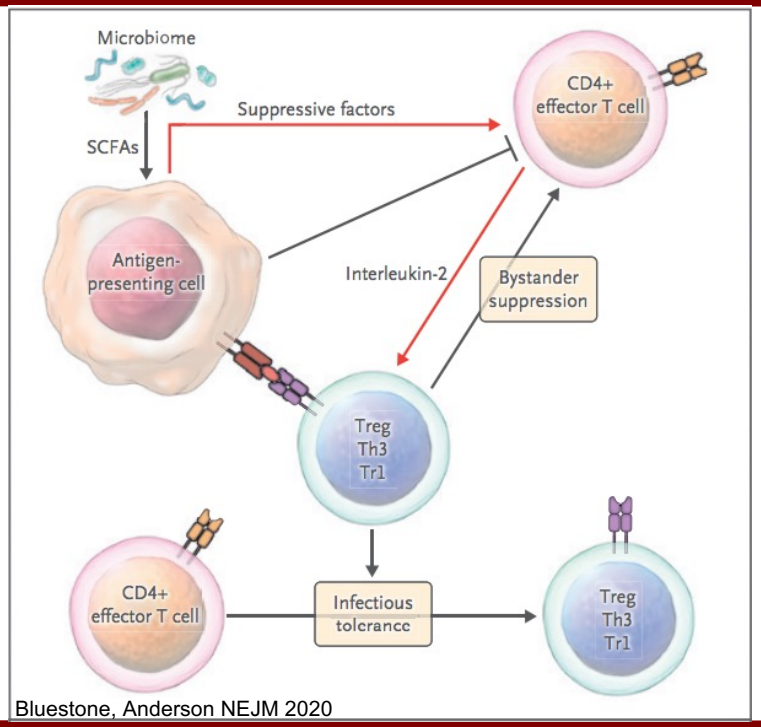

Bluestone, Anderson NEJM 2020

Immunological Tolerance and Autoimmunity

Abbas et al. Chapter 9

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Learning Objectives

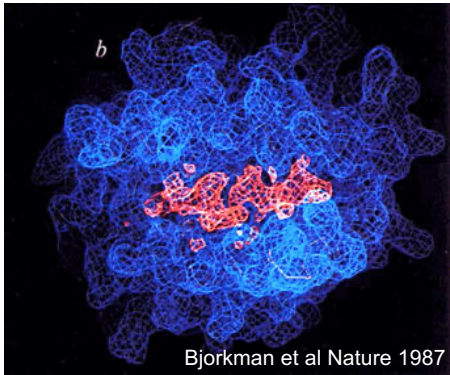
1. List the defining characteristics of immune tolerance
2. Compare mechanisms of central vs. peripheral tolerance with respect to location and timing in lymphocyte development
3. Differentiate molecular mimicry from bystander activation (the two main mechanisms for breaking tolerance)
4. Differentiate immune tolerance from immune privilege, and identify the immunologically privileged tissues in humans
5. Describe how each mechanism for B cell and T cell tolerance can fail and lead to autoimmunity
6. List genes associated with increased risk of autoimmune diseases
7. Describe two ways in which diet can affect autoimmunity

Outline

- Characteristics of self-tolerance
- Mechanisms that induce and maintain self-tolerance
- Self-tolerance failure = autoimmunity
- Risk factors and triggers for autoimmune disease
- Immune privilege vs. immune tolerance
- Autoimmune disease therapy

Self/nonself recognition

- The immune system can distinguish self from nonself
- Immune receptors for self antigens are not excluded
- Self antigens are seen by the immune system
- There must exist mechanisms that prevent reactivity to self antigens



Bjorkman et al Nature 1987

Immunological Tolerance

Definition: Unresponsiveness to an antigen induced by previous exposure to that antigen

- Function of the adaptive immune system
- The *same* antigen can induce immune tolerance or immune responsiveness depending on context (e.g. dose or method of delivery)

Immunogenic vs Tolerogenic

Parameter	Pro-immunogenic	Pro-tolerogenic
Location, or route of entry	subQ, intradermal, intramuscular, mucosal	Intravenous, oral
Dose	Small doses	High doses
Persistence	Brief – eliminated by immune response	Prolonged
Coincident with PAMPs, DAMPs, inflammation	Yes	No
Antigen characteristics	Polyvalent or aggregated	Monovalent

Most microbes check all the boxes for immunogenicity

Pro-immunogenic
subQ, intradermal, intramuscular, mucosal
Small doses
Brief – eliminated by immune response
Coincident with PAMPs, DAMPs, inflammation
Polyvalent or aggregated

Induce the innate immune system co-stimulatory signals

Polyvalent antigens can crosslink B-cell receptors, initiating antibody production

Tolerance is different from immunodeficiency or immunosuppression

Tolerance

- Antigen-specific
- Property of T and B lymphocytes
- Tolerance to self-antigens is an essential feature of the normal immune system
- Usually permanent

