T cell development and tolerance

Mark S. Anderson
Diabetes Center
UCSF

UCSF
University of California
San Francisco
advancing health worldwide™

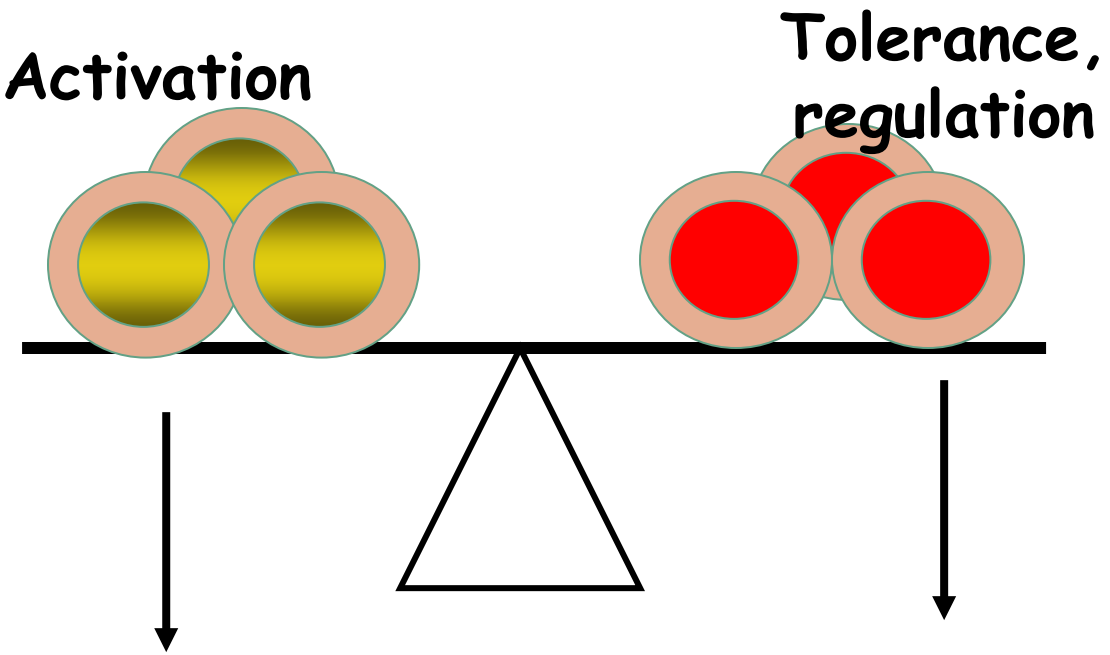
FOCiS


FOCiS®
ANNUAL MEETING
BOSTON-2023
JUNE 20-23

Lecture outline

- Central and peripheral tolerance
- Inhibitory receptors of T cells
- Regulatory T cells

The immunological equilibrium: balancing lymphocyte activation and control



Normal: reactions against pathogens, vaccines

No response to self and other harmless antigens

Immunological tolerance

- **Definition:**
  - unresponsiveness to an antigen induced by exposure of lymphocytes to that antigen; antigen-specific (unlike "immunosuppression")

- **Significance:**
  - All individuals are tolerant of their own antigens (self-tolerance); breakdown of self-tolerance results in autoimmunity
  - Preventing immune responses to commensal microbes, fetal antigens
  - **Therapeutic potential:** Inducing tolerance may be exploited to treat autoimmune and allergic diseases

THE PHENOMENON OF TOLERANCE:

Seminal observations in 1945 by **R.D. Owen**
that cattle dizygotic twins display red cell (chimerism/mosaicism)
in adult life.

Owen interpreted that placenta of cattle dizygotic twins
undergo anastomosis early in fetal life permitted blood
cells and their precursors to move from one twin to the other.

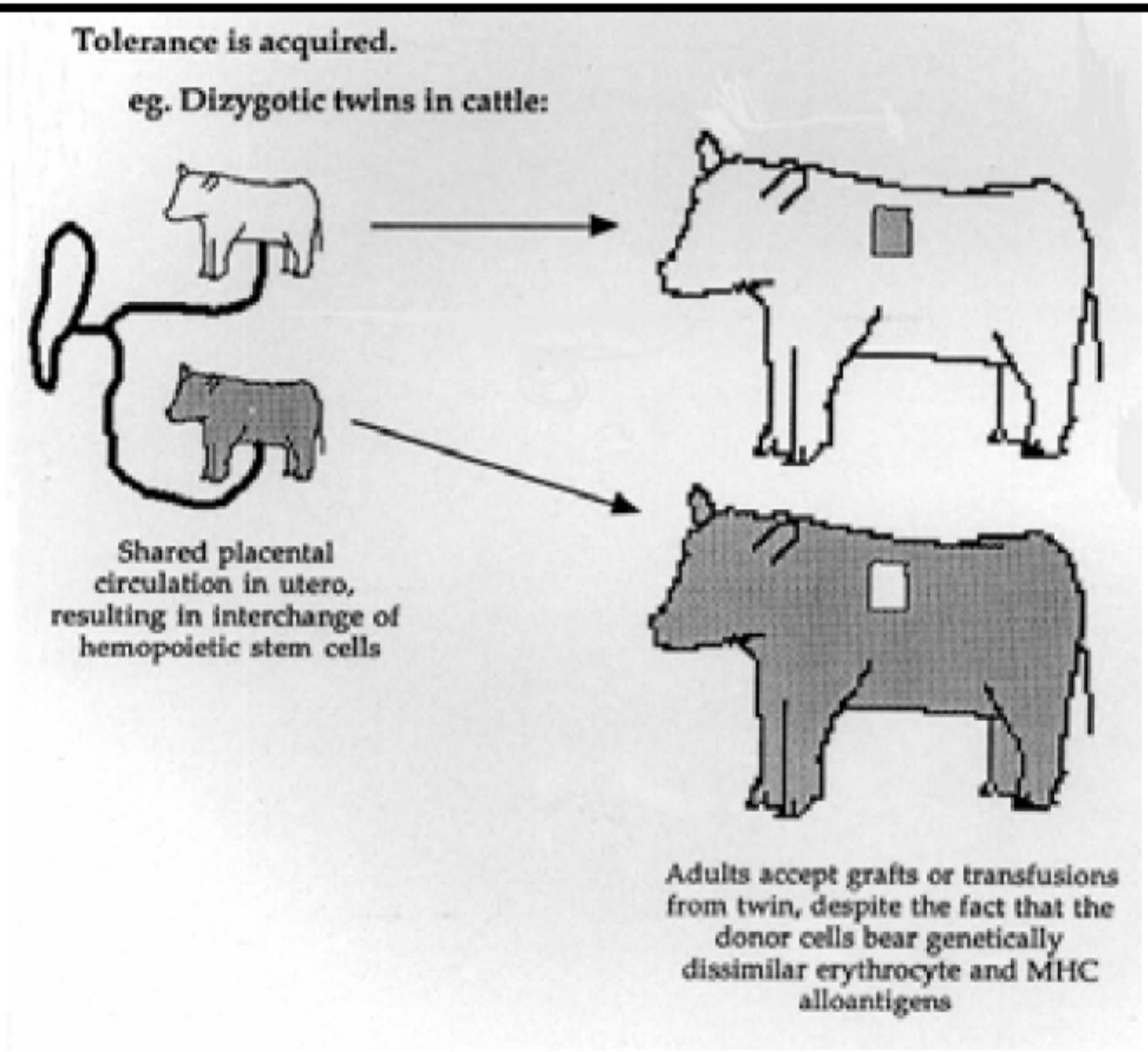


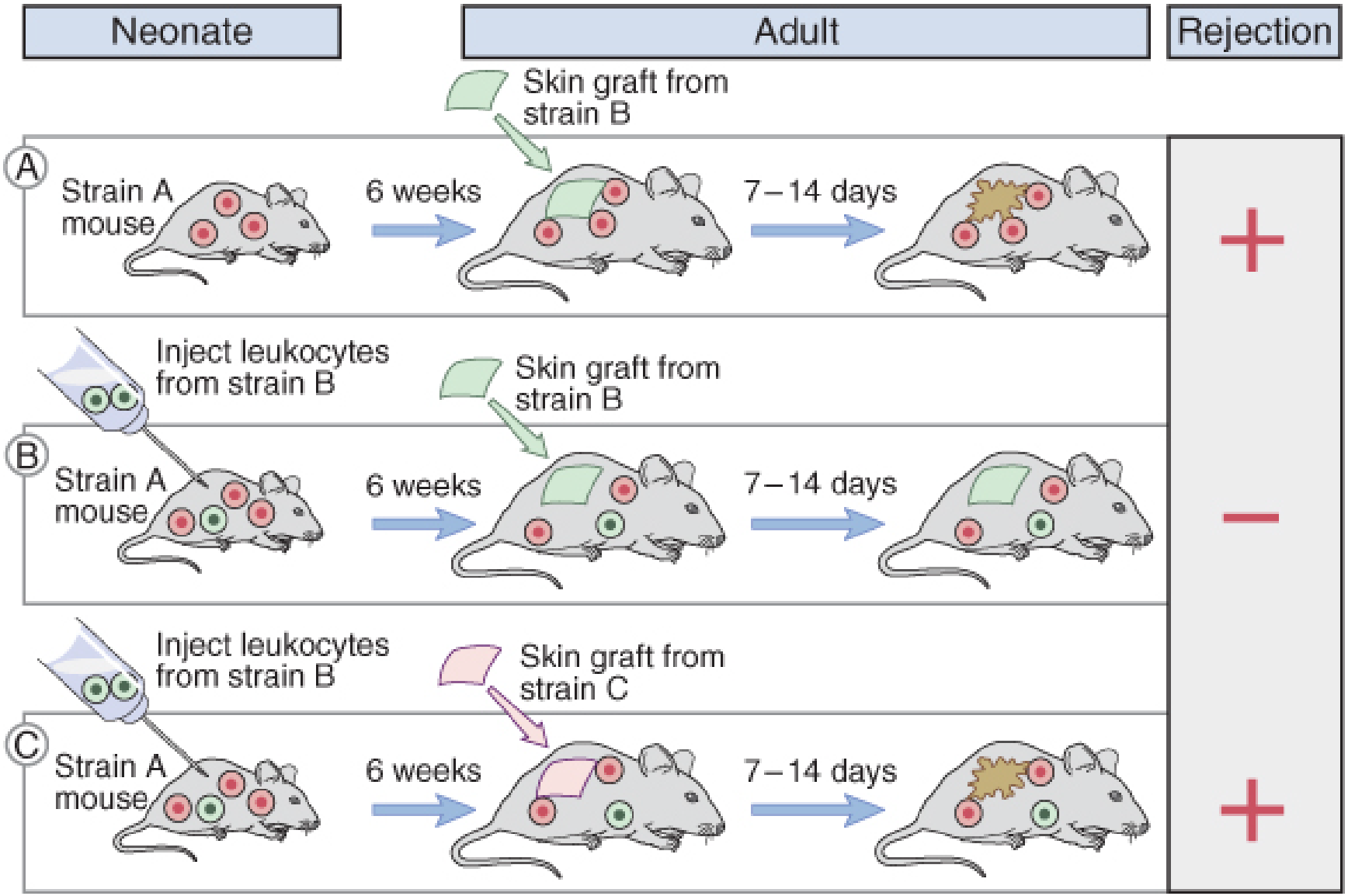
Fig. 2: Dizygotic twins in cattle.

