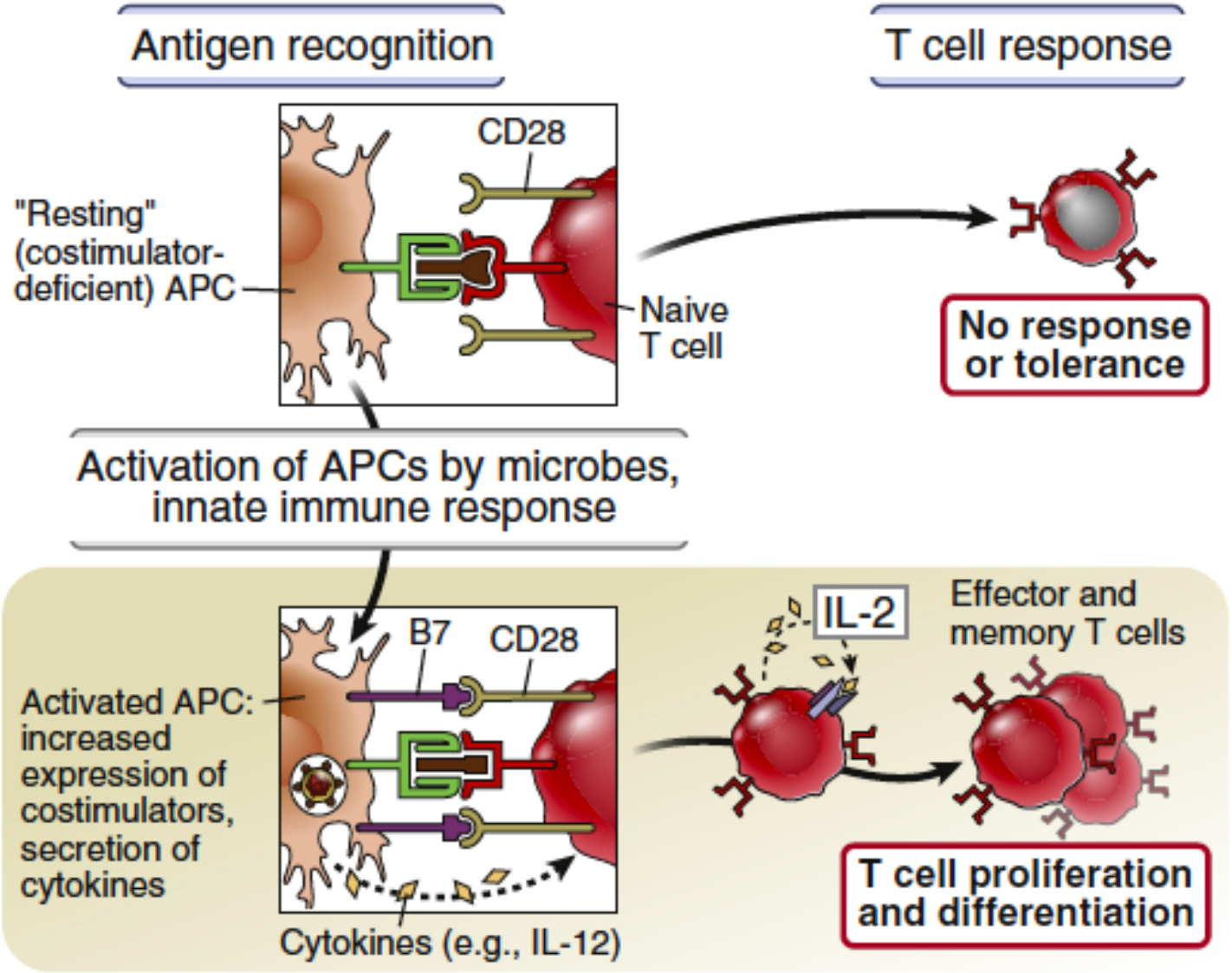


Costimulation: *signal(s) in addition to antigen that are needed to stimulate adaptive immune responses*

Costimulators are induced on APCs by microbes and by adjuvants administered in vaccines

Self antigens and other harmless substances fail to induce costimulators, do not elicit immune reactions and may induce tolerance

