T cell tolerance; Mechanisms and applications

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Lecture outline

- Self-tolerance; central and peripheral tolerance
- Thymic deletion
- Regulatory T cells
- Strategies for inducing tolerance
- Why tolerance fails: autoimmunity

The problem of self-nonself discrimination

- The immune system responds to many foreign (microbial) antigens but not to self antigens
- Developing lymphocytes express a large number of antigen receptors, not biased by specificity
- Therefore, all individuals produce lymphocytes with the ability to recognize self antigens
- Self antigens have access to the immune system

- Therefore, self-reactive lymphocytes must be selected against (eliminated or inactivated) to prevent autoimmunity

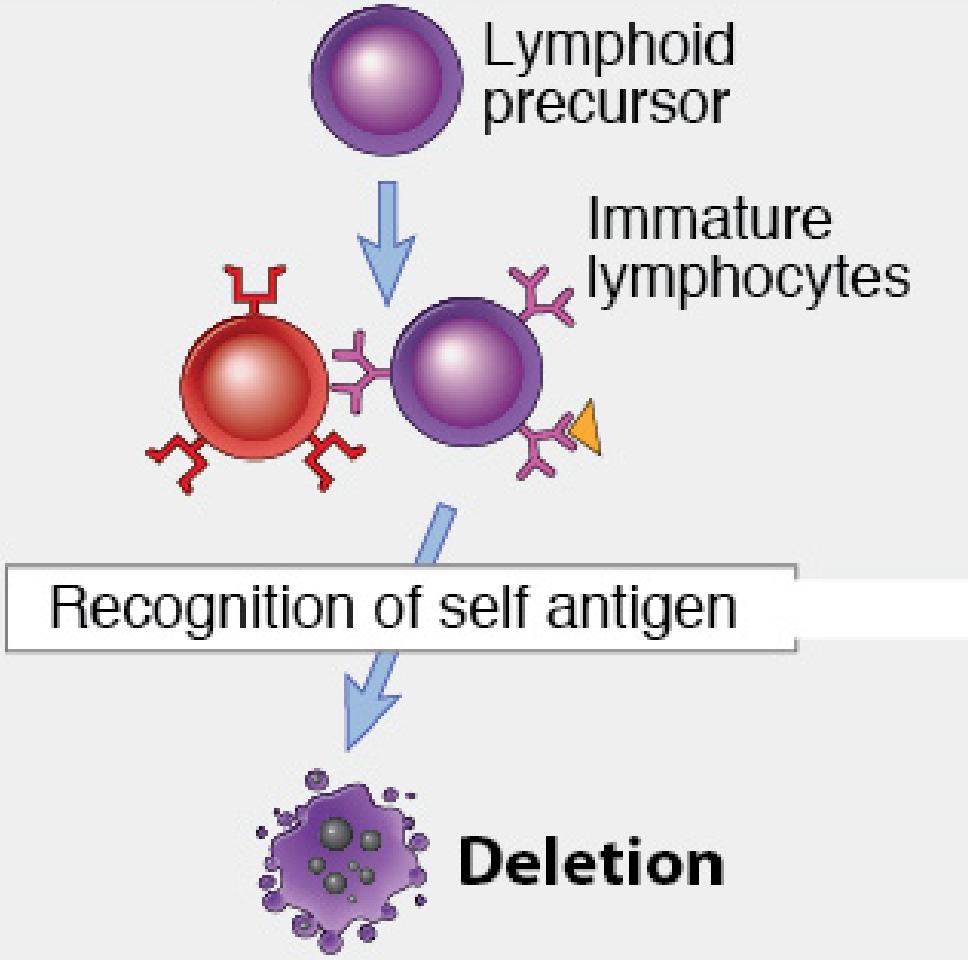
Immunological tolerance

- **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
  - **Therapeutic potential:** Inducing tolerance may be exploited to treat autoimmune and allergic diseases

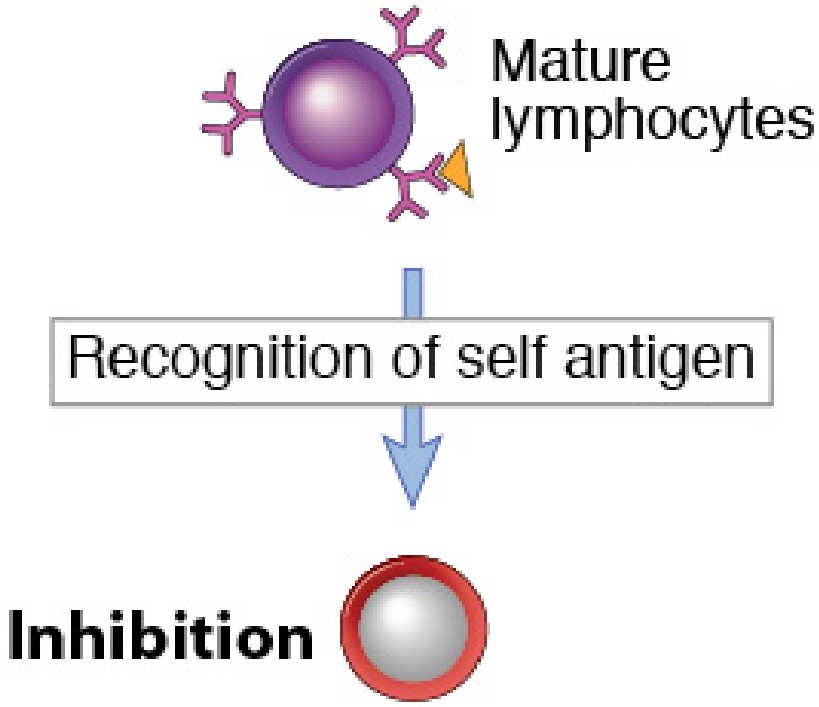
Where and when is self-tolerance induced?