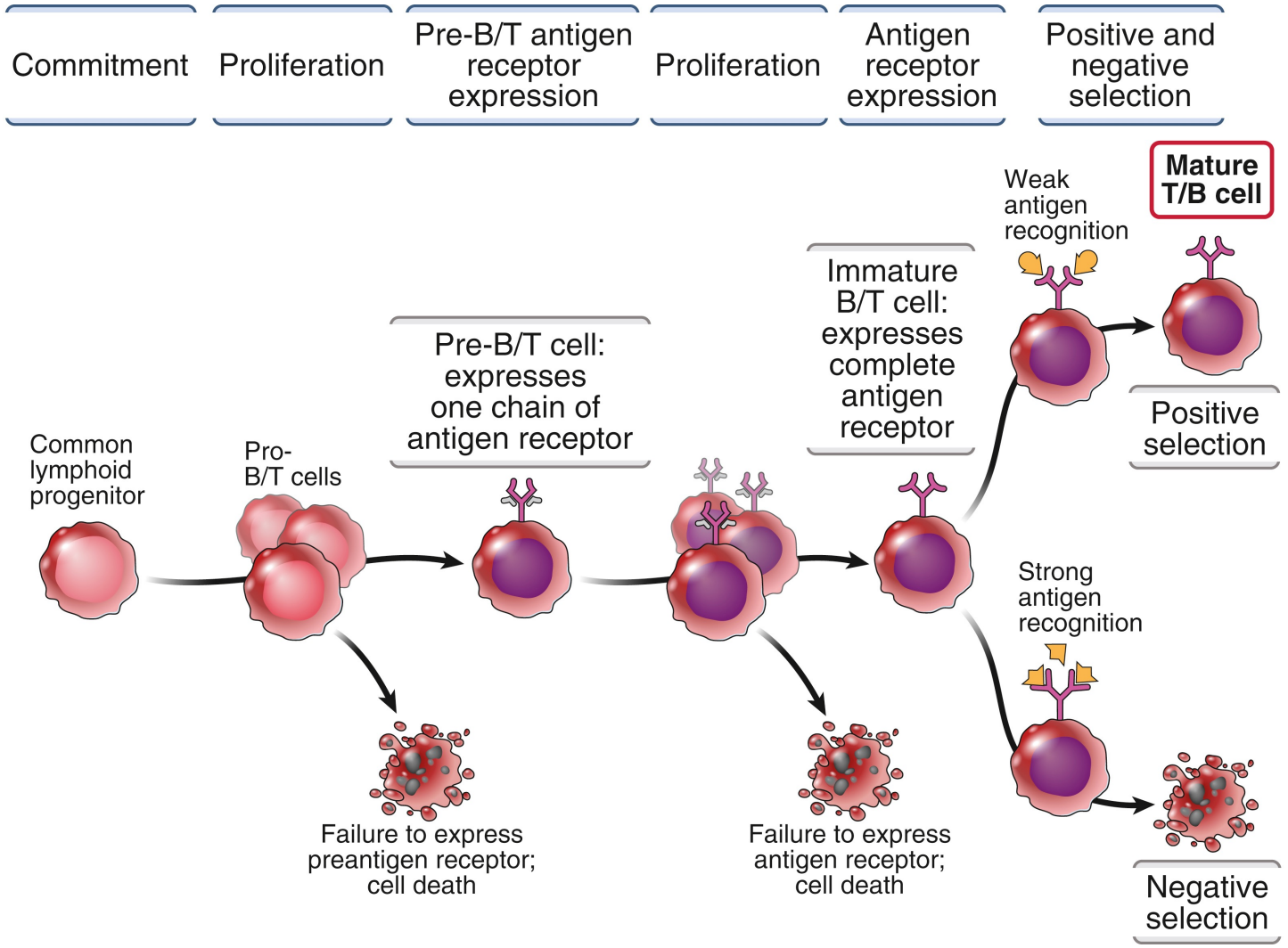
Immunodeficiency/ immunosuppression

- NOT antigen specific
- Can affect innate and adaptive immunity
- Usually indicative of a disease state
- Often reversible or transient

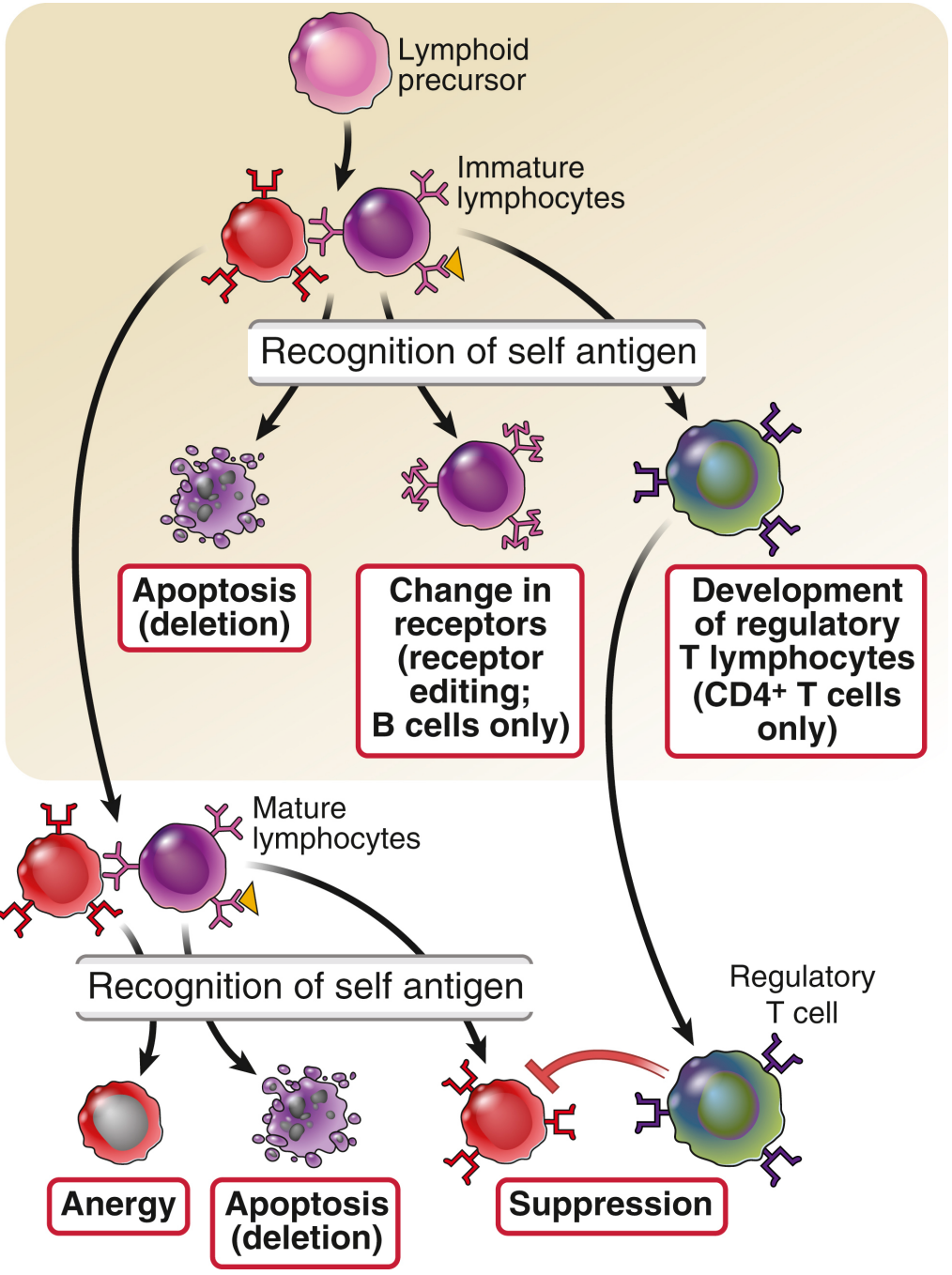
Importance of tolerance to medicine

- Tolerance to self-antigens is an essential feature of the normal immune system
- Failure of tolerance to self-antigens results in autoimmune diseases
- Tolerance to tumor antigens allows metastasis
- Ability to induce tolerance to foreign antigens would aid organ transplantation, gene therapy, and treatment of autoimmunity

Lymphocyte maturation



What are the mechanisms that induce immunological tolerance?