Role of costimulation in T cell activation



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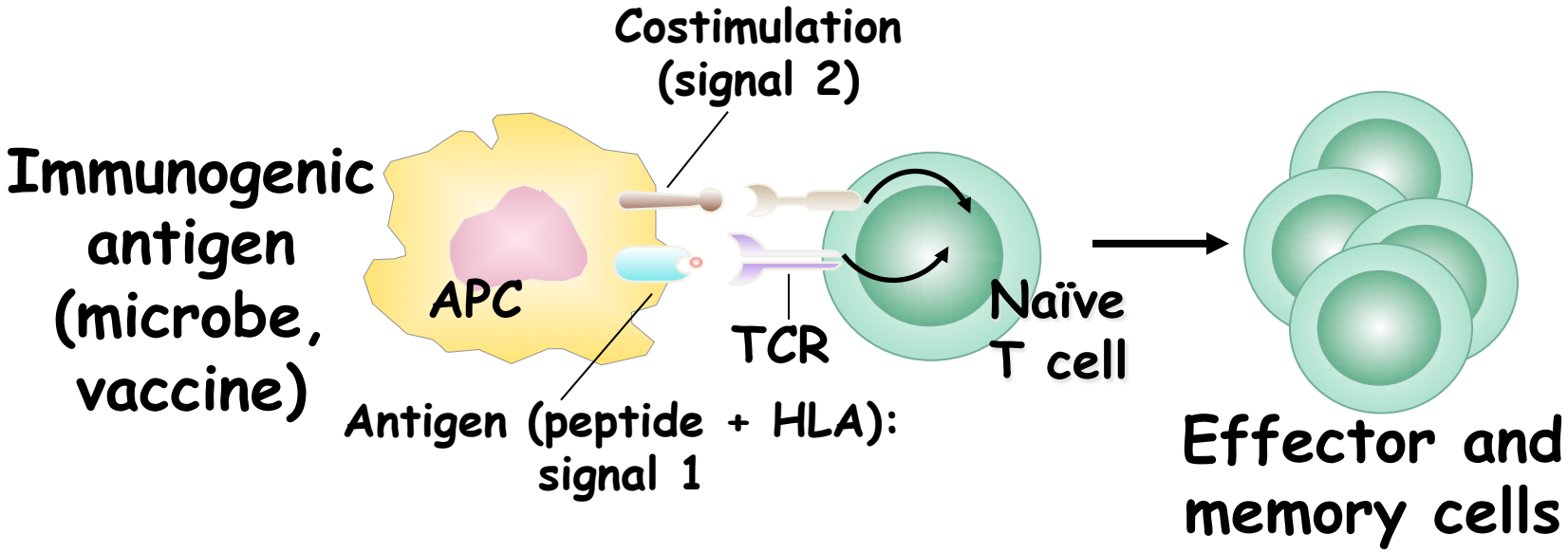
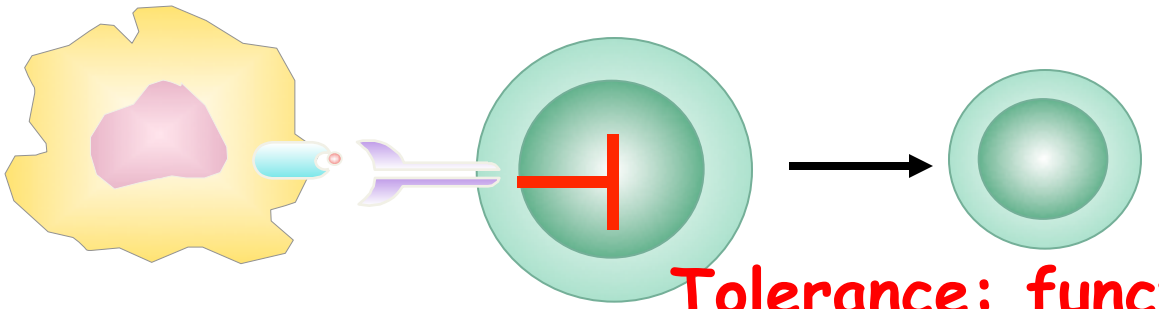
Costimulation determines the choice of activation vs tolerance