THE PHENOMENON OF TOLERANCE:

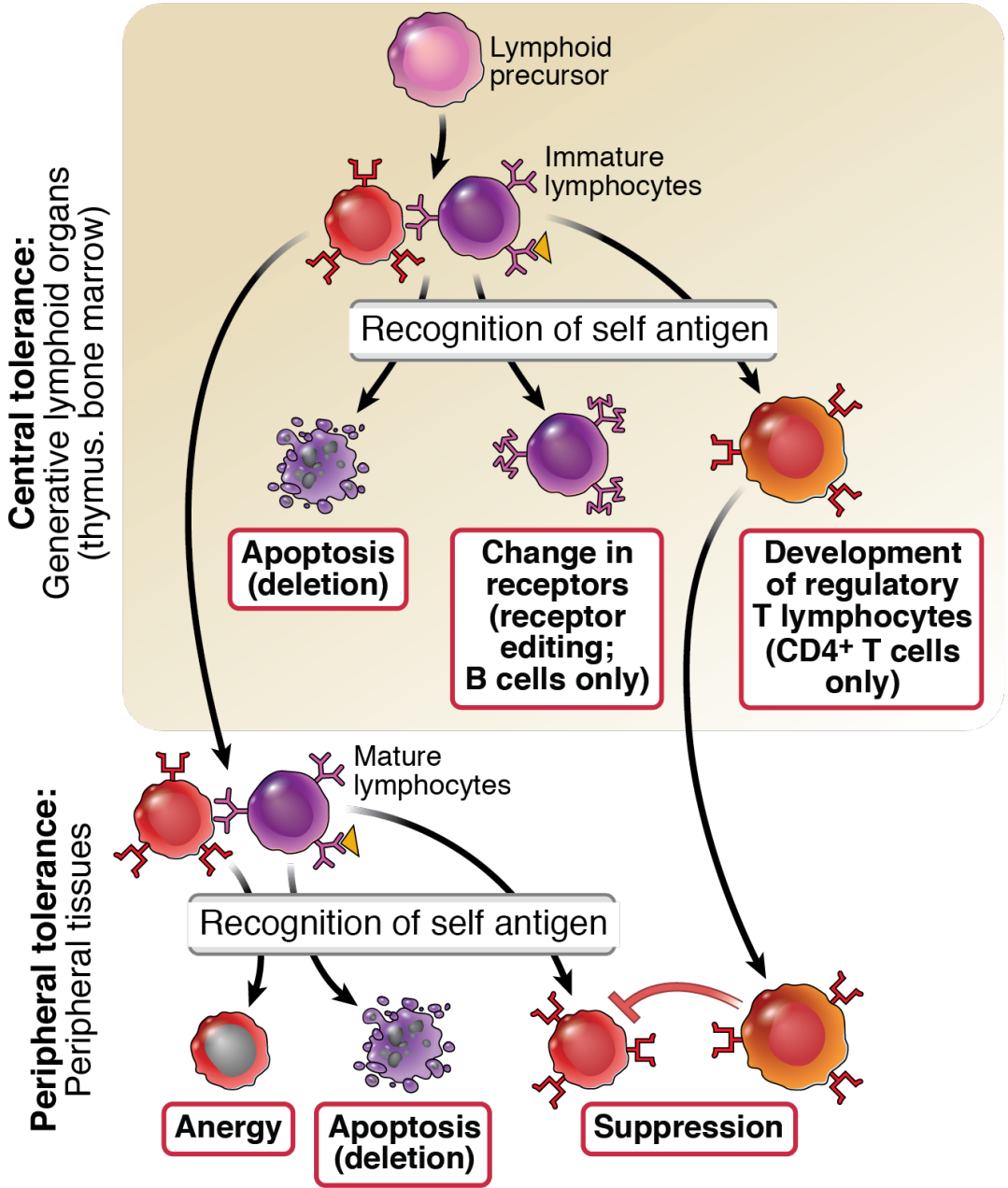
Seminal observations in 1945 by **R.D. Owen**
that cattle dizygotic twins display red cell (chimerism/mosaicism)
in adult life.

Owen interpreted that placenta of cattle dizygotic twins
undergo anastomosis early in fetal life permitted blood
cells and their precursors to move from one twin to the other.

In 1953, BILLINGHAM, BRENT, and MEDAWAR
demonstrated that immunological tolerance could be
acquired by introduction of donor cells during fetal
development - tolerance permanent and Ag specific



Central and peripheral tolerance to self



The principal fate of lymphocytes that recognize self antigens in the generative organs is death (deletion)

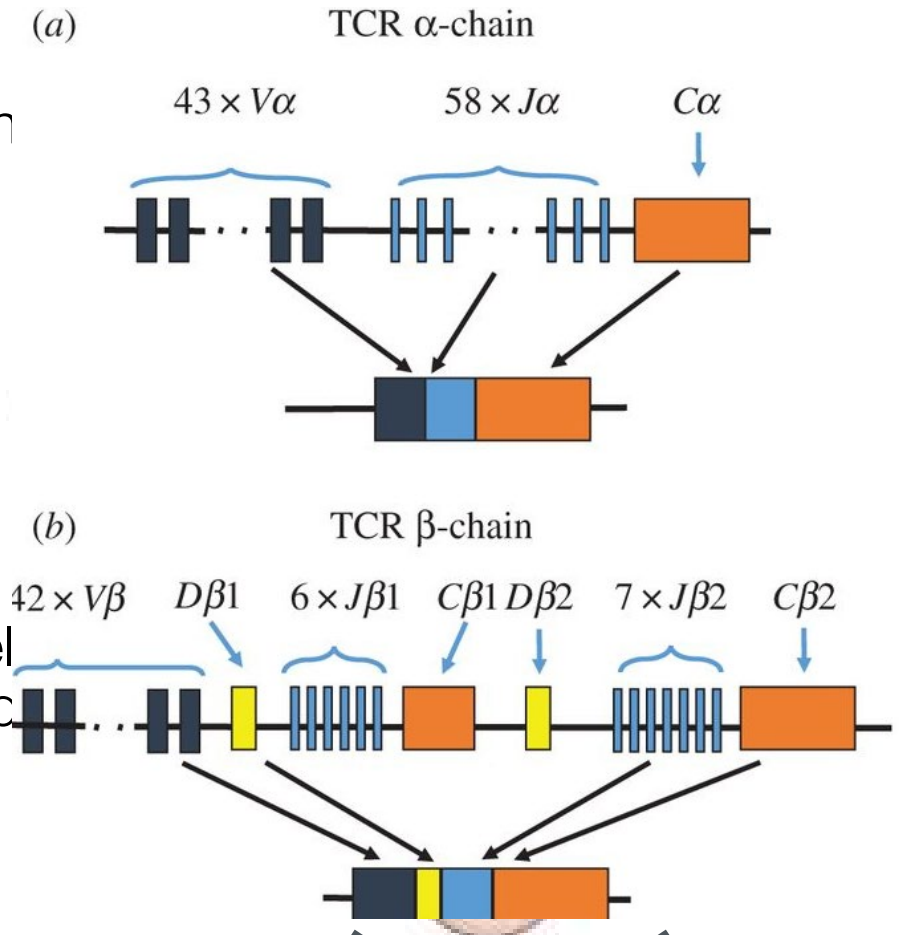
Some B cells may change their specificity (called "receptor editing")

Some T cells may differentiate into regulatory (suppressor) T lymphocytes

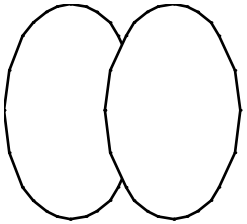
T cell tolerance: Diversity is a challenge