During lymphocyte maturation in thymus and bone marrow



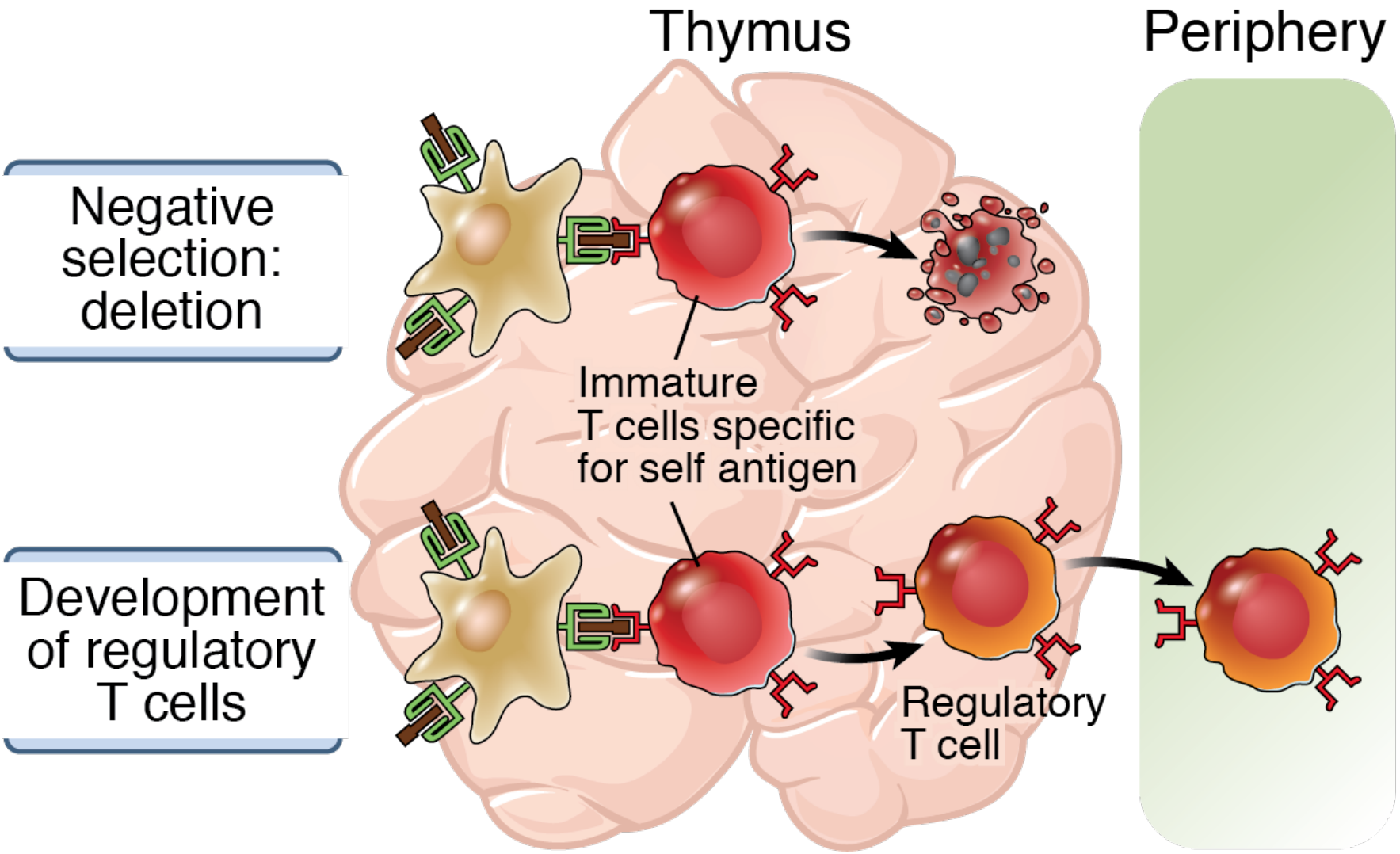
Central tolerance

After lymphocytes have matured, in peripheral tissues



Peripheral tolerance

Consequences of self antigen recognition in thymus



What self antigens are seen in the thymus?

- Ubiquitous cell-associated and circulating proteins

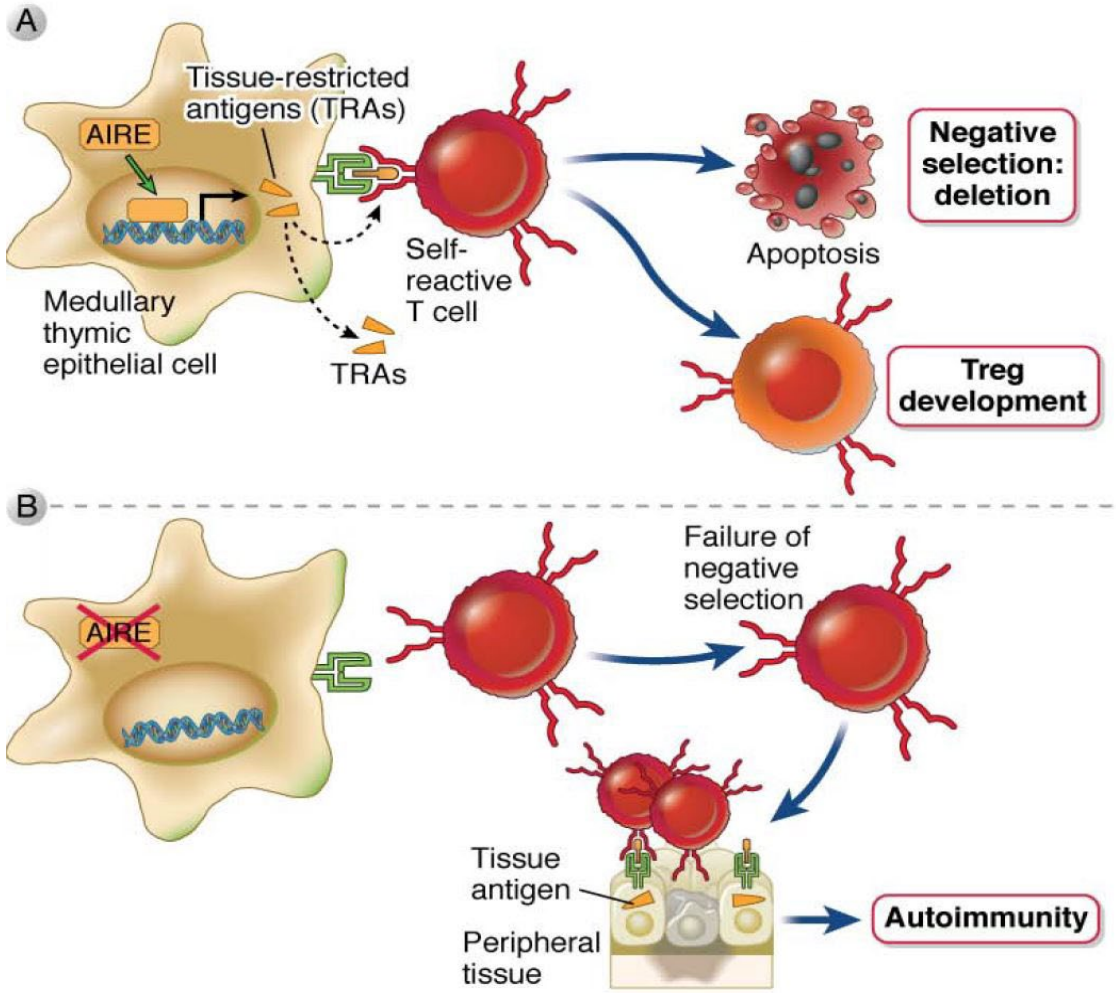
- The thymus has a special mechanism for displaying peripheral tissue antigens in thymic medullary epithelial cells, where they signal self-reactive thymocytes for death