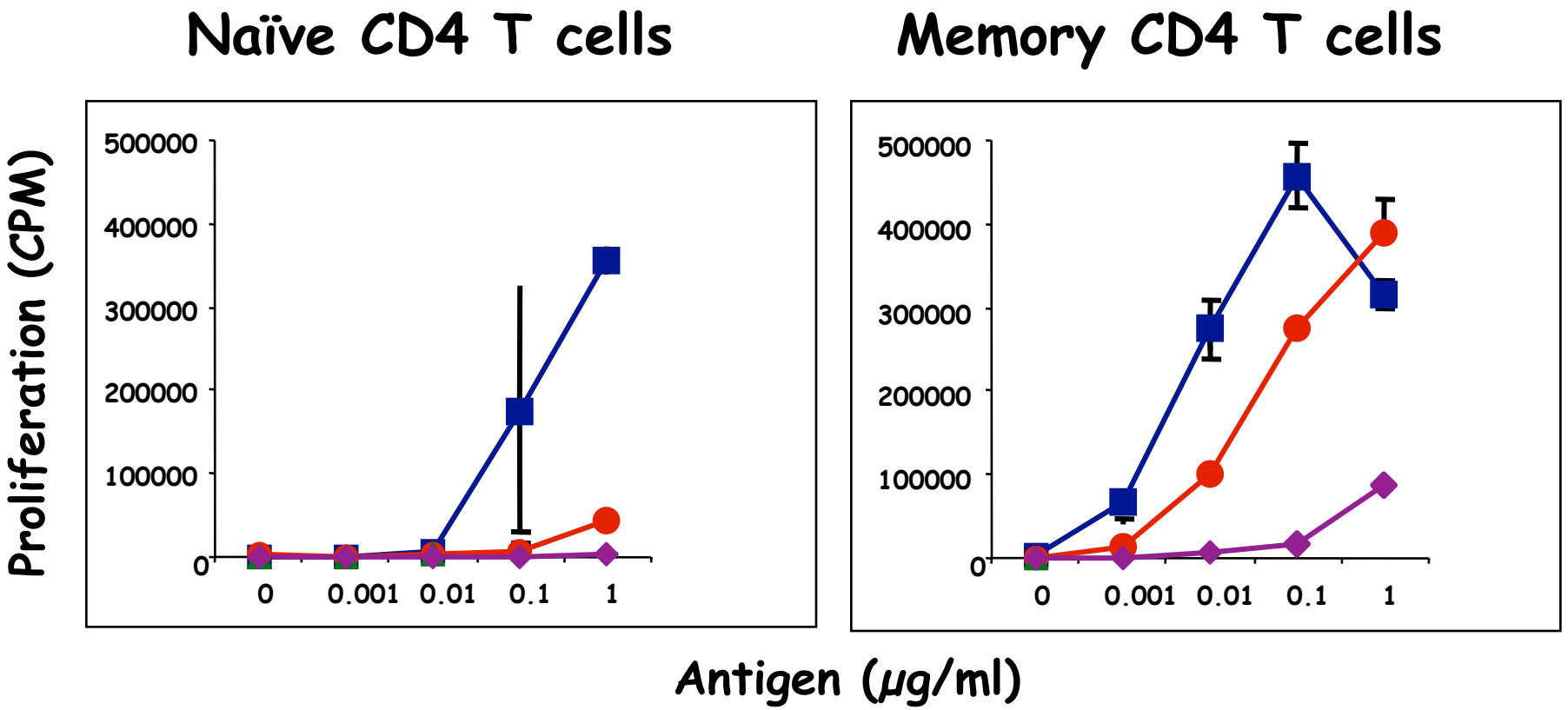


Tolerogenic antigen (e.g. self)



Tolerance: functional inactivation or cell death, or sensitive to suppression

Naïve T cells are more dependent on B7 costimulation than are memory cells



Costimulation

- Required for initiating T cell responses (activation of naïve T cells)
- Ensures that T cells respond to microbes (the most potent inducers of costimulators) and not to harmless antigens
  - Source of costimulation during responses to tumors, transplants, proteins given without adjuvants ?
- Memory cells are less dependent on costimulation than are naïve T cells

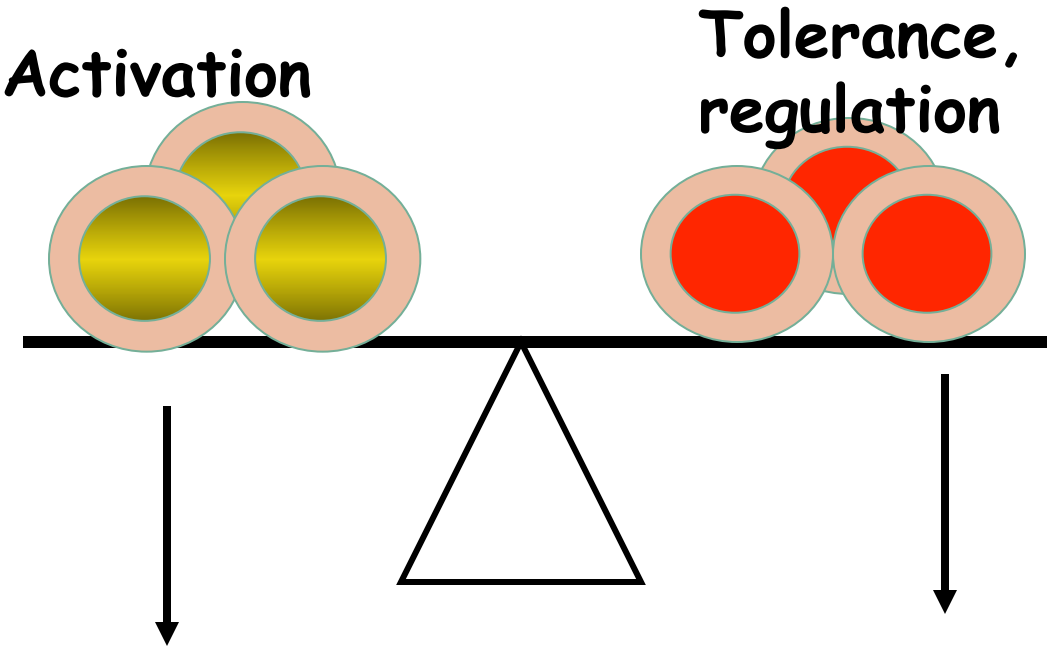
Possible host determinants of antibody responses to a biological

- Size of antigen-specific T cell pool
  - Pre-existing pool of naïve T cells
  - High number of memory cells induced by cross-reactive environmental antigens
- Inheritance of HLA alleles that present the antigen
  - Unlikely to be a key issue in an outbred population (many different HLA alleles present)
- High level of costimulation
  - Source? Unlikely to be provided by antigen itself
  - Activation state of dendritic cells?