Central tolerance
Primary lymphoid organs

- Apoptosis (deletion)
- Receptor editing (B-cells only)
- Development of Treg cells ($CD4^+$T-cells only)

Peripheral tolerance

- Anergy
- Apoptosis (deletion)
- Suppression by Tregs
- Regulation by inhibitory receptors

Tolerance is induced in two stages

	(stage 1) Central Tolerance	**(stage 2)** Peripheral tolerance
Lymphocyte stage affected	Immature lymphocytes	Mature lymphocytes
Location of tolerance induction	Primary lymphoid tissues	Peripheral lymphoid tissues

Central vs. Peripheral Tolerance

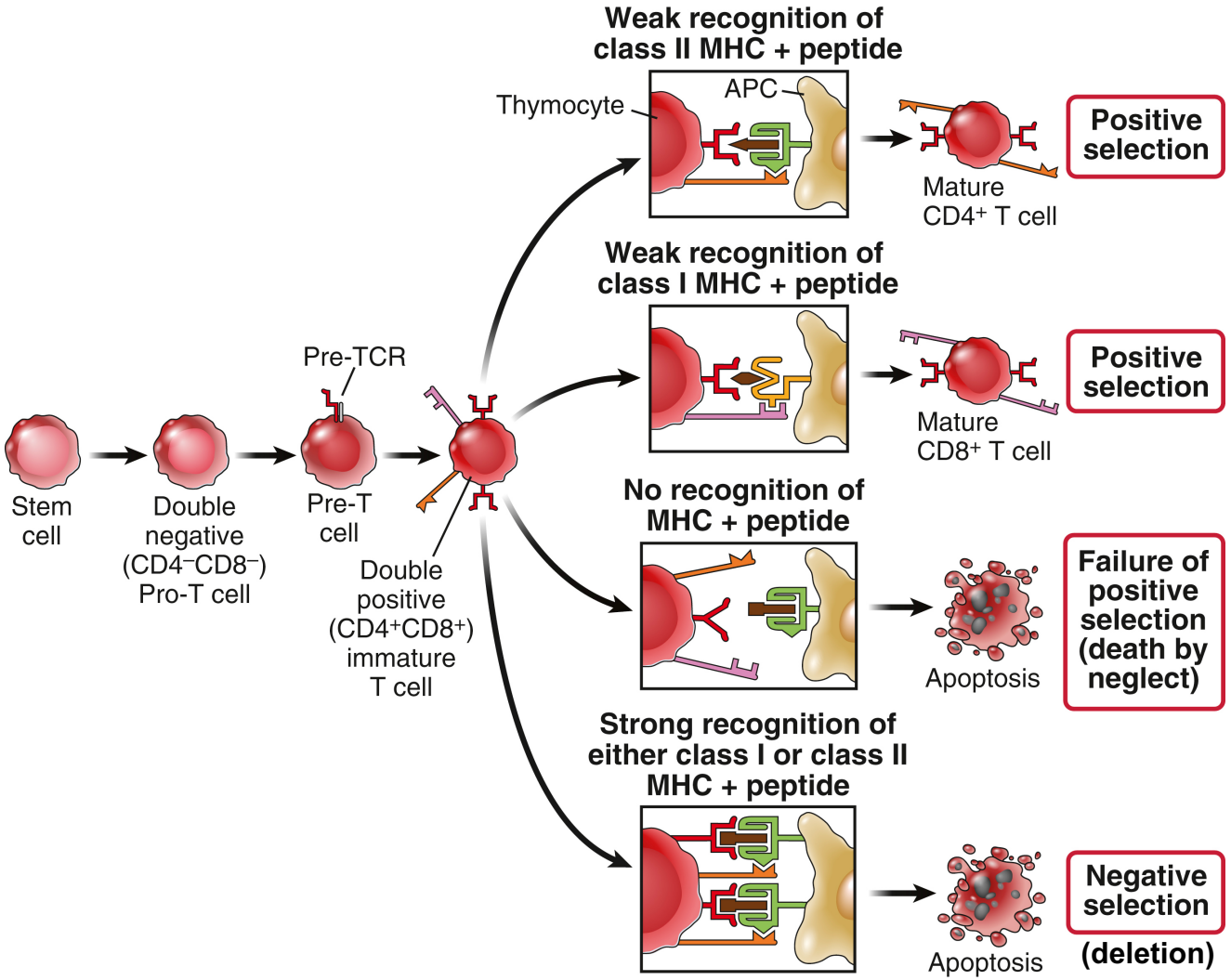
Central

- Primary lymphoid organs
  - Thymus for T-cells
  - Bone marrow for B-cells
- A self-antigen must be present in primary lymphoid organs to induce tolerance