Consequences of AIRE mutation

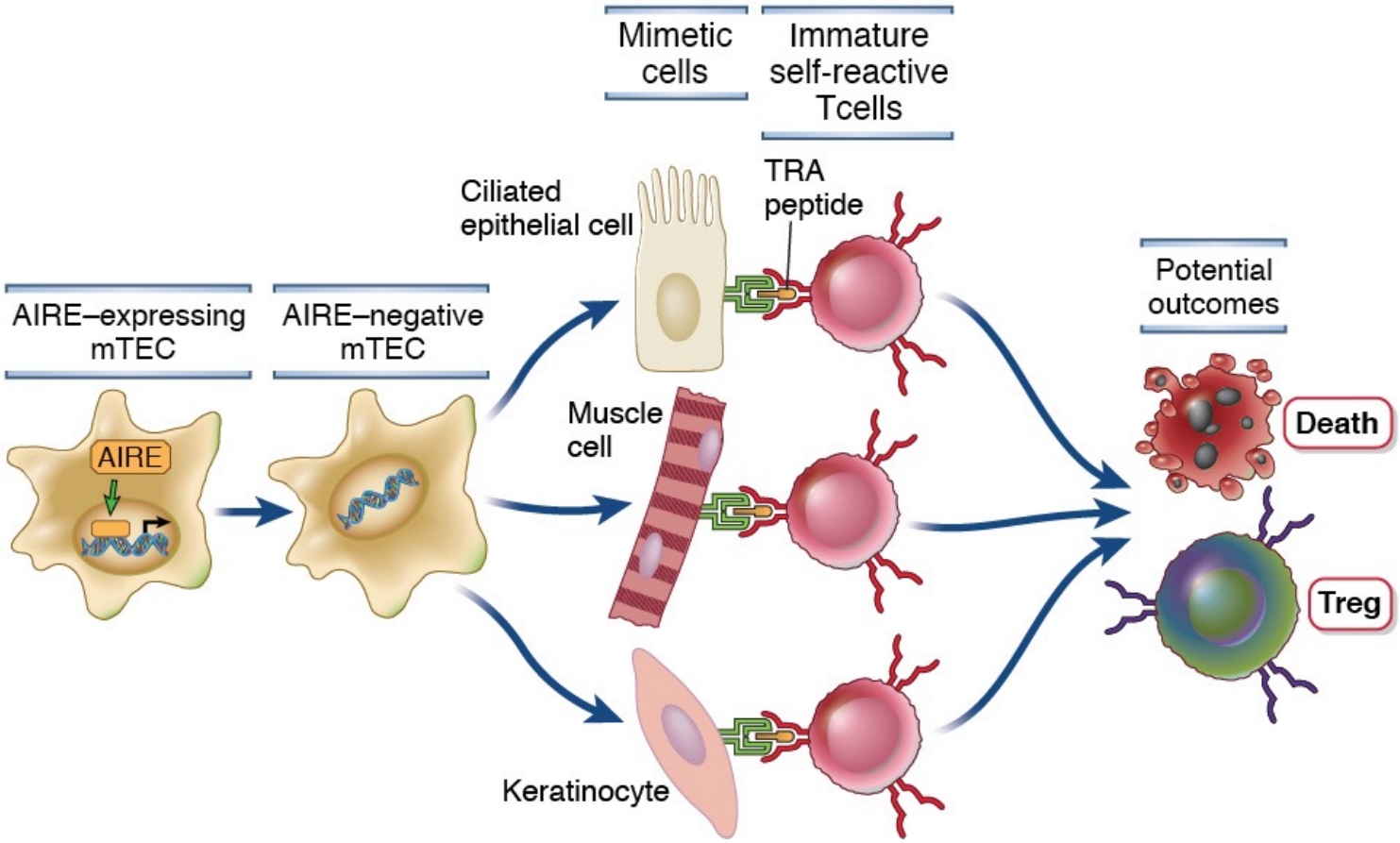
- Human disease: autoimmune polyendocrinopathy with candidiasis and ectodermal dysplasia (APECED), also called autoimmune polyglandular syndrome (APS-1)
  - Associated gene identified by positional cloning, named *AIRE* ("autoimmune regulator")

- Mouse knockout: autoantibodies against multiple endocrine organs, retina
  - Failure to express many self antigens in the thymus (revealed by transcriptome analysis of normal vs AIRE-/- thymic epithelial cells)