Exploiting absence of costimulation to inhibit immune responses

- Costimulatory blockade (blocking B7-CD28, CD40 pathways)
- Peptide administration (repeated low doses, modeled after desensitization for allergies)
  - Peptide-coupled PBLs (spleen cells in mice)

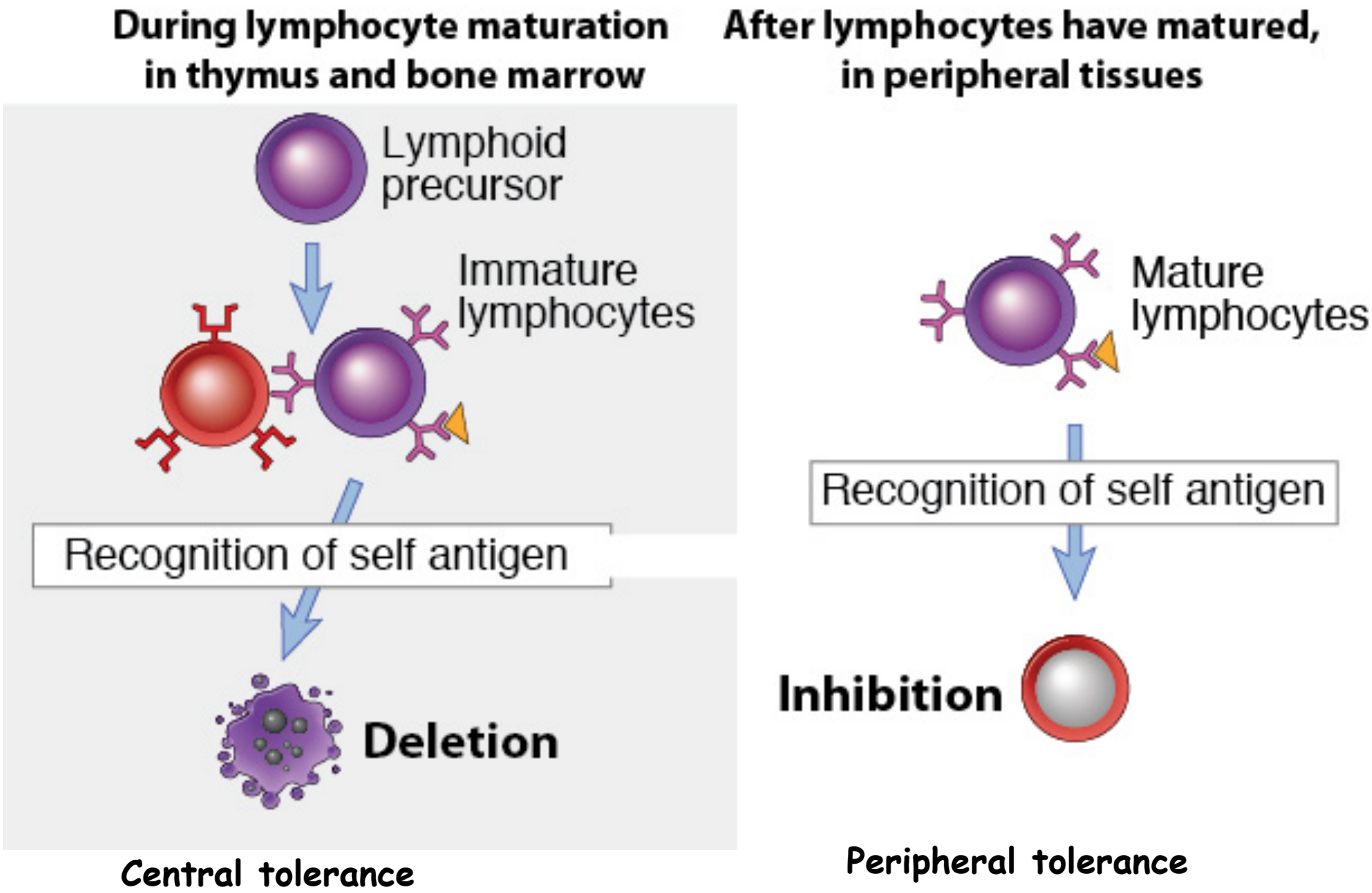
The immunological equilibrium: balancing lymphocyte activation and control



Normal: reactions against pathogens

No response to self and other harmless antigens

Where is tolerance induced?