Peripheral

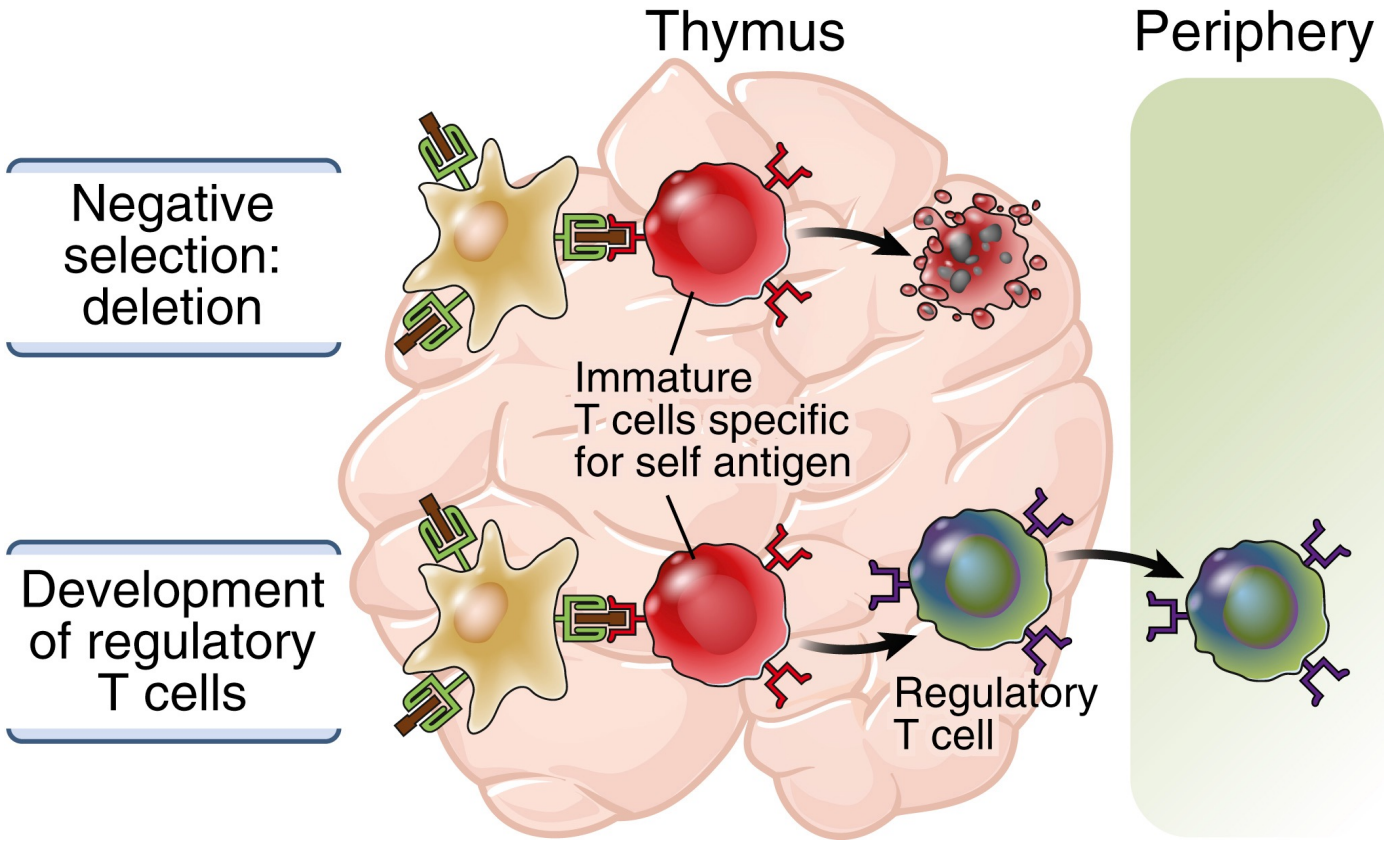
- Peripheral lymphoid sites
- Mature self-reactive cells that leave the thymus or bone marrow can be made tolerant
- Tolerance to self-antigens not in primary lymphoid tissues

T lymphocyte maturation



This selection process occurs in the thymus.

Central tolerance induction: T cells



Negative selection acts on $CD4^+$ and $CD8^+$ cells

Only $CD4^+$ cells can become Tregs in the thymus

Peripheral tolerance induction: T cells

- Necessary because not all self-antigens are expressed in the thymus to induce central tolerance
  - AIRE (autoimmune regulator) causes *some* peripheral tissue antigens to be expressed in the thymus (thymic epithelial cells)
  - AIRE gene defects result in autoimmune polyendocrine syndrome (a rare disease)
- Three mechanisms of T-cell peripheral tolerance
  - Anergy
  - Suppression by Tregs
  - Deletion/ Activation-induced cell death

Anergy

Definition:

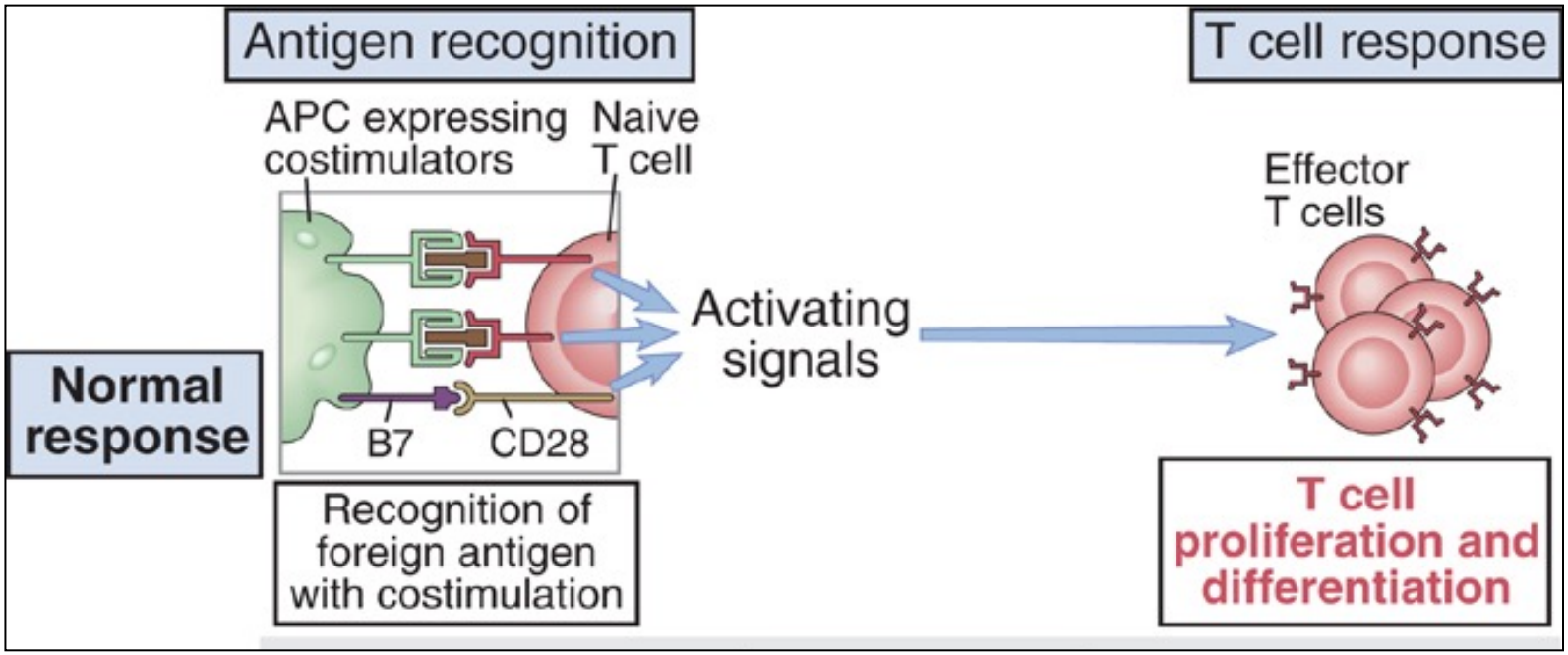
Long-lived state of unresponsiveness to antigenic stimulation in lymphocytes.