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



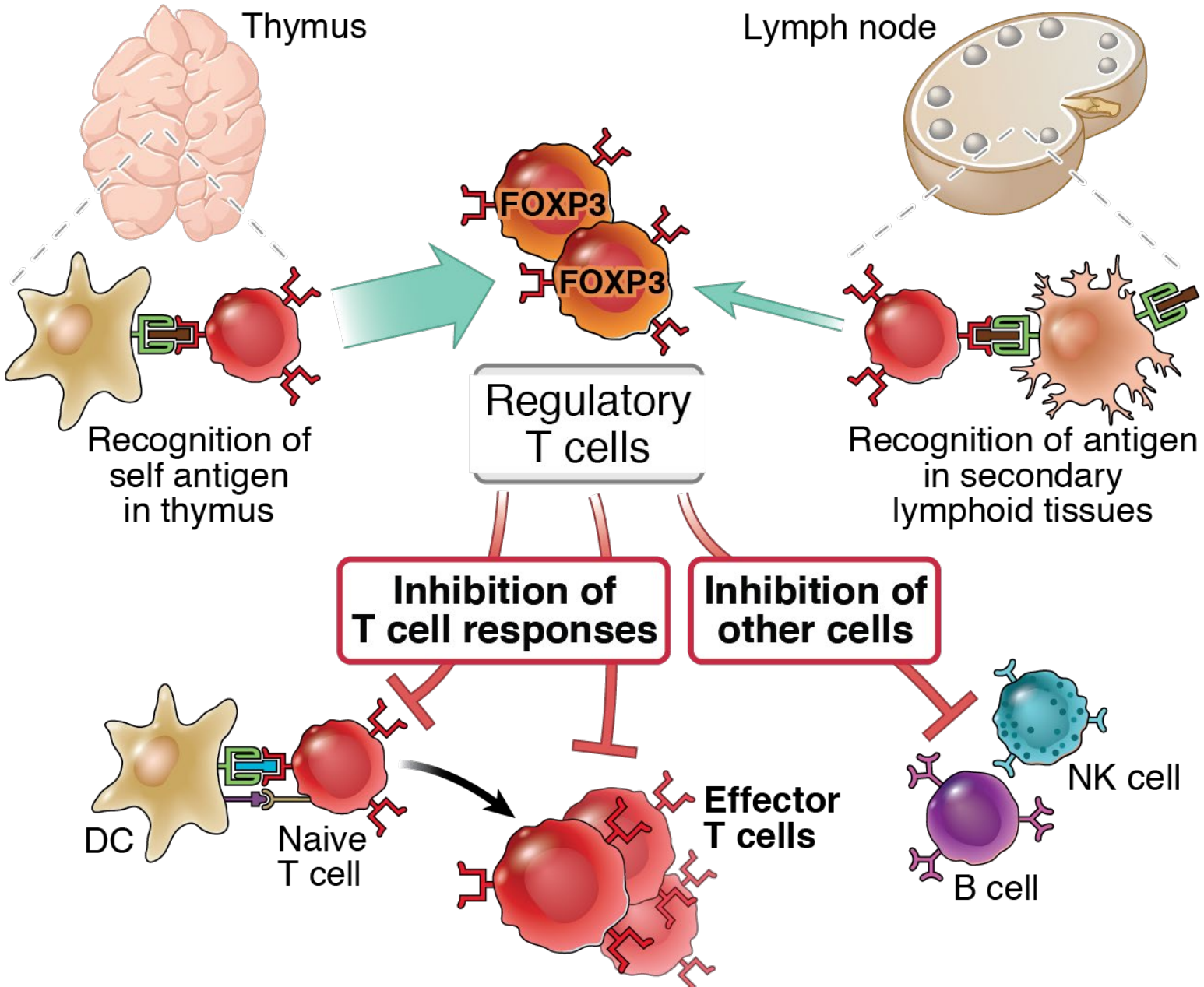
Thymus contains cells that mimic peripheral different tissue cells



Mechanisms of peripheral T cell tolerance

- Anergy: Failure of T cells to respond
  - Signaling block or engagement of inhibitory receptors
  - Lack of costimulation
- Suppression by regulatory T cells
- Deletion: death of self-reactive T cells

Regulatory T cells



Historical background

- 1970s: search for cells that controlled immune responses
-
- 1980s: explosion of publications on "suppressor T cells"
  - Failure to define cells or mode of action
- 1995: discovery of CD25+ Tregs (Sakaguchi)
  - Limitations of CD25 as the marker
- 2000s: identification of Foxp3 as the essential Treg transcription factor
  - Scurfy mice (Ramsdell), IPEX patients (Ochs, Chatila), knockout and over-expression (Sakaguchi, Rudensky)

Properties of regulatory T cells

- **Phenotype:** CD4+, Foxp3 transcription factor high IL-2 receptor (CD25), CTLA-4; other markers

- Heterogeneity
  - Multiple subsets described; functional significance unclear
  - Natural (thymic) and induced (peripheral)
  - Stages of activation

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 or cancer progression?
  - Inconsistent and variable data

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

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)**

- **Consumption of IL-2**
- **Many others reported**

"Non-immune" functions of tissue Tregs

- Tregs in adipose tissue regulate lipid metabolism
- Tregs in muscle and other tissues produce growth factors that promote repair (after trauma, infections, degenerative diseases)
- Tregs in skin stimulate cycling and differentiation of hair follicle stem cells

- Do Tregs adapt to their environment, or do distinct subsets exist that populate different tissues?

Regulatory T cells

- Explosion of information about the generation, properties, functions and significance of these cells

- Will therapeutic manipulation of Tregs become a reality?

- Therapeutic goal: induction or activation of Treg in immune diseases, deletion or suppression in cancer