Best defined mechanisms of peripheral T cell tolerance

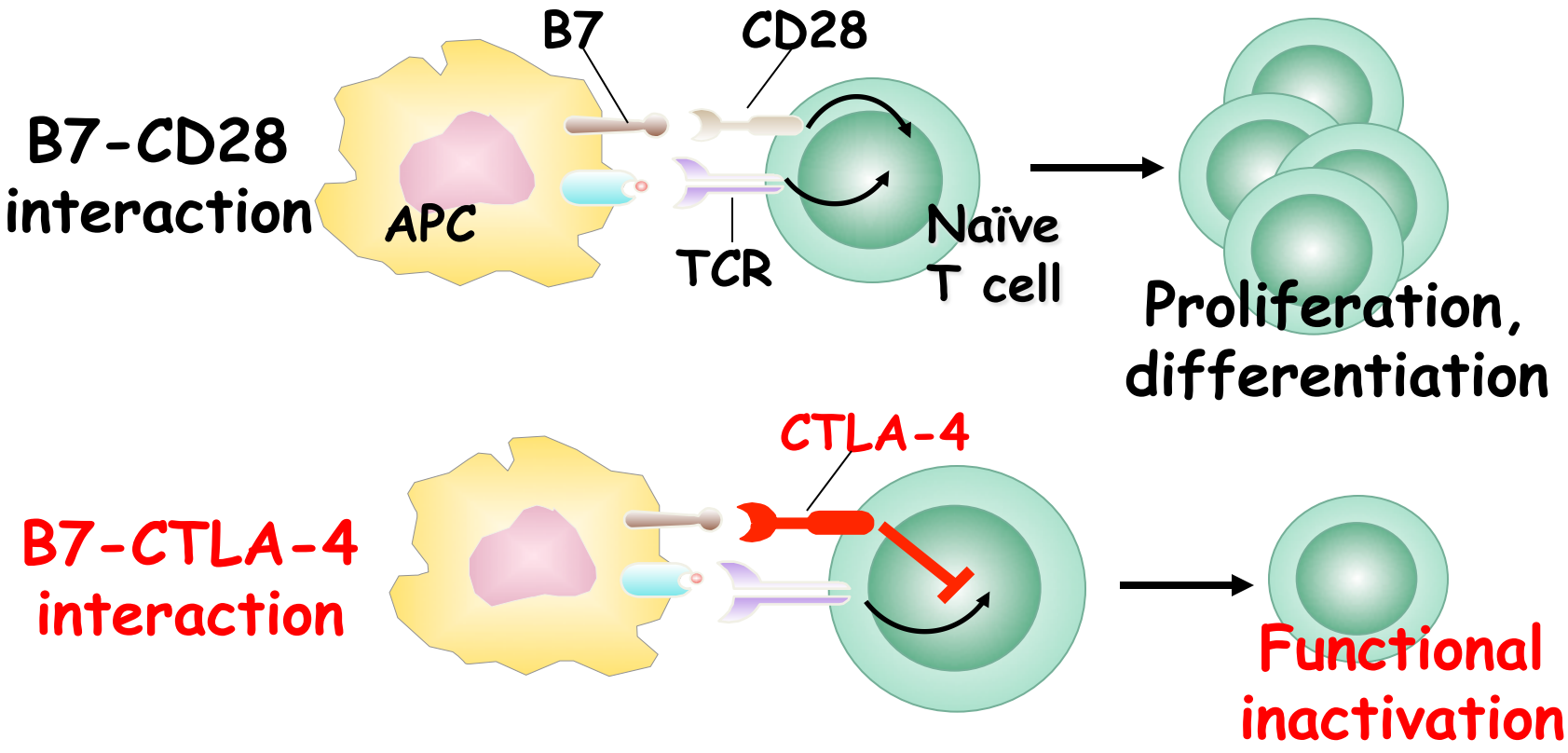
- Engagement of inhibitory receptors
- Suppression by regulatory T cells (Tregs)

Inhibitory receptors of the immune system

- One mechanism by which the system maintains a balance between activation and inhibition is to use different receptors for different outcomes

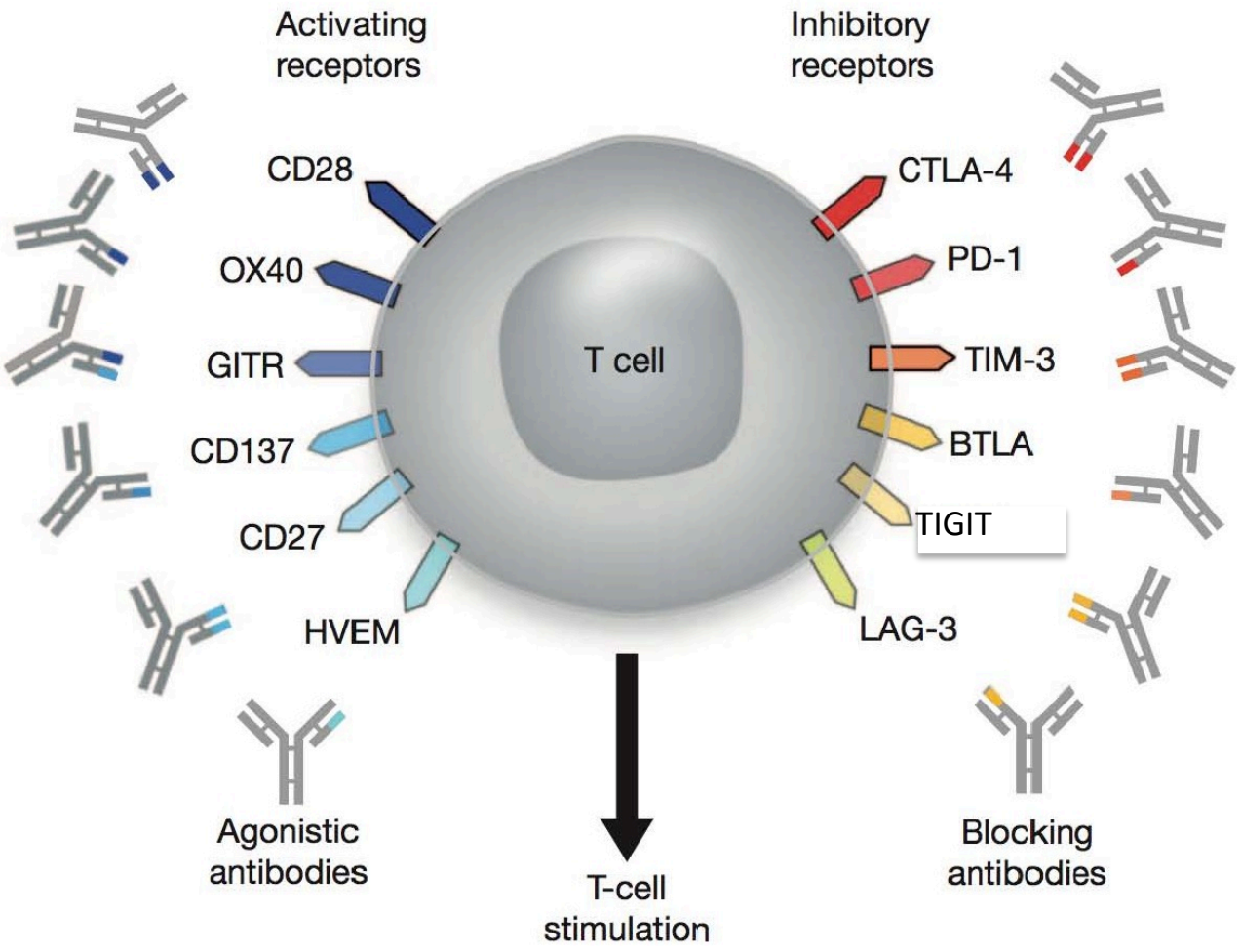
- Inhibitory receptors are present in NK cells, T cells and B cells
  - Best defined activating and inhibitory receptors of T cells are members of the CD28 family