DP th

Pre-sel
reperto

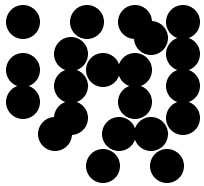


Death b
neglect



Thymus

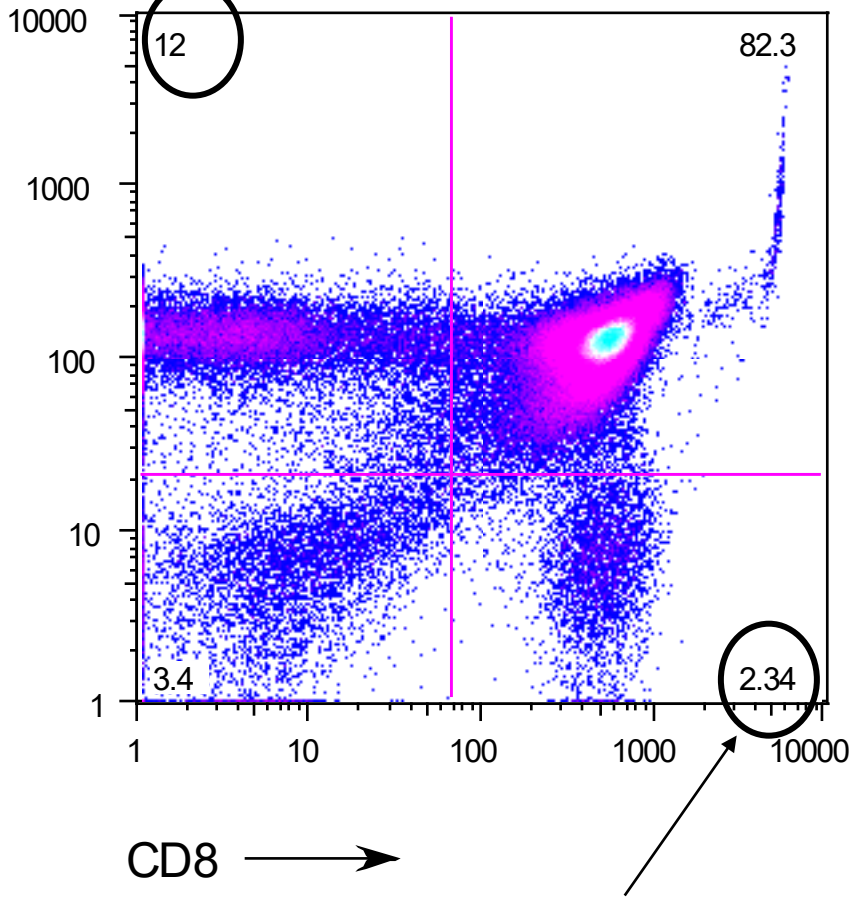
Squish to release cells



Coat with red-anti-CD4 and green anti-CD8

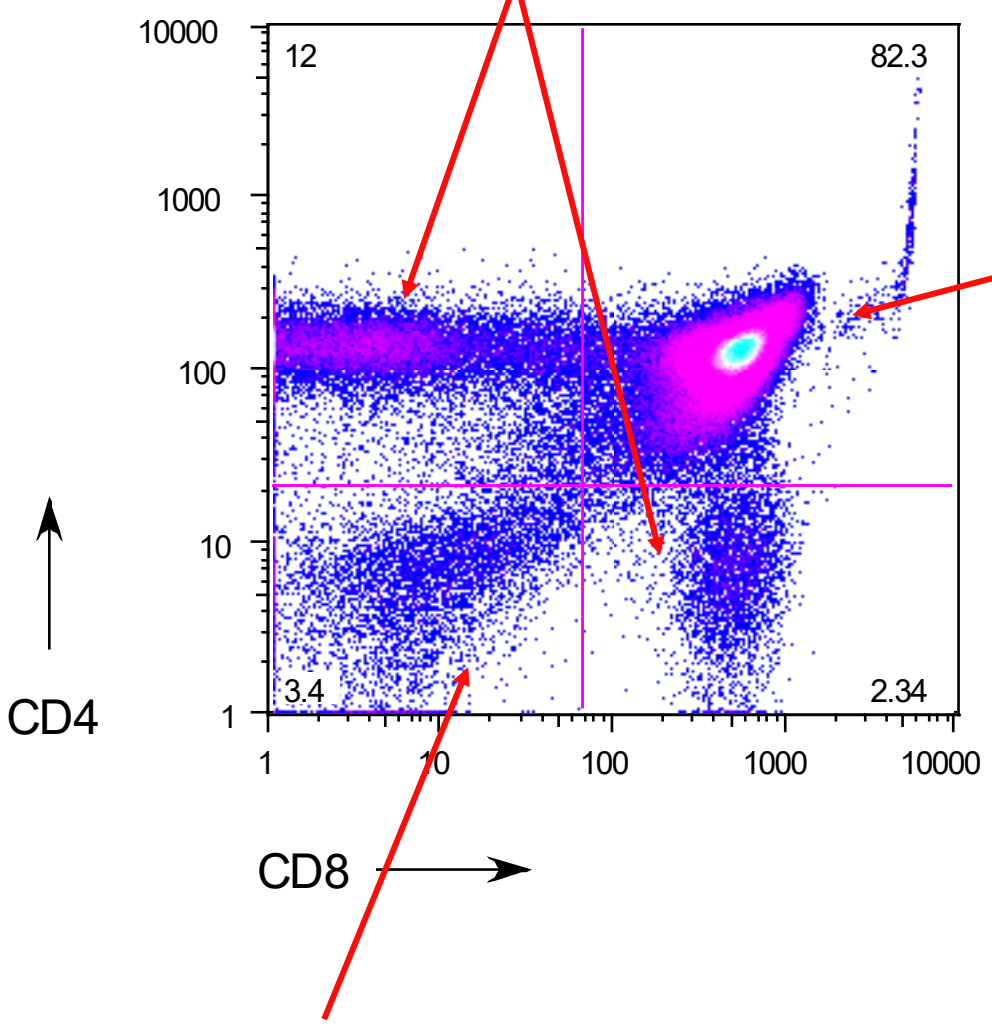
Analyze using the flow cytometer (aka the FACS)

12% of the cells fall in this quadrant (these are CD4+CD8-)



2.34% of the cells fall in this quadrant (these are CD4-CD8+)

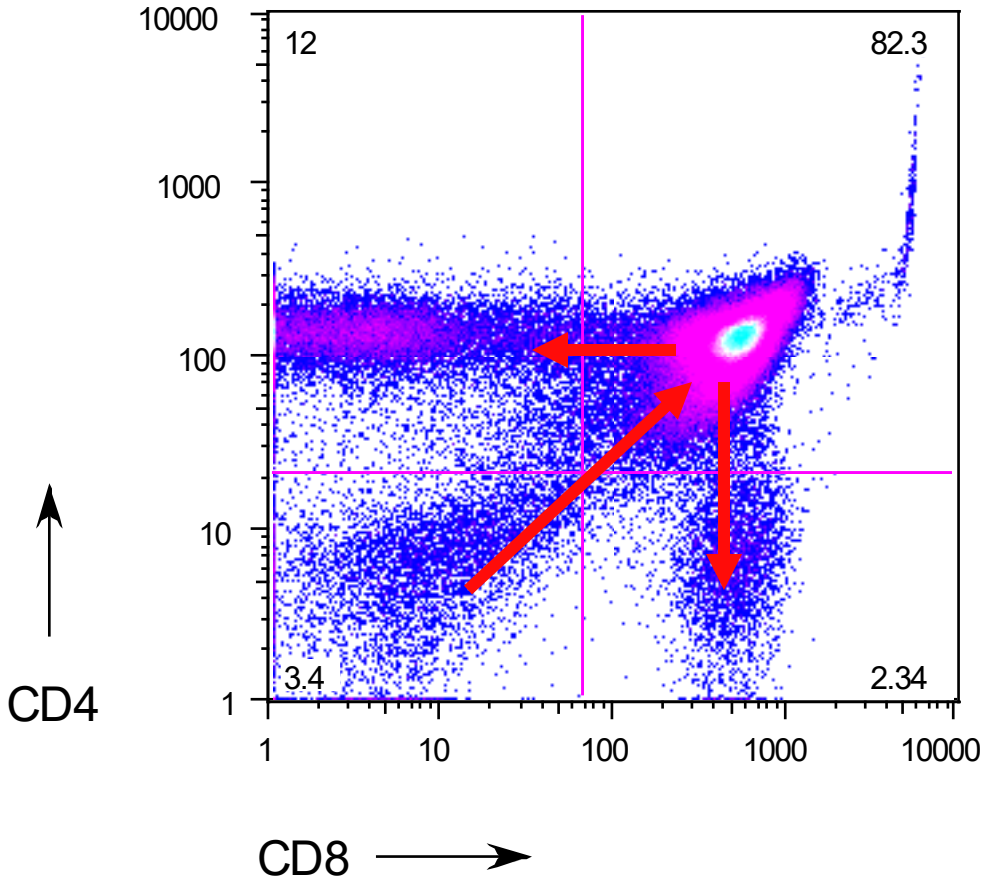
Most mature cells (CD4+CD8- or CD4-CD8+)
- called "single positive" because they express either CD4 or