The therapeutic potential of regulatory T lymphocytes

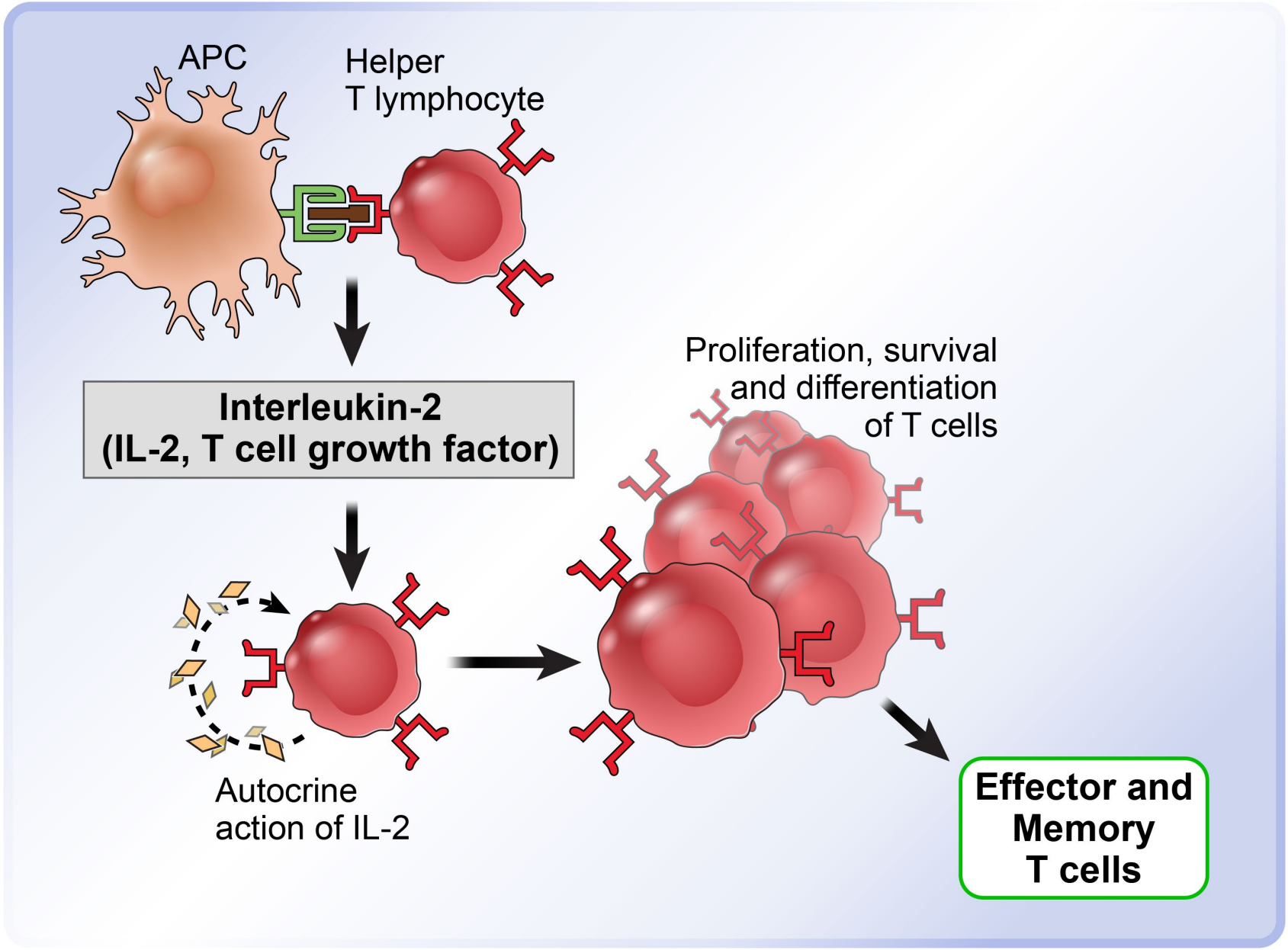
- Cell transfer of autologous Tregs to suppress immune responses
  - Grow up patient's Tregs ex vivo
  - Ongoing clinical trials in graft rejection, T1D show it is safe
  - Very little efficacy data
  - Technically difficult, individualized

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



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

- Prediction: what will be the consequence of eliminating IL-2 or the IL-2 receptor?

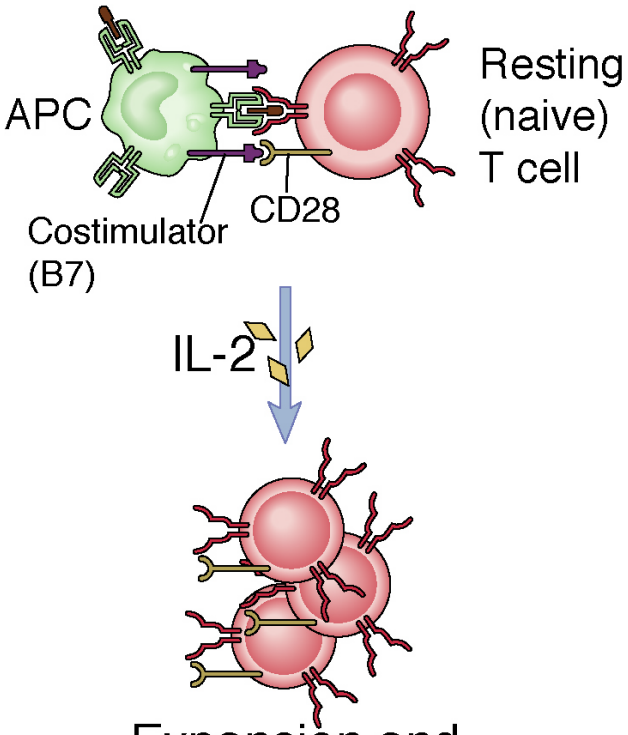
The unexpected biology of IL-2

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

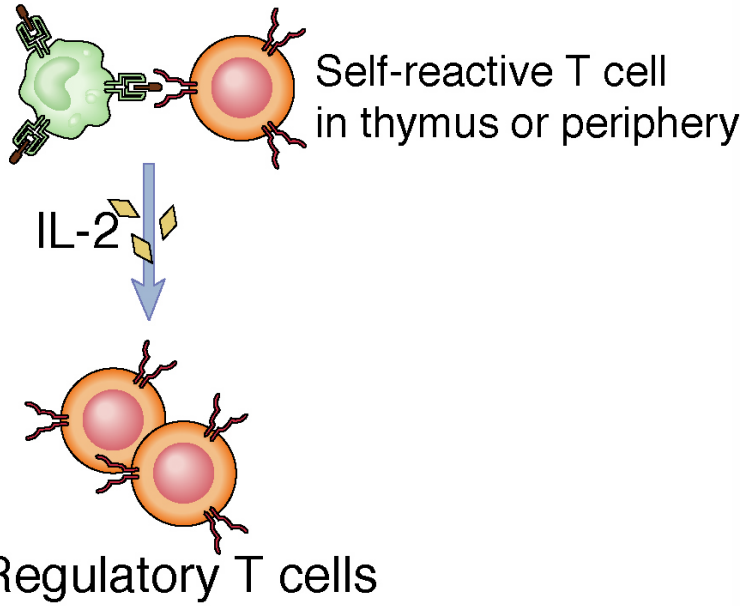
- BUT: knockout of IL-2 or the α or β chain of the IL-2R results not in immune deficiency but in systemic autoimmunity and lymphoproliferation

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

IL-2 and Tregs

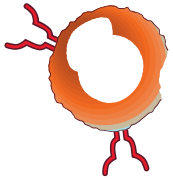
- IL-2 is a survival factor for Tregs and maintains their functional competence
- Tregs do not make IL-2; what is the source of IL-2 for activating Tregs?