The opposing functions of CD28 and CTLA-4



Knockout of CTLA-4 in mice and mutation in humans results in immune dysregulation (lymphoproliferation, multi-organ inflammation)

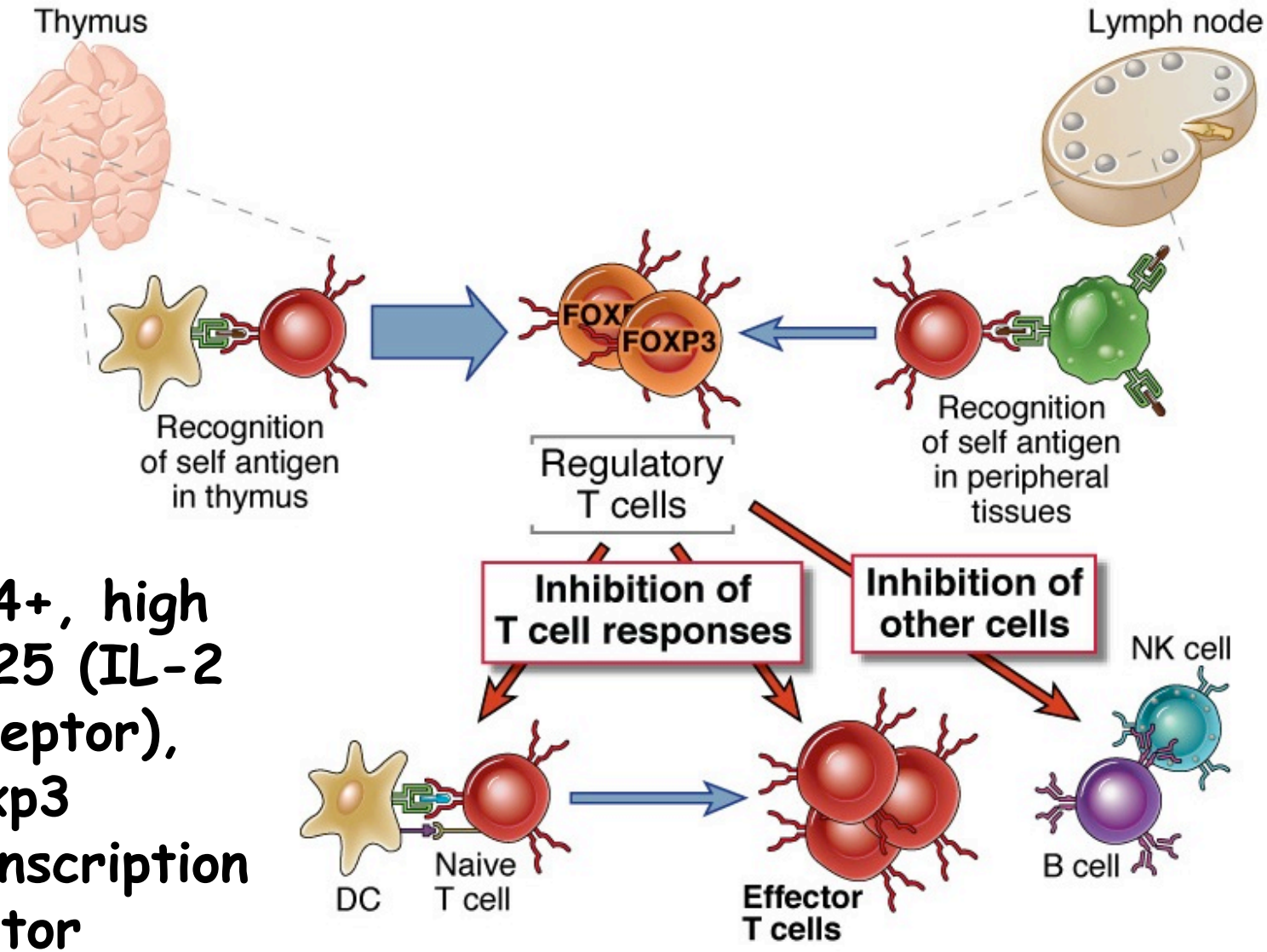
The landscape of T cell activating and inhibitory receptors