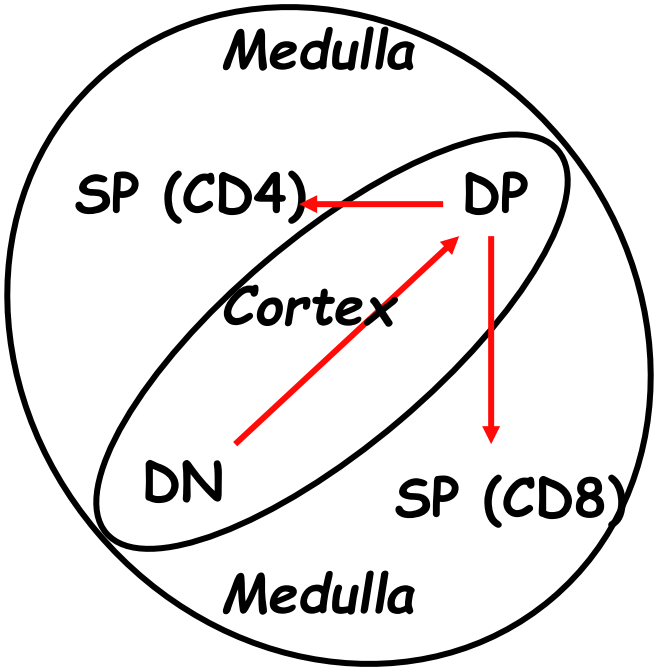


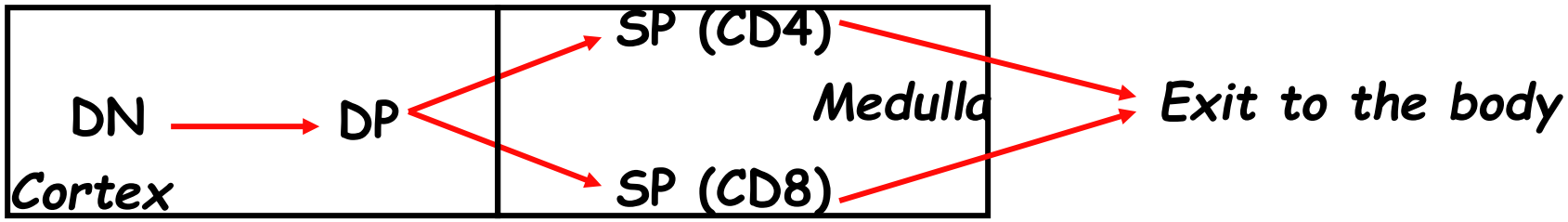
Intermediate cells
(CD4+CD8+)
- called "double positive" (
because they express
both CD4 and CD8 cells
[these are the most
numerous cells in the thym

Most immature cells
(CD4-CD8-)

ed "double negative" (DN) because they express neither CD4 r


10000
1000
100
10
1
12
82.3
3.4
2.34
1
10
100
1000
10000
CD4
CD8


Medulla
SP (CD4)
DP
Cortex
DN
SP (CD8)
Medulla


DN
DP
Cortex
SP (CD4)
Medulla
SP (CD8)
Exit to the body

T cell development in the thymus

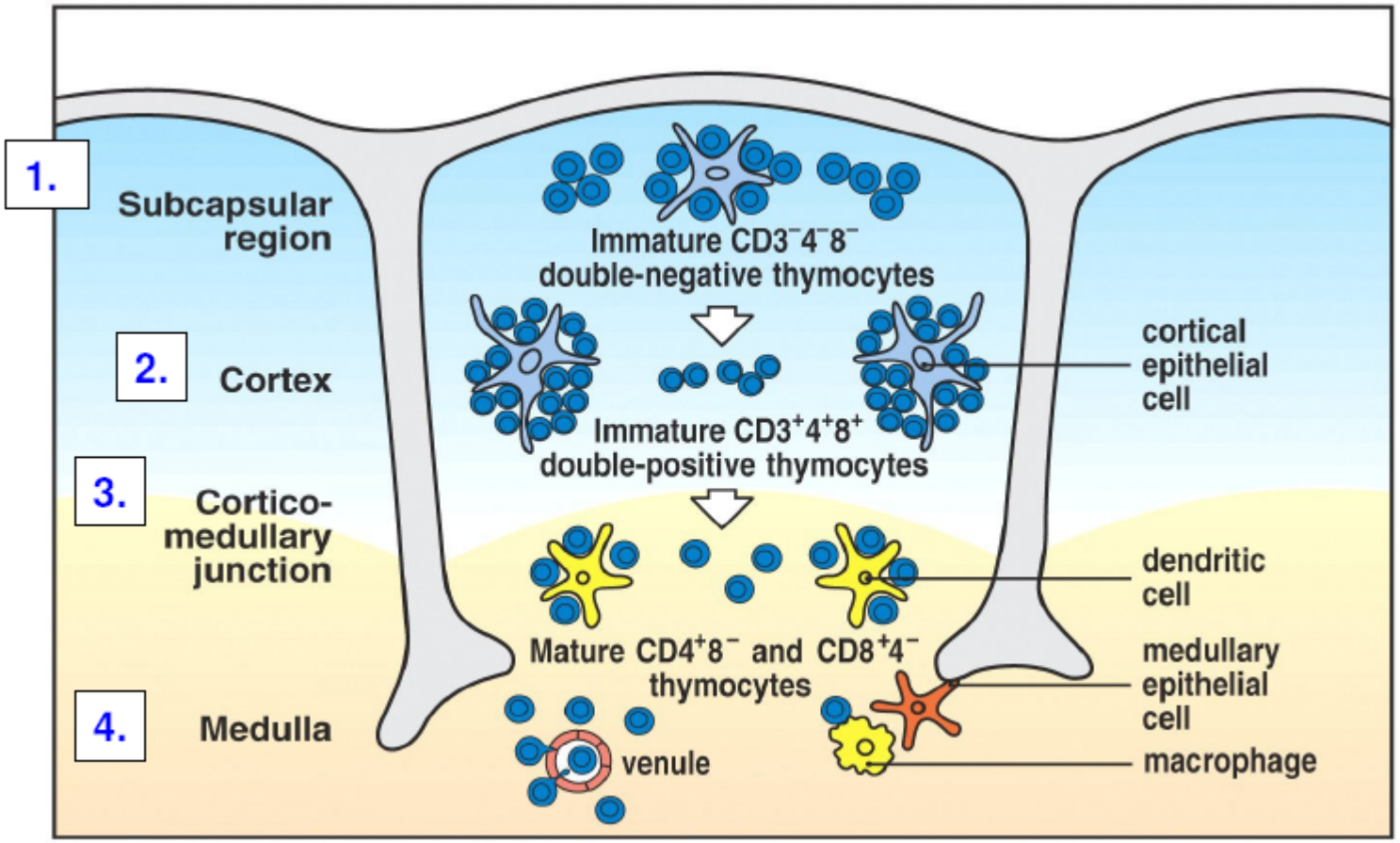
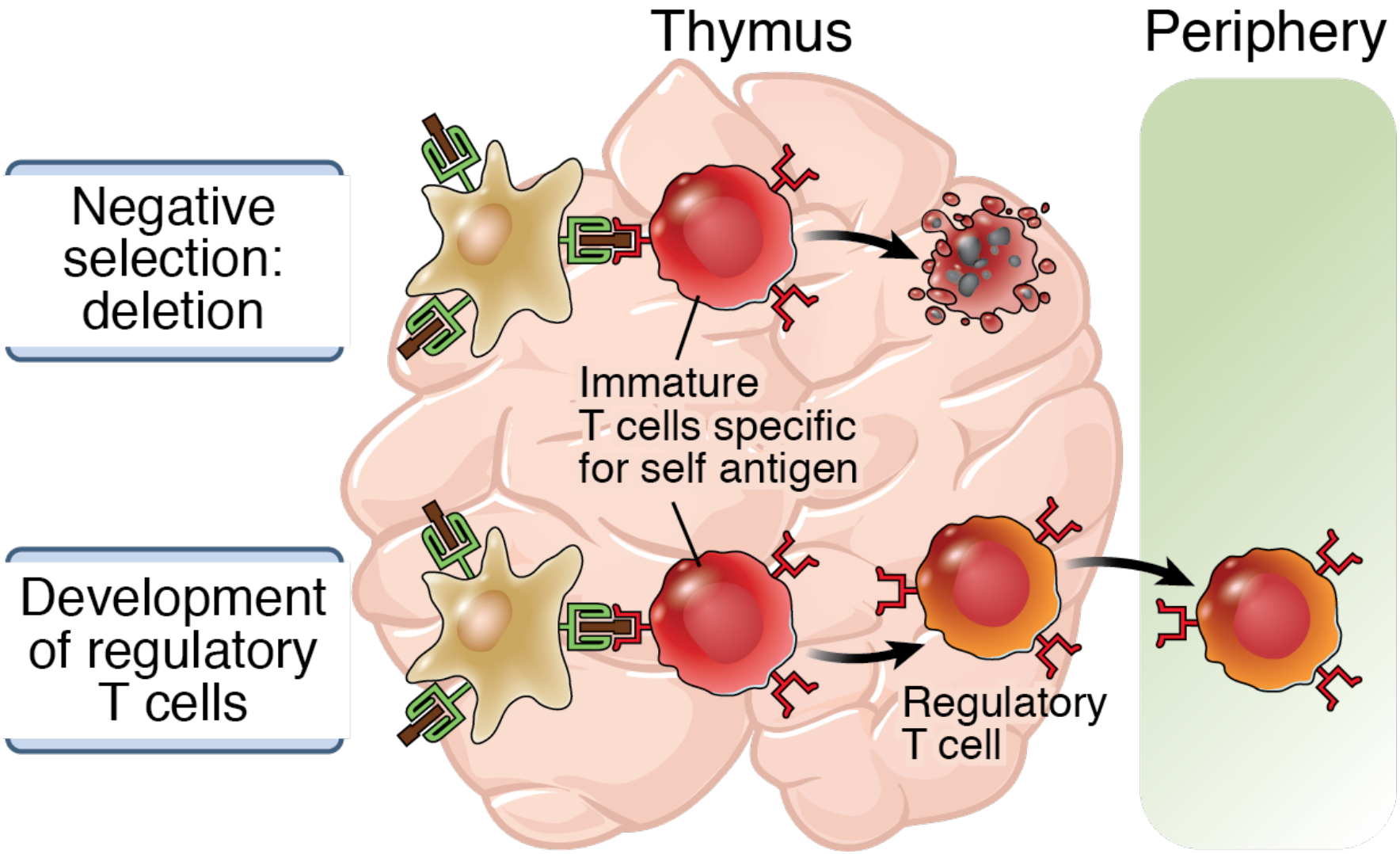


Figure 7-14 Immunobiology, 6/e. (© Garland Science 2005)

Consequences of self antigen recognition in thymus



What self antigens are seen in the thymus?