Mechanisms of anergy induction in T-cells:

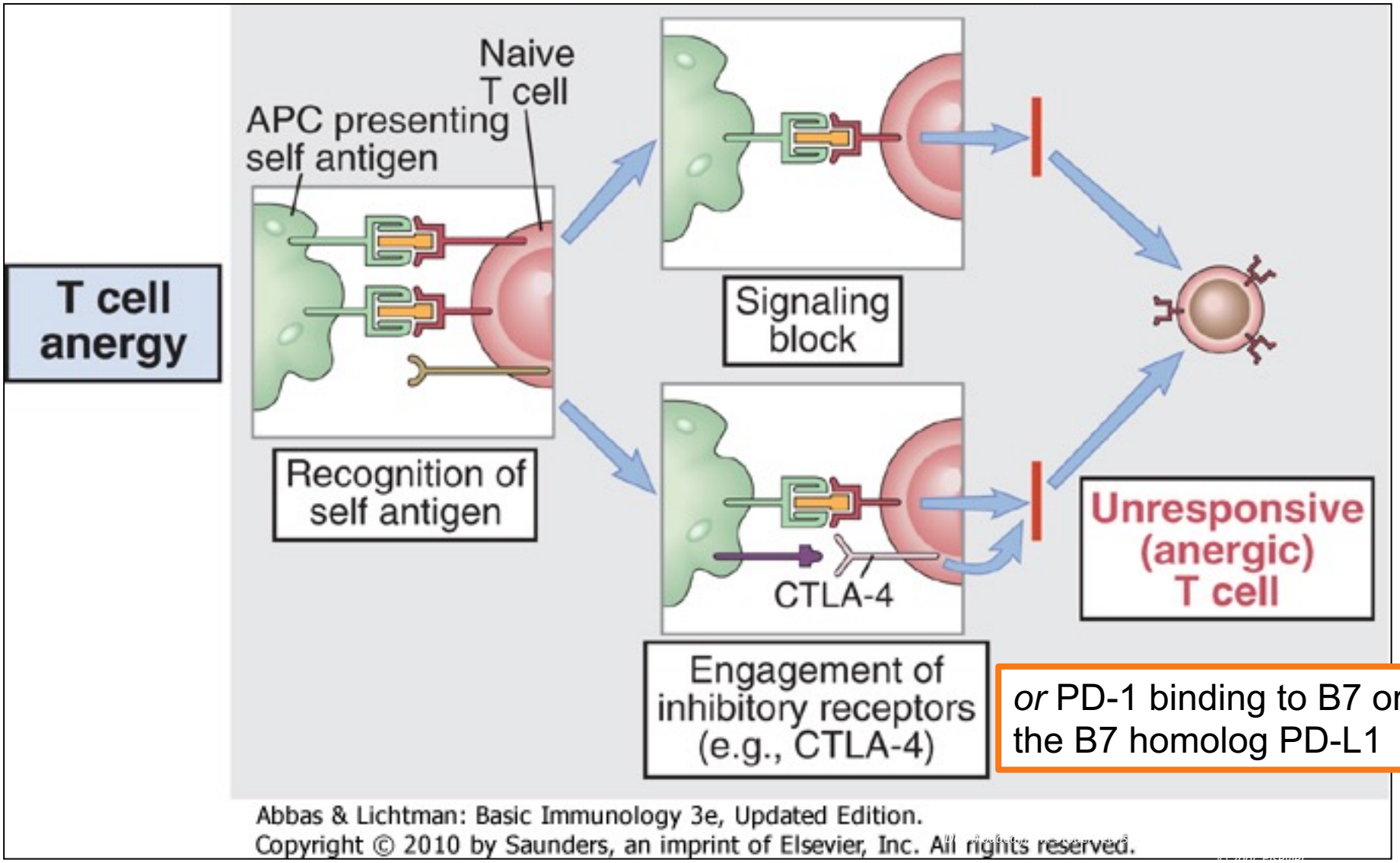
- Antigenic signaling in the absence of co-stimulation
- Antigenic signaling with engagement of inhibitory receptors CTLA-4 or PD-1

Activation vs. Anergy in T-cells

- Recall that T-cell **activation** requires two signals
  - 1. Antigen via TCR/CD3 complex
  - 2.Costimulation by B7 (CD80/86) via CD28