Engaging inhibitory receptors for inducing tolerance

- Can inhibitory immune receptors be triggered to shut off immune responses?
  - Problem of reliably producing agonistic antibodies against cellular receptors
- Blocking inhibitory receptors has revolutionized cancer immunotherapy
  - "Checkpoint blockade" stimulates anti-tumor immune responses

Regulatory T cells



CD4+, high CD25 (IL-2 receptor), Foxp3 transcription factor

Abbas, Lichtman and Pillai. *Cellular and Molecular Immunology, 8th edition, 2014, Elsevier*

The significance of Foxp3+ Tregs

- Genetic evidence: Foxp3 mutations --> autoimmune disease (IPEX); in mice, disease can be corrected by providing normal Foxp3+ cells

- Do defects in Foxp3+ Tregs or resistance to Treg-mediated suppression contribute to common autoimmune diseases?
  - Inconsistent and variable data
  - By definition, any abnormal immune response reflects an imbalance of activation vs control

The therapeutic potential of regulatory T lymphocytes

- **Cell transfer of autologous Tregs to suppress immune responses**
  - Grow up patient's Tregs ex vivo
  - Ongoing clinical trials in graft rejection, T1D show it is safe
  - Very little efficacy data

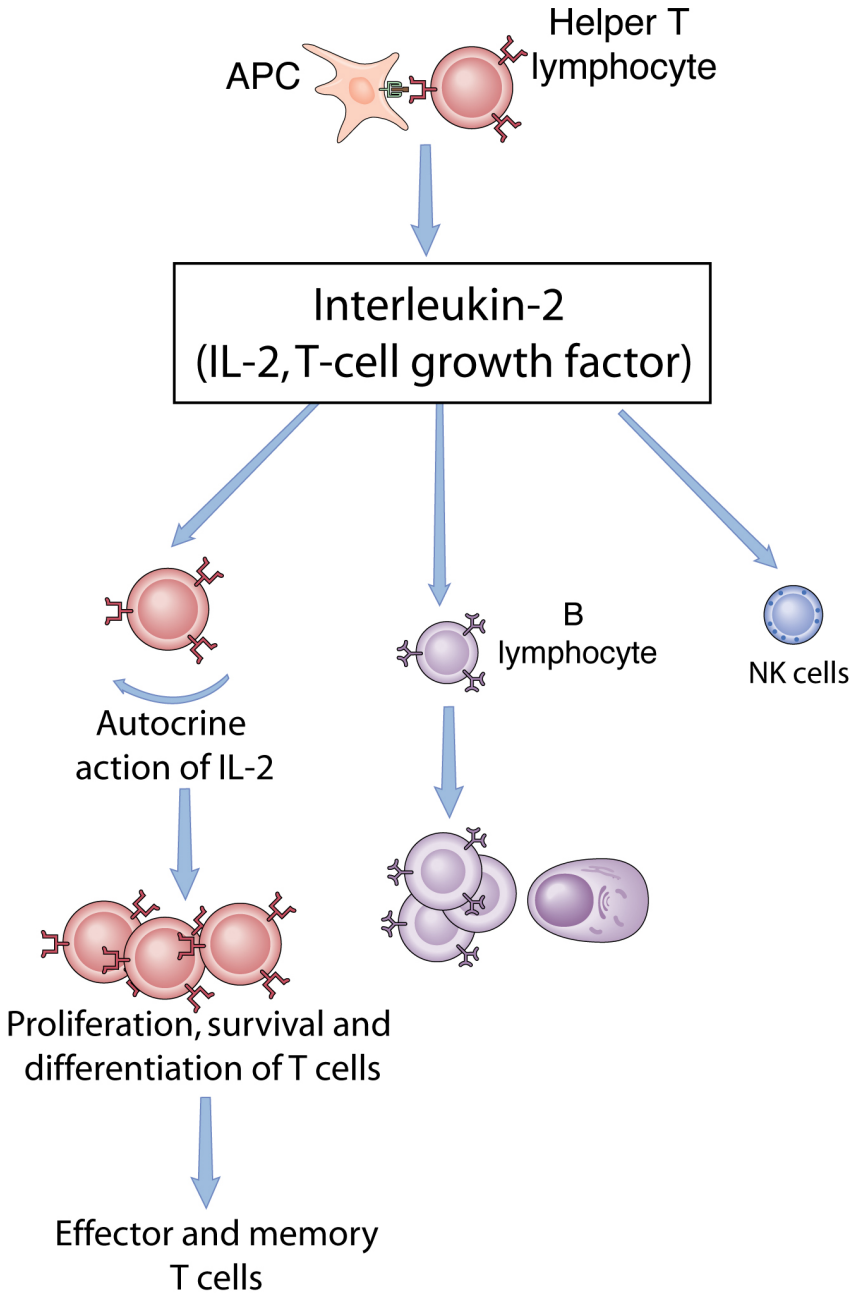
The therapeutic potential of regulatory T lymphocytes