- Ubiquitous cell-associated and circulating proteins

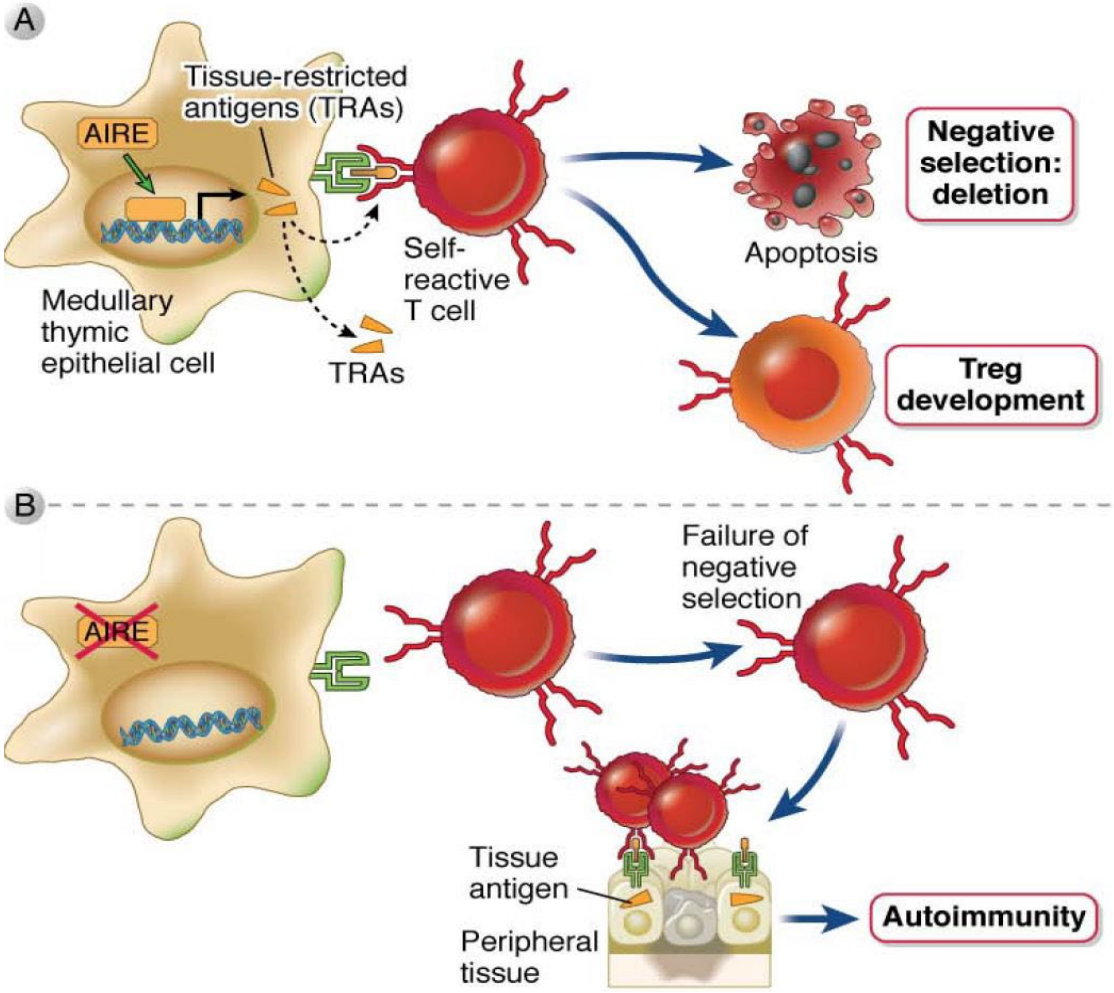
- The thymus has a special mechanism for displaying peripheral tissue antigens in thymic medullary epithelial cells, where they signal self-reactive thymocytes for death

Consequences of AIRE mutation

- Human disease: autoimmune polyendocrinopathy with candidiasis and ectodermal dysplasia (APECED), also called autoimmune polyendocrine syndrome (APS-1)
  - Associated gene identified by positional cloning, named *AIRE* ("autoimmune regulator")

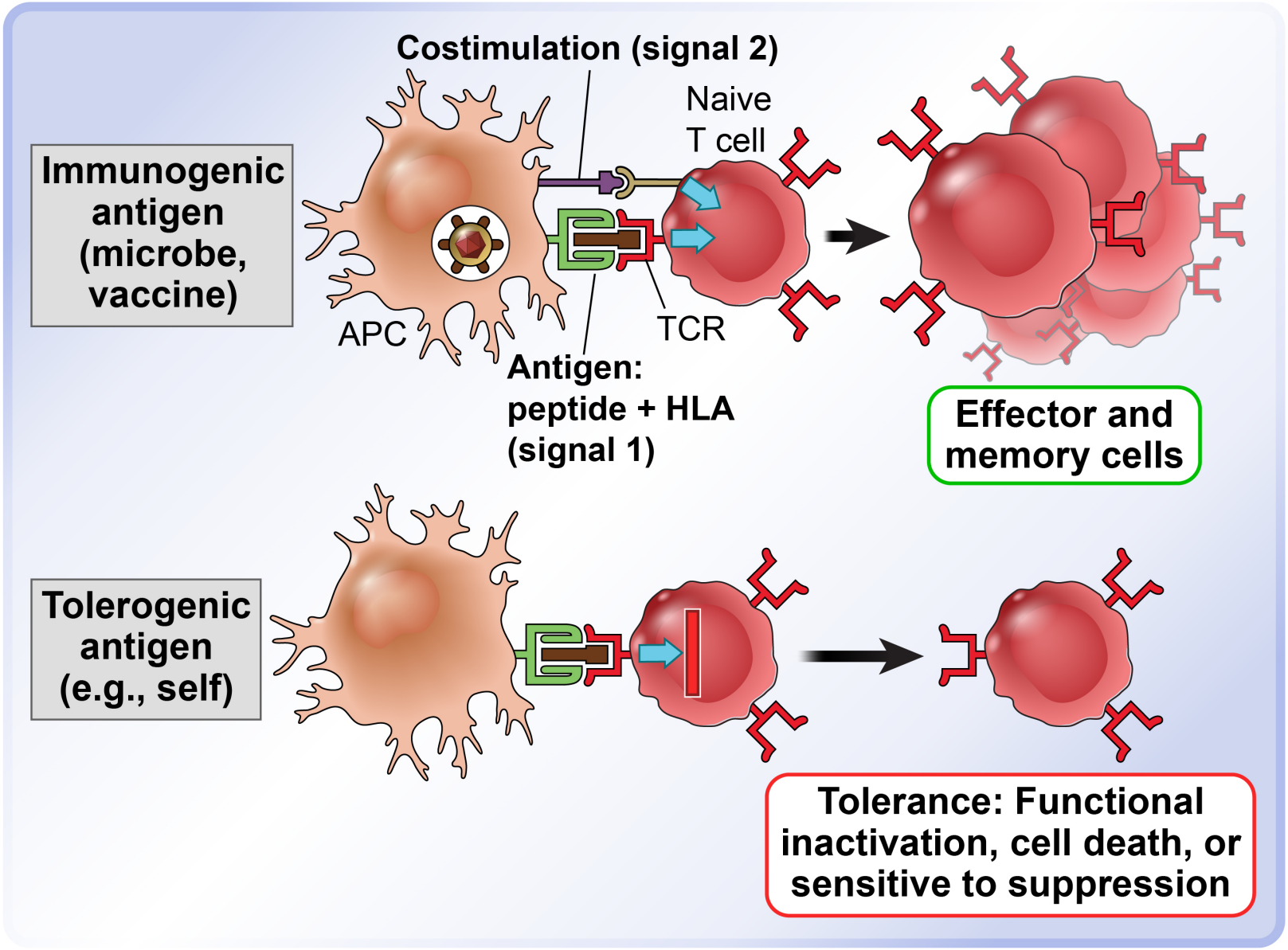
- Mouse knockout: autoantibodies against multiple endocrine organs, retina
  - Failure to express many self antigens in the thymus --> failure of negative selection

Deletion of self-reactive T cells in the thymus: how are self antigens expressed in the thymus?