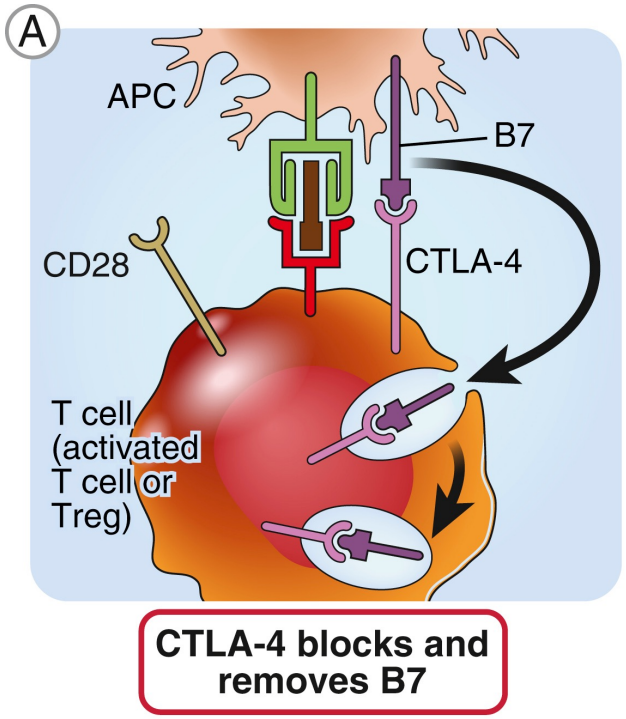


No signal 2 *or* CTLA-4 binding → Anergy



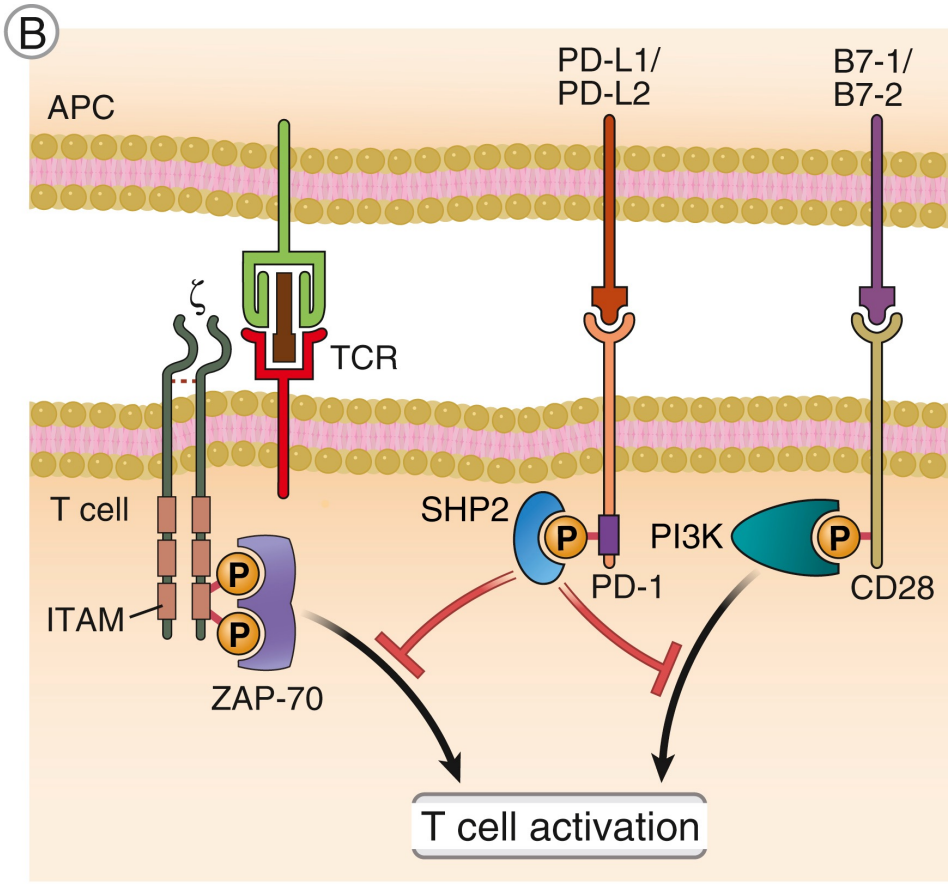
or PD-1 binding to B7 or the B7 homolog PD-L1

Mechanisms of action of CTLA-4 and PD-1



CTLA-4 blocks and removes B7

All T-cell types begin to express CTLA-4 about 48 h after activation by antigen + costimulatory signals



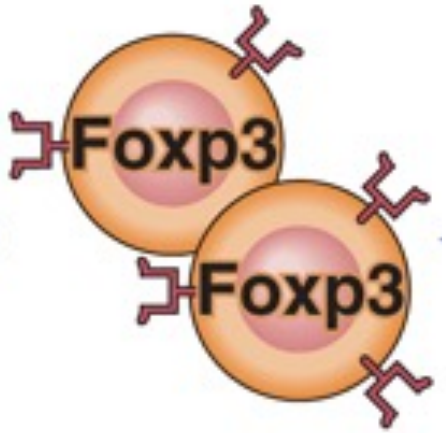
PD-1 inhibits signals from the TCR complex and CD28

CTLA-4 and PD-1 have different roles

	CTLA-4	PD-1
Major site of action	Secondary lymphoid organs	Peripheral tissues
Stage of immune response that is inhibited	Induction (priming)	Effector phase
Cell type that is inhibited	$CD4^+$ same as or more than $CD8^+$	$CD8^+ > CD4^+$
Cellular expression	Tregs, activated T cells	Mainly activated T cells
Main signals inhibited	Competitive inhibitor of CD28 costimulation by binding to B7 with high affinity and removing B7 from APCs	Signaling inhibitor of CD28 and TCR: inhibits kinase-depending signals by activating phosphatase
Role in Treg-mediated suppression of immune responses	Yes	No

Peripheral Immune suppression by Tregs

- Develop mostly in thymus but also periphery
- Treg markers
  - CD4
  - CD25 (high)
  - Foxp3
- Dependent on IL-2 for maintenance
- Tregs produce memory cells just like other T cell subsets