- Cell transfer of autologous Tregs to suppress immune responses

- **Challenges:**
  - **Technically difficult, individualized**

- **Administer antigen or antigen mimic in ways that preferentially induce Tregs?**
  - **Weak stimulus (peptide antigen, anti-CD3); + IL-2?**

Functions of Interleukin-2: the dogma



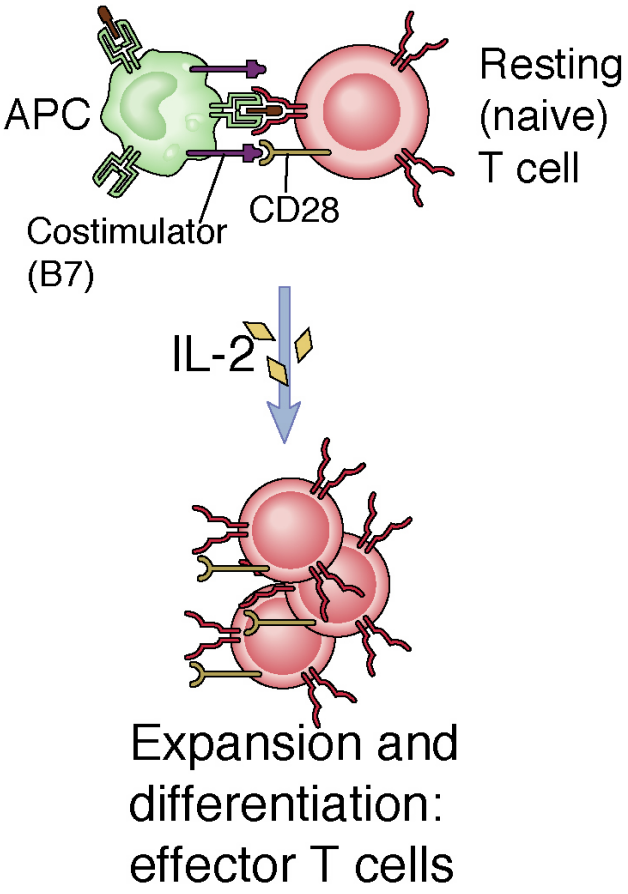
The unexpected biology of IL-2

- Interleukin-2 is the prototypic T cell growth factor (TCGF), required for initiating clonal expansion of T cells in response to antigen

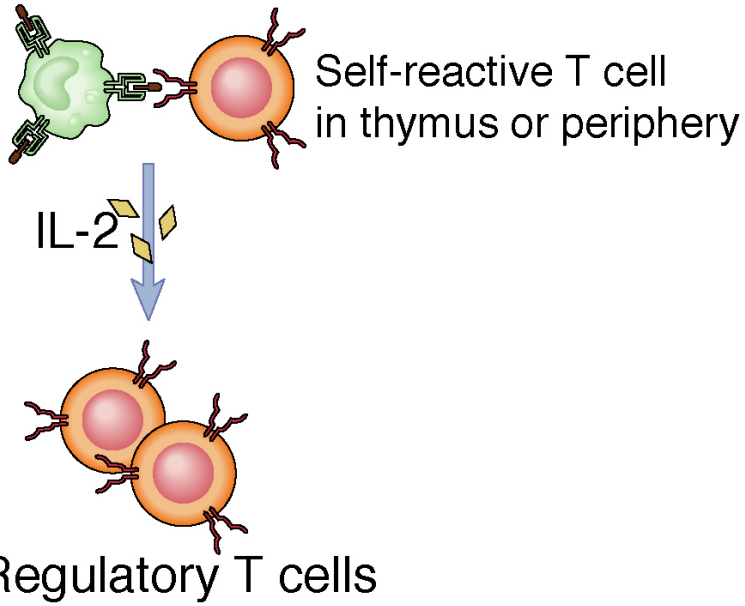
- BUT: knockout of IL-2 or the α or β chain of the IL-2R results not in immune deficiency but in systemic autoimmunity and lymphoproliferation

Dual roles of IL-2 in T cell responses

Induction of immune response



Control of immune response



Surprising conclusion from knockout mice: the non-redundant function of IL-2 is in controlling immune responses

Therapeutic potential of IL-2: a revision

- IL-2 was originally used to boost immune responses in cancer, HIV infection (promoting effector and memory T cells)
  - Inconsistent clinical results

- IL-2 treatment can increase number and functional activity of Tregs
  - Low-dose IL-2 to treat steroid-resistant chronic GVHD, vasculitis
  - More recent clinical trials ongoing in type 1 diabetes, SLE, graft rejection

Antigen-specific lymphocytes

- Are antigen-specific T (and B) cells present before treatment? Are they altered by treatment?
  - Frequency of naïve T cells predicts magnitude of response following antigen exposure

- What are the phenotypic and functional characteristics of antigen-specific lymphocytes?

Need for more sophisticated analyses of antigen-specific lymphocytes

Strategies for restoring tolerance

- Administration of antigen in tolerogenic form
  - Repeated doses of peptides without adjuvants
- Blocking costimulation
- Engaging inhibitory receptors
- Treg targeted therapies:
  - Treg cell transfer
  - IL-2