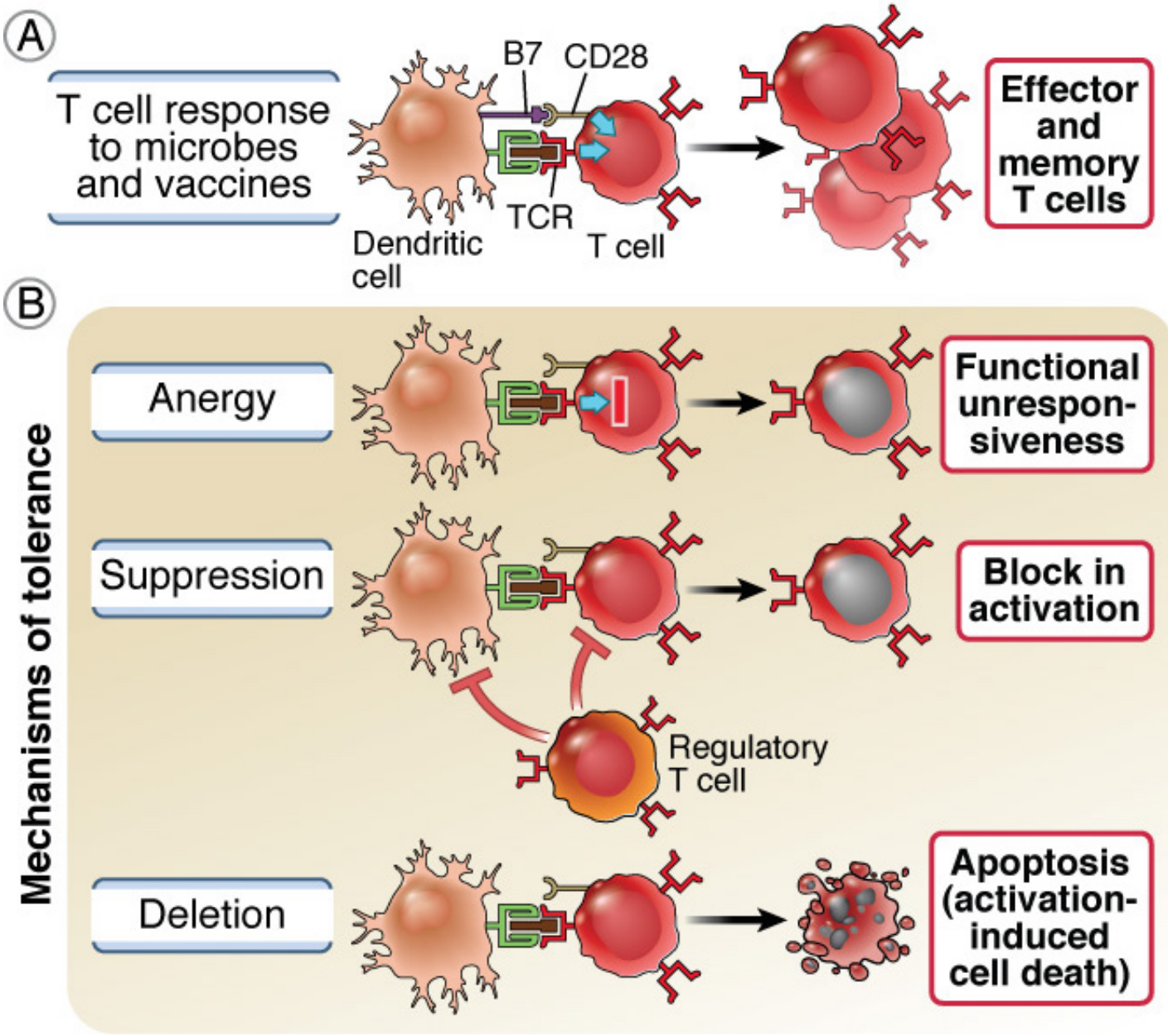


AIRE (autoimmune regulator) is a regulator of gene transcription that stimulates thymic expression of many self antigens which are largely restricted to peripheral tissues