IL-2 dependent activation of regulatory T cells suppresses effector responses: a classical negative feedback loop

Antigen recognition

IL-2 secretion by Teffs

Activation of Tregs



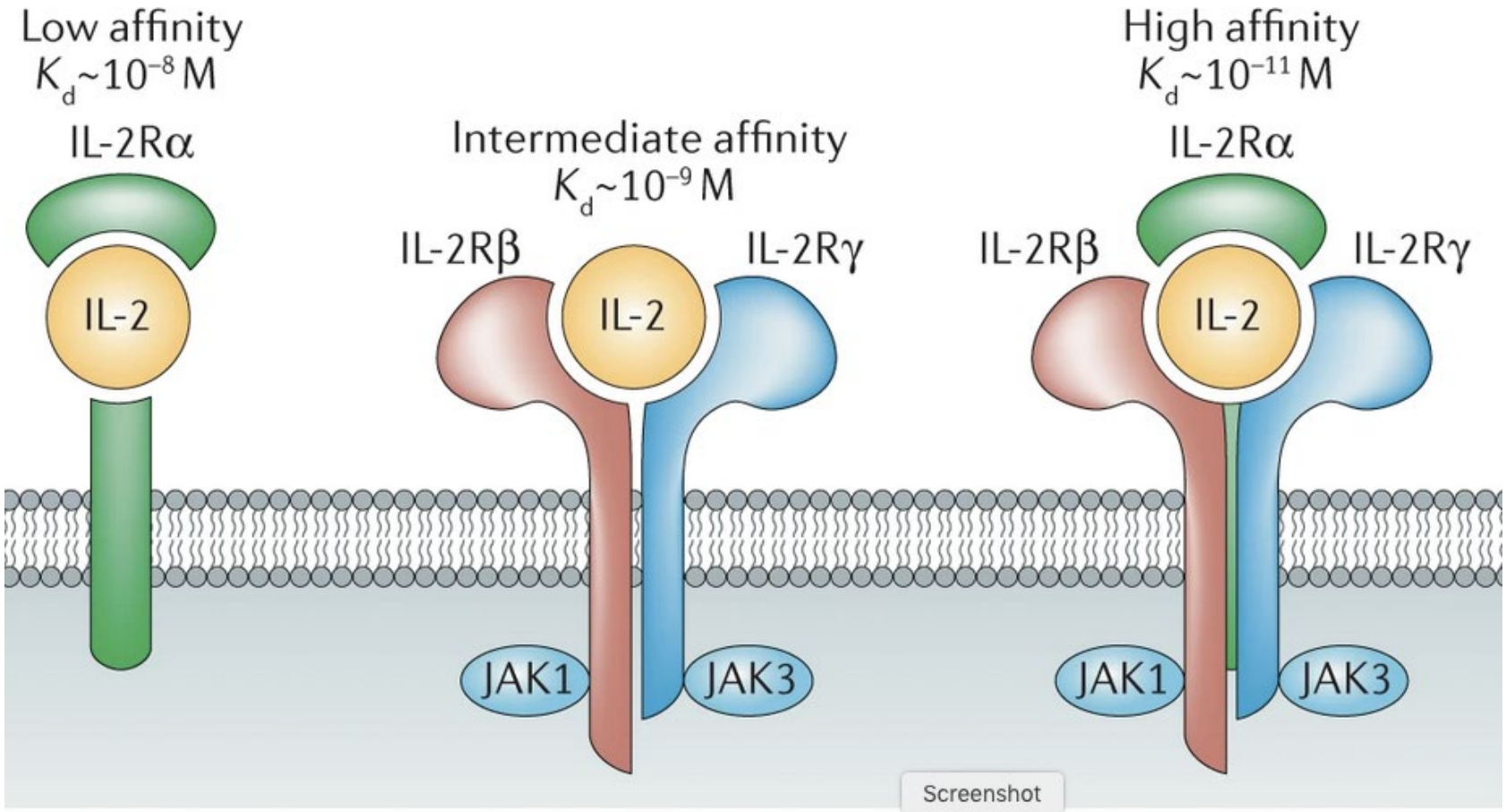
antigen

IL-2

IL-2 and Tregs

- Tregs are much more sensitive to IL-2 than conventional (responder or effector) T cells

IL-2 receptor determines dual role of IL-2 in T cell responses



Effector T cells
Memory T cells
NK cells

Recently activated T cells (transient)
Tregs (always)

From Spolski et al 2018

Therapeutic potential of IL-2

- Designing IL-2 to stimulate or inhibit immune responses

- IL-2 to stimulate effector T cells for cancer immunotherapy
  - Muteins that bind preferentially to β chain of IL-2R ("no α IL-2")
  - IL-2 targeted to CD8 or antigen-specific T cells

- IL-2 to boost Tregs for autoimmune diseases
  - Low-dose IL-2
  - Muteins that binds preferentially to CD25

Regulating immune responses

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

- Autoimmunity is caused by a failure of tolerance

- Can tolerance be induced to prevent autoimmune and allergic diseases and to permit transplants, gene and cell therapy?

Autoimmunity

- Definition: immune response against self (auto-) antigen

- General principles:
  - Significant health burden, 5% of population
  - Multiple factors contribute to autoimmunity, including genetic predisposition, infections
  - Fundamental problem is the failure of self-tolerance

- Problems:
  - Failure to identify target antigens, heterogeneous disease manifestations, disease usually presents long after initiation

Examples of autoimmune disease

Graves' Disease (thyrotoxicosis)

Hashimoto's thyroiditis

pernicious anaemia

Addison's disease

insulin dependent diabetes mellitus

Goodpasture's syndrome

myasthenia gravis

multiple sclerosis(?)