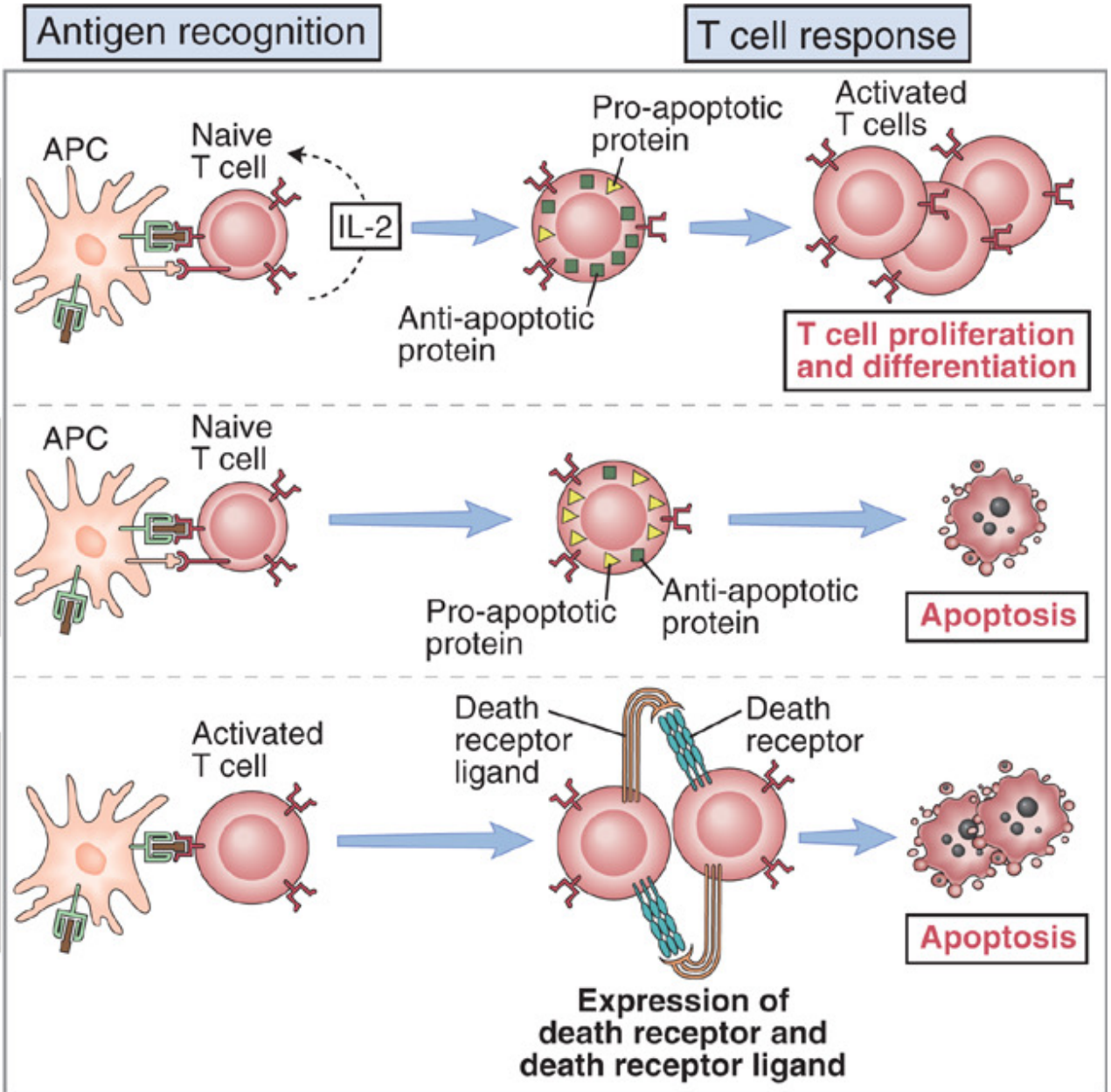


Regulatory T cells

Peripheral Immune suppression by Tregs

- Treg mechanisms of action (>12) are not entirely understood and are context-specific. Some of them are:
  - Cytokine secretion that blocks activation of macrophages and other lymphocytes
    - IL-10
    - TGF-β
    - IL-35
  - Cell-cell contact with APC: CTLA-4 removes B7 molecules (CD80/86) from APC by trans-endocytosis
  - Depleting IL-2 and inflammatory cytokines (TNF, IL-1)

Peripheral T cell **deletion**: Activation-induced cell death (AICD)



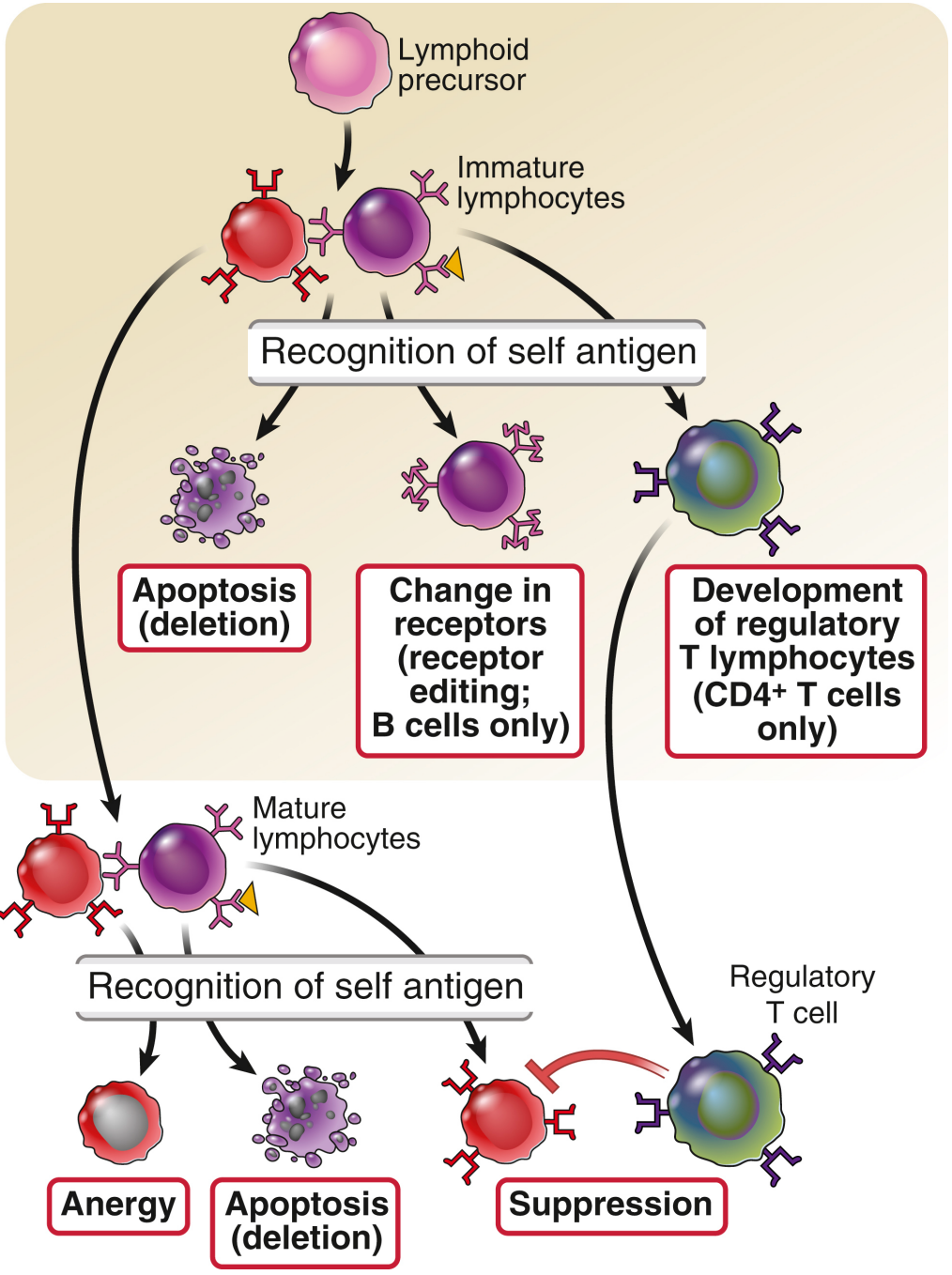
Normal

- Antigen stimulates apoptotic proteins
- IL-2 stimulates anti-apoptotic proteins

Low or no costimulation

- AICD: apoptosis by mitochondrial pathway
- AICD: apoptosis by FasL extrinsic pathway

What about B-cell tolerance mechanisms?