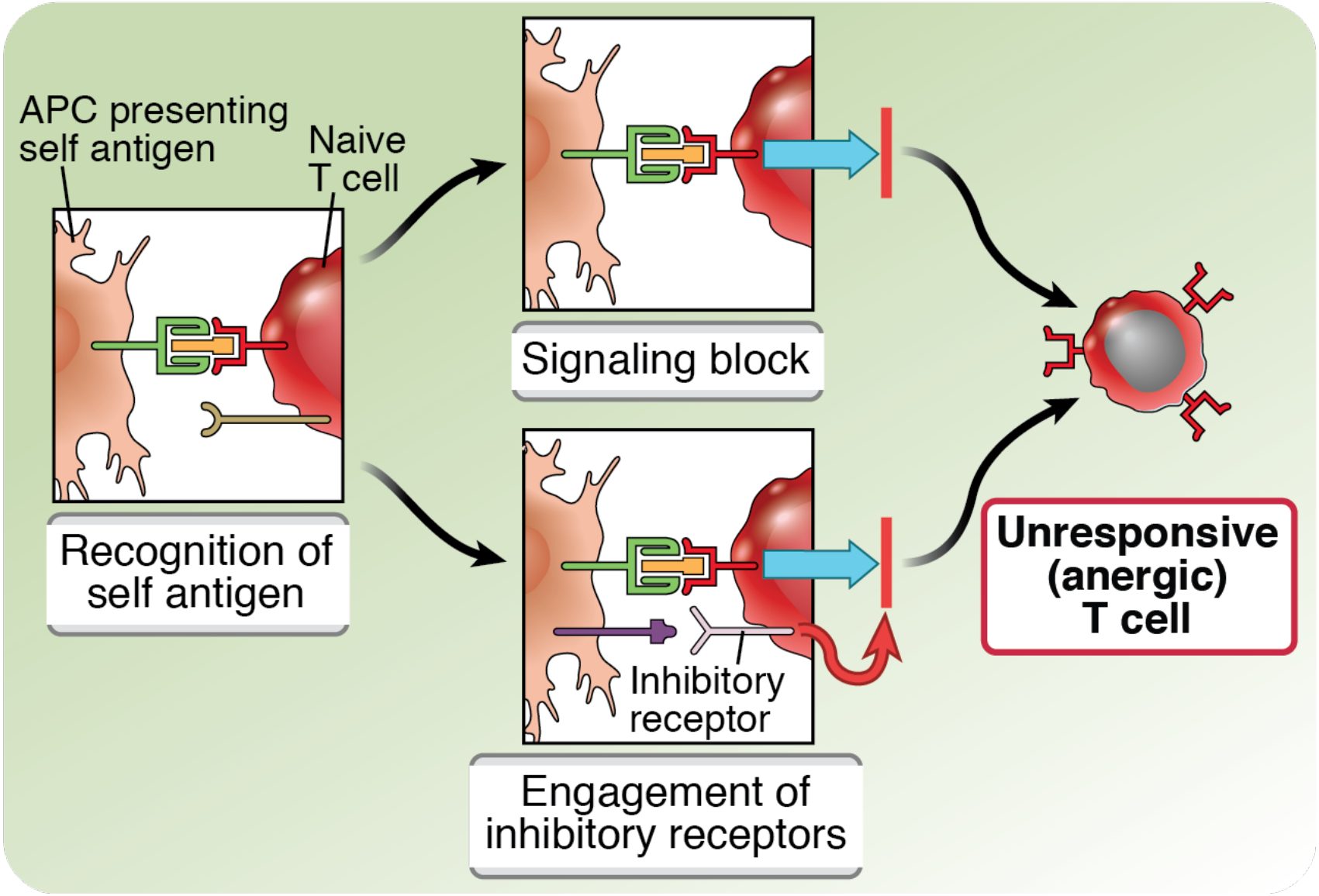
Peripheral tolerance



Peripheral T cell tolerance



T cell anergy

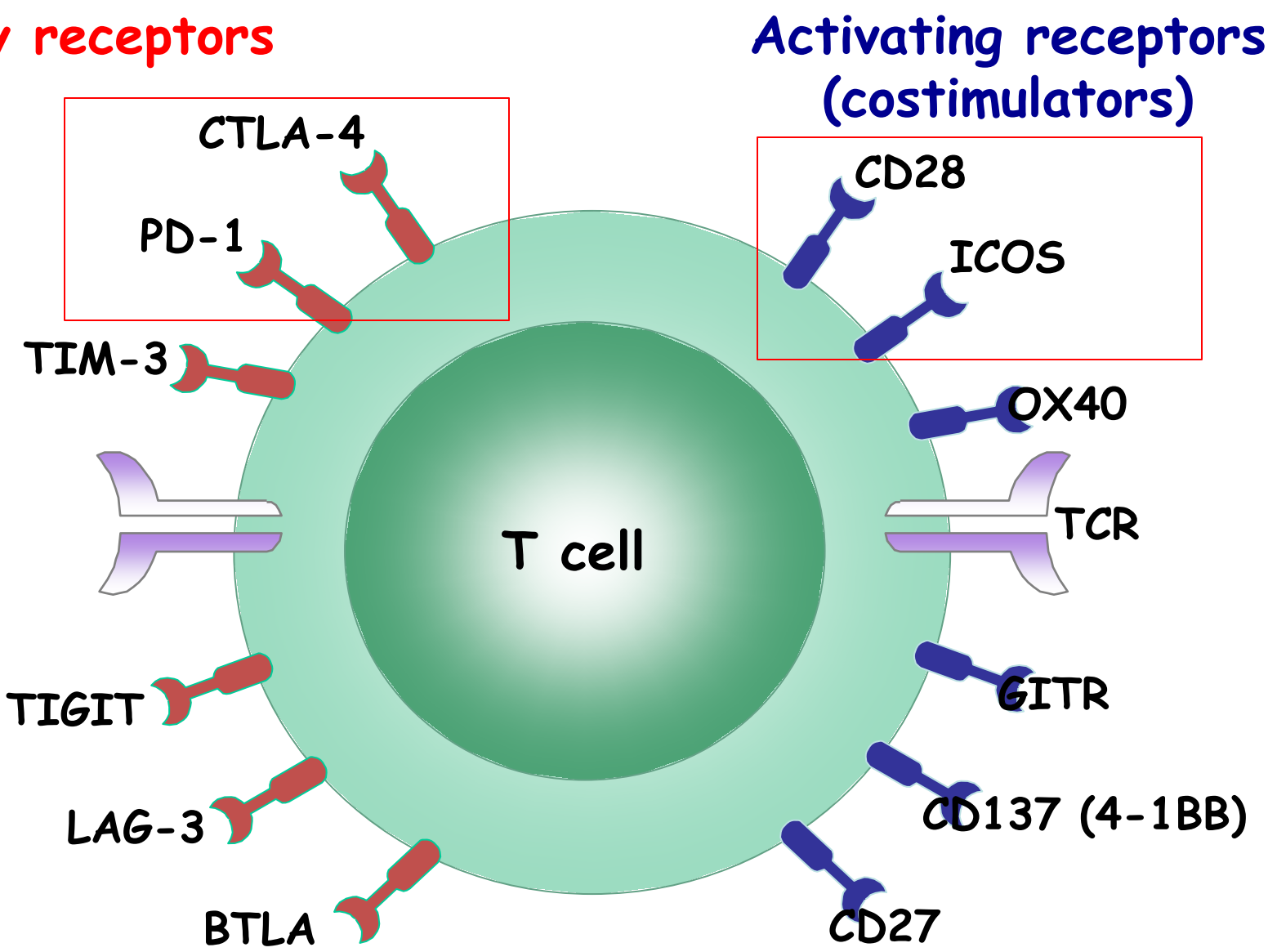


Inhibitory receptors of T cells

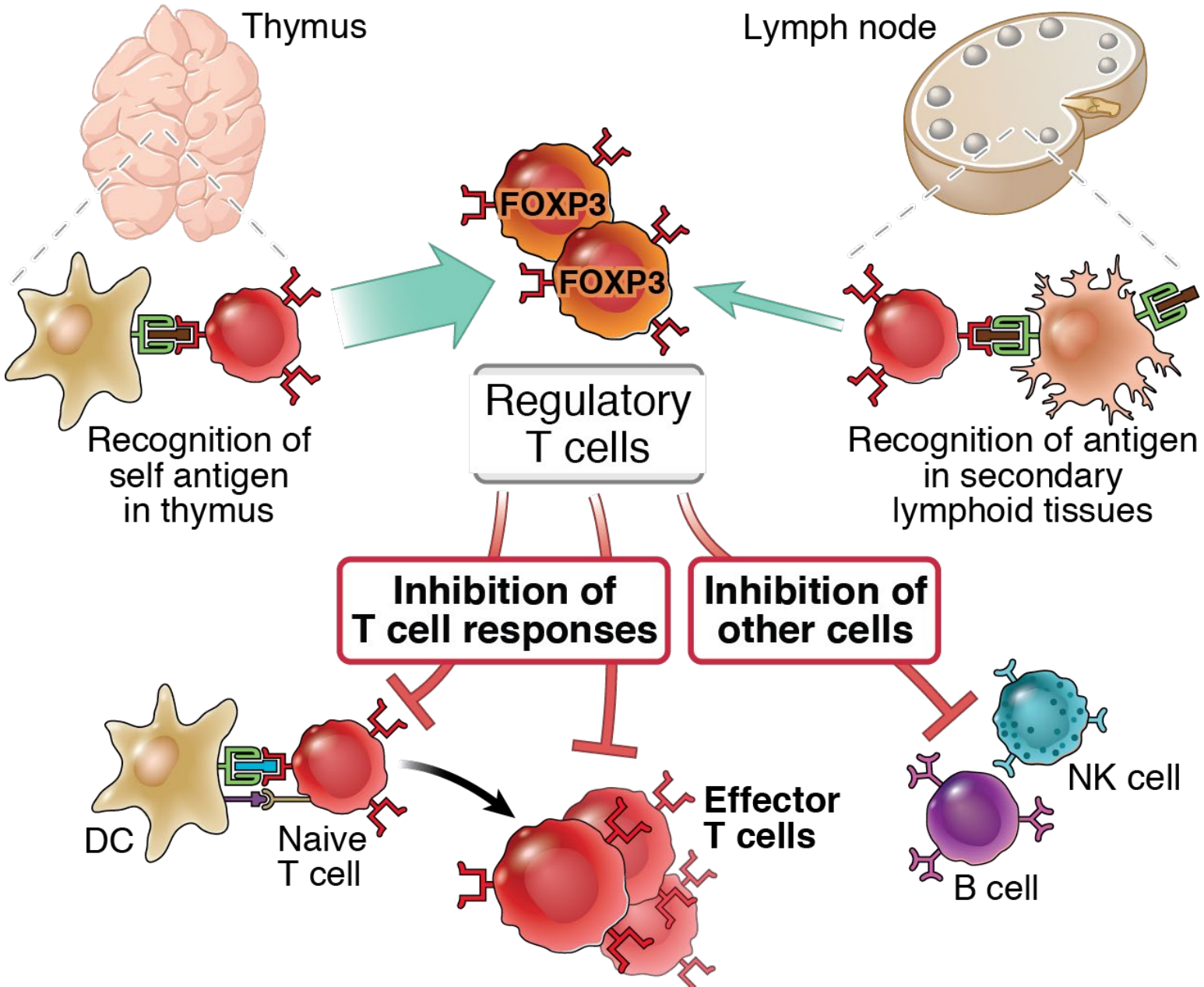
- Prevent reactions against self antigens (their physiologic function)
  - Deletion or blockade of these receptors results in autoimmune diseases

- Suppress immune responses to some tumors, chronic infections (HCV, HIV)
  - Therapeutic application: checkpoint blockade for cancer immunotherapy

T cell activating and inhibitory receptors



Regulatory T cells



Properties of regulatory T cells

- **Phenotype:** CD4+, high IL-2 receptor (CD25), Foxp3 transcription factor; other markers

- How do we define Tregs:
  - In vitro suppression assays
  - In vivo suppression assays (mice)
  - Cells that prevent autoimmunity

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
  - Treg assays are not standardized

Populations of Tregs

- Thymic, tTreg ("natural")
  - Induced by self antigen recognition during T cell maturation
- Peripheral, pTreg ("adaptive")
  - In response to antigen exposure in the periphery; contribution to preventing inflammatory disease?
- In vitro induced, iTreg (sometimes called Tr1)
  - Culture with TGFβ + IL-2; therapeutic options
- There may not be reliable markers for distinguishing these Tregs in a "bulk" population

Mechanisms of action of Foxp3+ Tregs

- CTLA-4 on Tregs removes B7 on APCs, reduces CD28 engagement and T cell activation
  - Genetic deletion of CTLA-4 in Foxp3+ cells results in severe systemic autoimmunity and lymphoproliferation
  - Commonly used assay for Treg function in humans bypasses the need for B7 and cannot measure this activity

Mechanisms of action of Foxp3+ Tregs

- CTLA-4 on Tregs removes B7 on APCs, reduces CD28 engagement and T cell activation

- **Inhibitory cytokines produced by Tregs (IL-10, others?) suppress immune responses (DCs, Macs, T cells)**
  - **IL-10 is especially important for regulating mucosal immune responses (deletion of IL10 in Foxp3+ cells results in colitis)**

Mechanisms of action of Foxp3+ Tregs

- CTLA-4 on Tregs removes B7 on APCs, reduces CD28 engagement and T cell activation

- Inhibitory cytokines produced by Tregs (IL-10, others?) suppress immune responses (DCs, Macs, T cells)

- **Consumption of IL-2**
  - **Deletion of IL-2R α or β chain in Foxp3+ cells causes autoimmunity**

- **Many others reported**

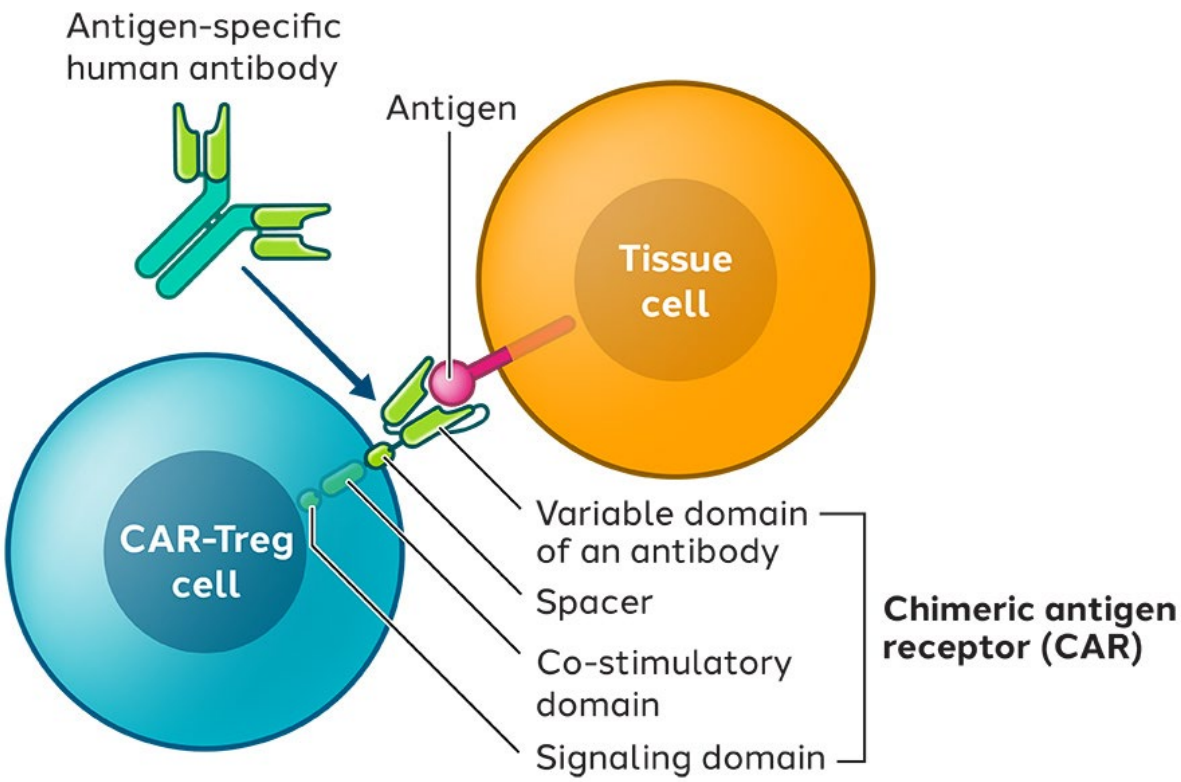
Regulatory T cells

- Explosion of information about the generation, properties, functions and significance of these cells
- Will cellular therapy with ex vivo expanded Treg become a reality?
- Therapeutic goal: induction or activation of Treg in immune diseases

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
  - Technically difficult, individualized
  - Heavy new investment: >$1 Billion invested in Treg companies in last few years