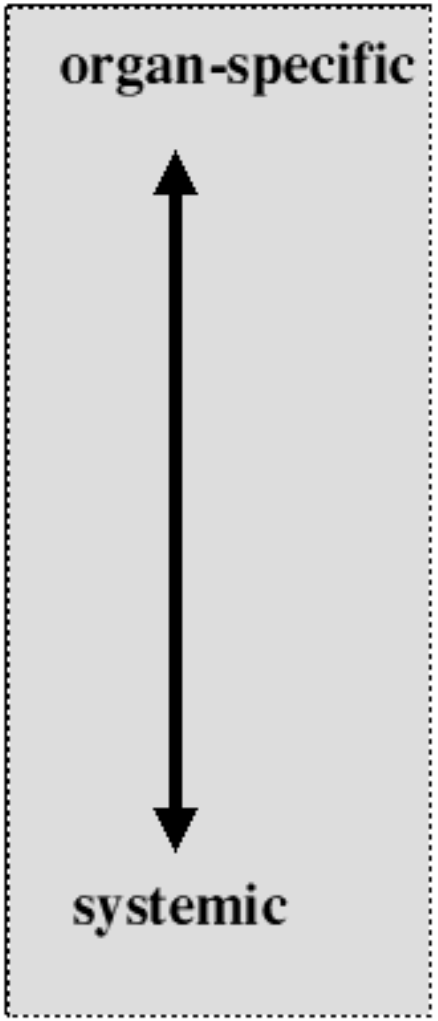
autoimmune haemolytic anaemia

idiopathic thrombocytopenic purpura

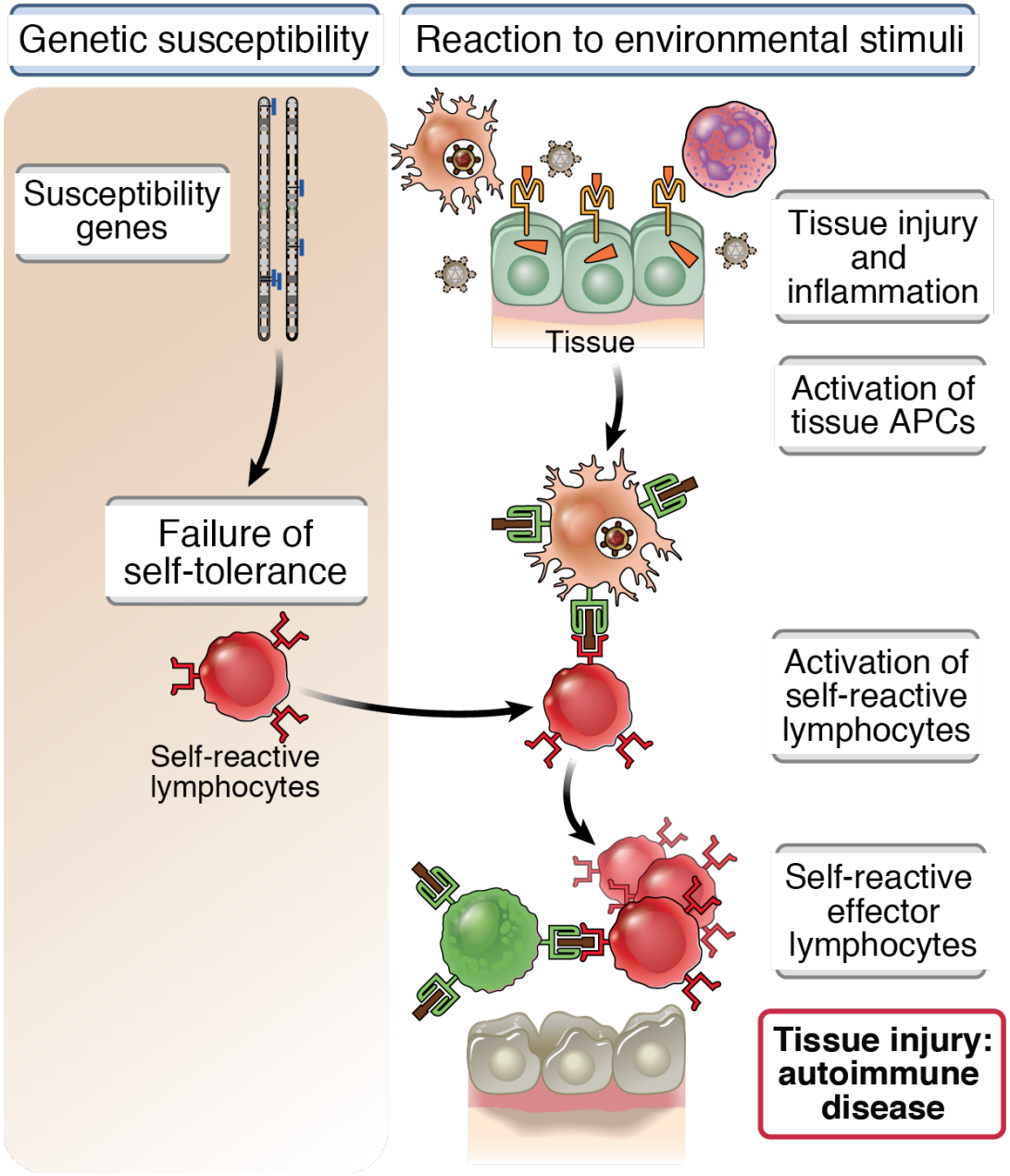
rheumatoid arthritis

scleroderma

systemic lupus erythematosis (SLE)



Pathogenesis of autoimmunity



Because Autommunity is so complex, how can we figure out how it happens?

Answer:

1) Use genetics
2) Animal models

Genetic basis of autoimmunity

- **Genetic predisposition of autoimmune diseases**
  - Increased incidence in twins
  - Identification of disease-associated genes by breeding and genomic approaches
- **Multiple genes are associated with autoimmunity**
  - No single mutation causes autoimmunity
- **MHC genes**
  - Major genetic association with autoimmune diseases (relative risk)
  - Disease-associated alleles may be found in normal individuals
- **Non-MHC genes**
  - Many loci identified by genomic methods, animal studies
  - Mutations in complement genes predispose to lupus

HLA (or MHC) is the strongest genetic factor for susceptibility to autoimmune disease

HLA-associated risk factors for autoimmune disease

Disease	HLA allotype	Frequency (%)		Relative risk
		Patients	Control	
Ankylosing spondylitis	B27	> 95	9	> 150
Narcolepsy	DQ6	> 95	33	> 40
Celiac disease	DQ2 and DQ8	95	28	30
IDDM	DQ8 and DQ2	81	23	14
Subacute thyroiditis	B35	70	14	14
Multiple sclerosis	DQ6	86	33	12
Rheumatoid arthritis	DR4	81	33	9
Juvenile rheumatoid arthritis	DR8	38	7	8
Psoriasis vulgaris	Cw6	87	33	7
Addison's disease	DR3	69	27	5
Graves' disease	DR3	65	27	4
Myasthenia gravis	DR3	50	27	2
IDDM	DQ6	< 0.1	33	0.02

Figure 11-23 The Immune System, 2/e (© Garland Science 2005)

How does MHC predispose?

Animal models of autoimmunity

- NOD mouse- model of type 1 diabetes
- NZBxNZW mouse-model of Lupus
- KBxN mouse-model of rheumatoid arthritis
- EAE- induced model of multiple sclerosis whereby disease is induced by injecting proteins of the myelin sheath with adjuvant
- Knockouts that get autoimmunity

Recent work in this model suggests Th17 cells are important!

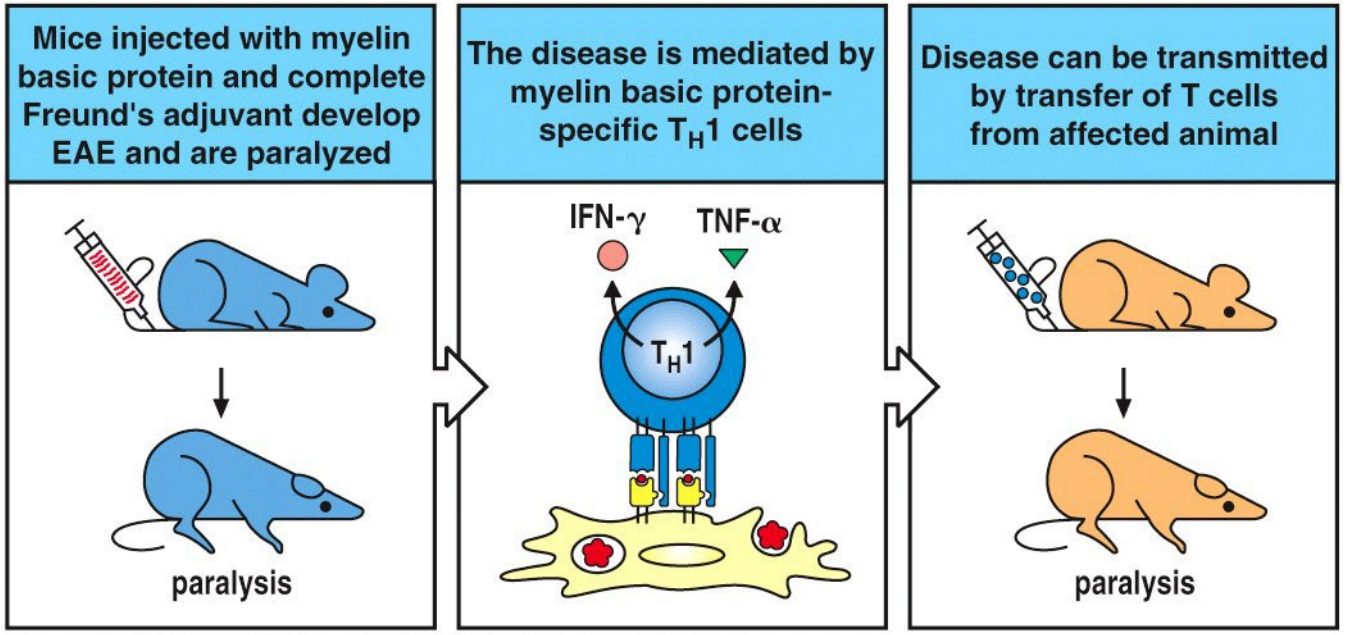
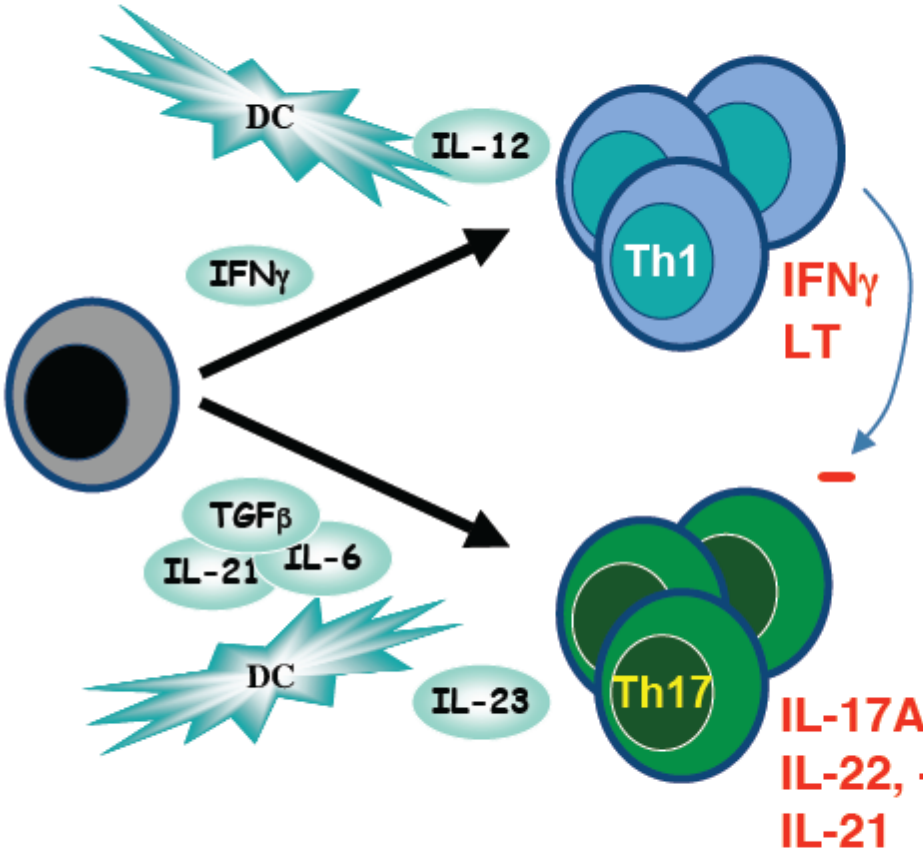


Figure 13-3 Immunobiology, 6/e. (© Garland Science 2005)

Th17 cells mediate neutrophil inflammation



Type 1

Systemic Immunity

- Phagocyte activation
- Opsonizing Ab
- CTL, NK cells

Antigen Elimination

Type 17

Inflammatory Immunity

- Neutrophils
- Monocytes

Acute Inflammation

Therapeutic tolerance induction

- Treat autoimmunity and allergy
  - Autoimmunity results from a failure of tolerance
- Antigen-specific, therefore does not disable immune responses
  - All cytokine antagonists and migration inhibitors suppress host defense against infections
- Prevent reactions to viral vectors for gene therapy, rejection of allogeneic cells (e.g., stem cells)

Strategies for inducing tolerance

- Administration of antigen in tolerogenic form
  - Aqueous peptides
  - Nanoparticles?
- Blocking costimulation
- Engaging inhibitory receptors, e.g., PD-1
- Treg targeted therapies:
  - Treg cell transfer
  - IL-2