Chimeric Antigen Receptor (CAR) for Treg's

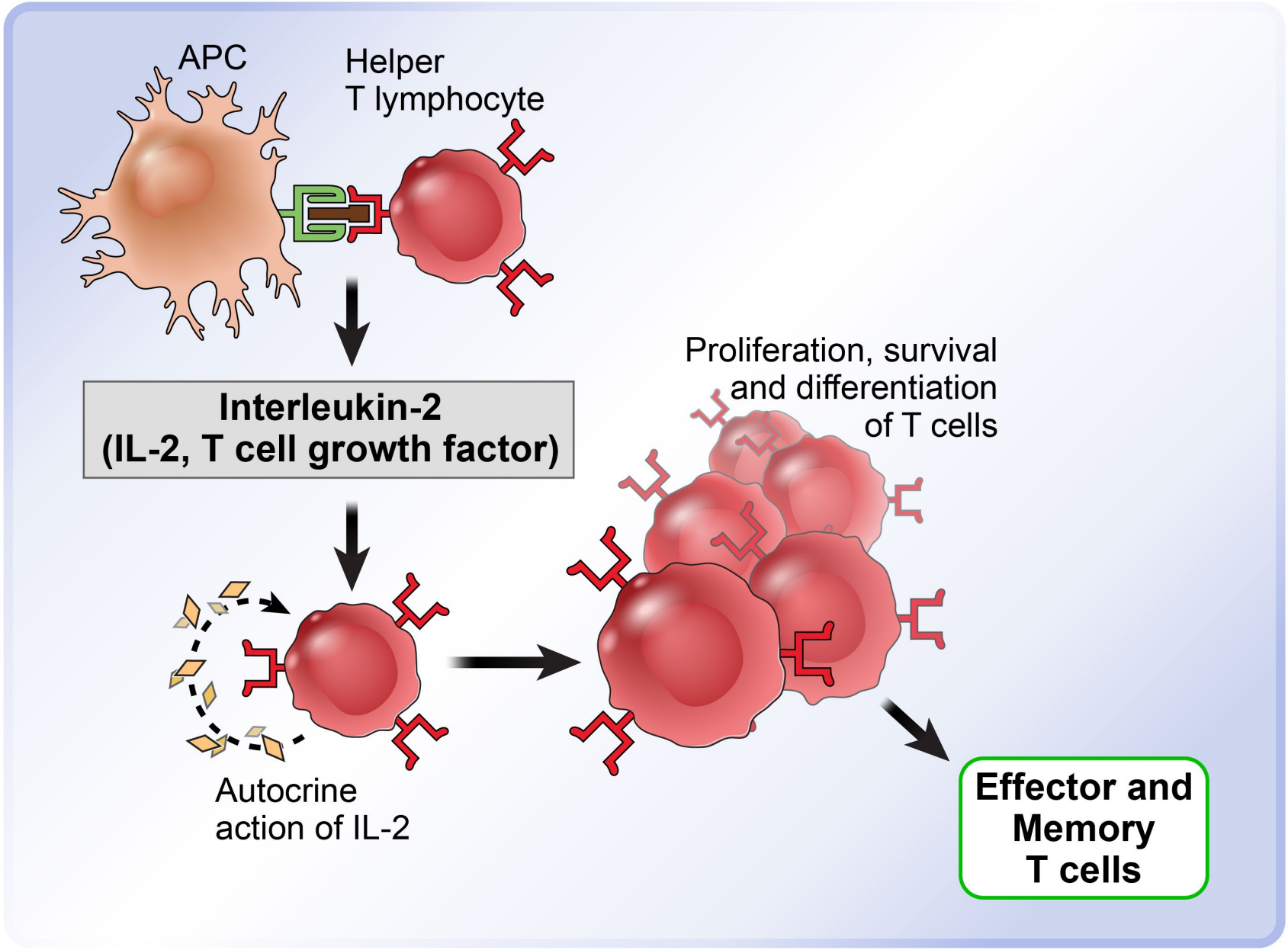


The therapeutic potential of regulatory T lymphocytes

- Cell transfer of autologous Tregs to suppress immune responses

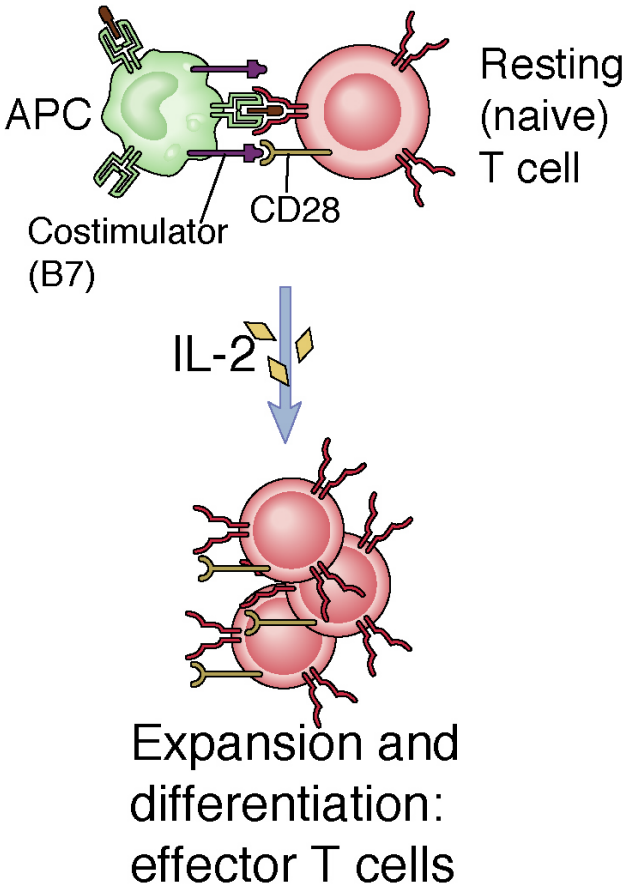
- **Administer antigen or cytokine in ways that preferentially induce Tregs?**
  - **IL-2**

Functions of Interleukin-2: the dogma

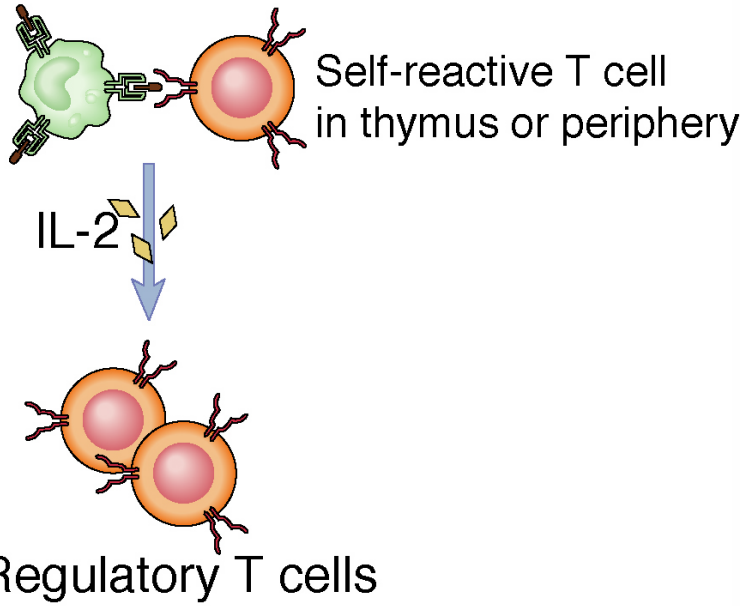


Dual roles of IL-2 in T cell responses

Induction of immune response



Control of immune response



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

Sensitivity of conventional (responding) and regulatory T cells to IL-2

- **Tregs are more sensitive to IL-2 because –**

1. Tregs express higher levels of CD25 and therefore the high-affinity IL-2 receptor
2. Tregs have a larger intracellular pool of IL-2R chains and can replenish the receptor following ligand-induced internalization
3. Tregs maintain higher levels of the IL-2-induced transcription factor p-STAT5 and for longer periods

The role of IL-2 in the lives of regulatory T cells

- IL-2 is a survival factor for maintaining Tregs in the periphery
  - Source of IL-2 is T cells that respond to antigens (microbes, other environmental antigens?)

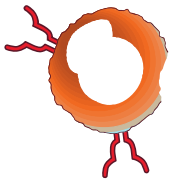
- IL-2 is also required for the functional competence of Tregs
  - Promotes FoxP3+ CTLA-4+ T cells

IL-2 dependent activation of regulatory T cells suppresses effector responses

Antigen recognition

IL-2 secretion by Teffs

Activation of Tregs



antigen

IL-2

Tregs do not make their own IL-2

Therapeutic potential of IL-2: a revision

- IL-2 was originally used to boost immune responses in cancer, HIV infection (enhancing effector and memory T cells)
  - IL-2 treatment can increase number and functional activity if Tregs
- Use of IL-2 to boost Tregs: design IL-2 to bind to high-affinity CD25
  - Low-dose IL-2
  - Mutant IL-2 that binds preferentially to CD25

Role of dendritic cells in self-tolerance

- **Mature DCs** (activated during innate immune responses to PAMPs and DAMPs) express costimulators, secrete cytokines, and initiate immune responses

- **Immature ("resting") DCs** live in tissues, display self antigens and maintain tolerance (by inducing anergy, deletion, and/or Tregs)

The potential of "tolerogenic antigen-presenting cells"

- Exploiting antigen-pulsed DCs to induce tolerance
  - Phase 1 trial of DCs pulsed with citrullinated peptides in RA published in 2015
  - Maintaining DCs in tolerogenic state?

- Can DCs be modified to make them tolerogenic?
  - Expression of costimulator antagonists, immunosuppressive cytokines, other inhibitors: being tried in animal models of graft rejection

Strategies for inducing tolerance

- Administration of antigen in tolerogenic form
  - Repeated doses of peptides without adjuvants
  - Only approach for antigen-specific tolerance
  - Understanding mechanism requires assays for antigen-specific lymphocytes
- Triggering inhibitory receptors
  - Risky, difficult to make antibodies that activate and not block receptors
- Treg targeted therapies:
  - Treg cell transfer
  - IL-2

Regulating immune responses: where are we?

- Elucidating the mechanisms of immune regulation is one of the dominant themes of modern Immunology; obvious relevance to immune-mediated inflammatory diseases, therapeutics, vaccines

- Already leading to new therapeutic strategies

- Continuing challenge is to determine which mechanisms of tolerance fail in different autoimmune diseases