Central tolerance

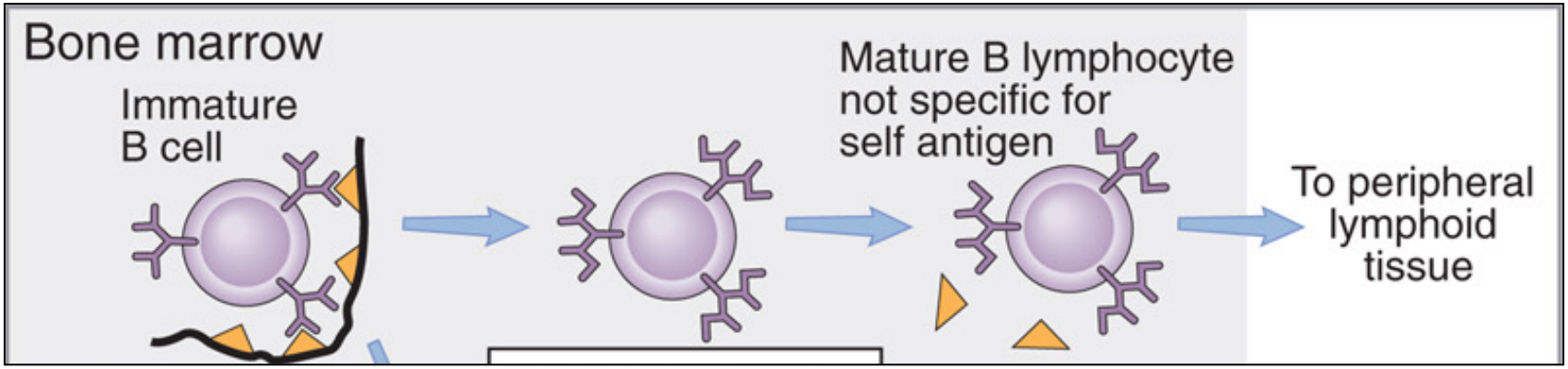
Primary lymphoid organs

- Apoptosis (deletion)
- Receptor editing (B-cells only)
- Development of Tregs

Peripheral tolerance

- Anergy
- Apoptosis (deletion)
- Suppression by Tregs (Bregs)
- Regulation by inhibitory receptors

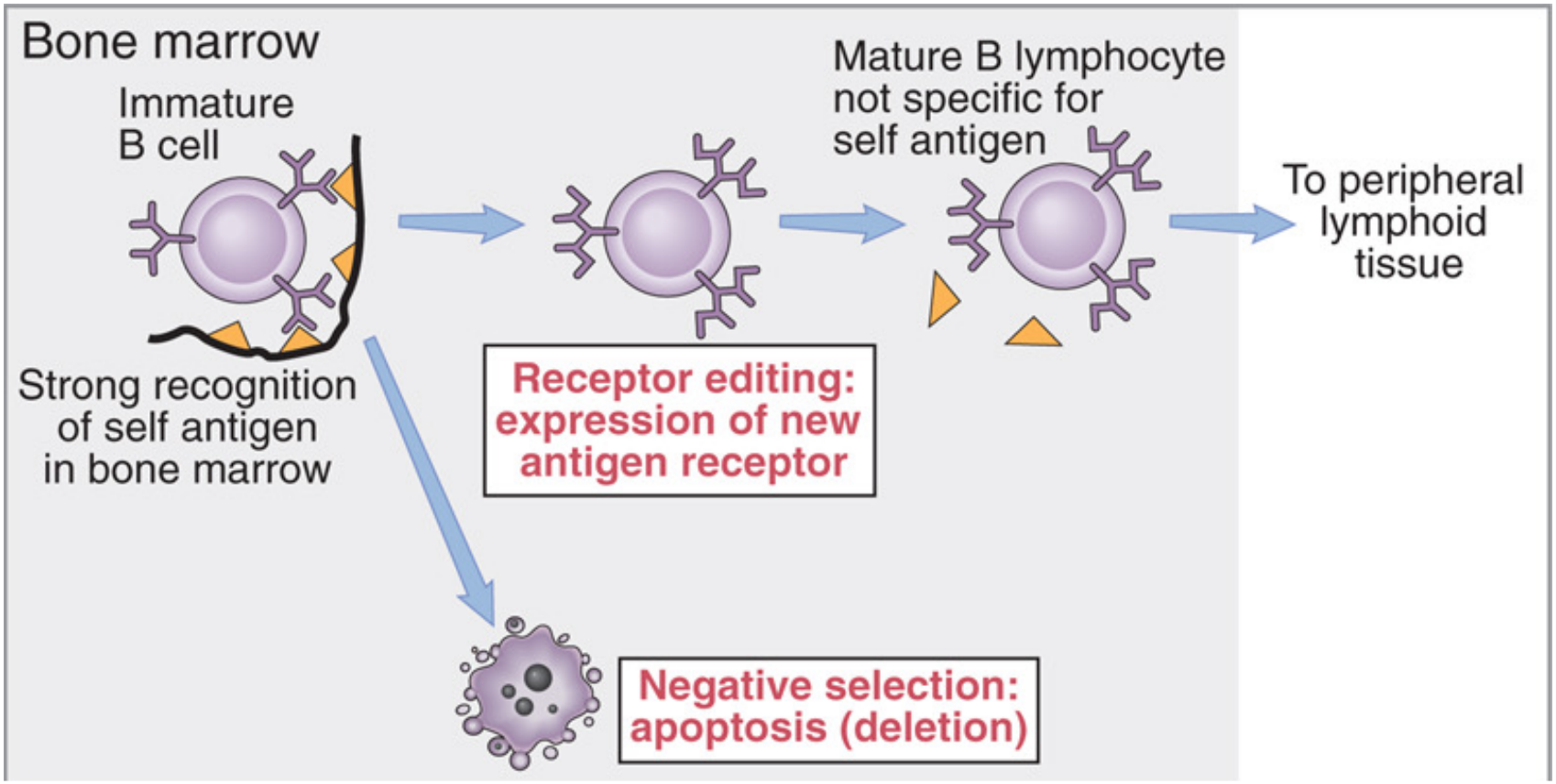
Central tolerance induction: B cells



Strongly self-reactive B-cells can undergo **receptor editing**:

- Reactivate RAG gene and rearrange a new light chain.
- Old heavy chain + new light chain = new specificity
- 25-50% of mature B-cells have undergone receptor editing

Central tolerance induction: B cells

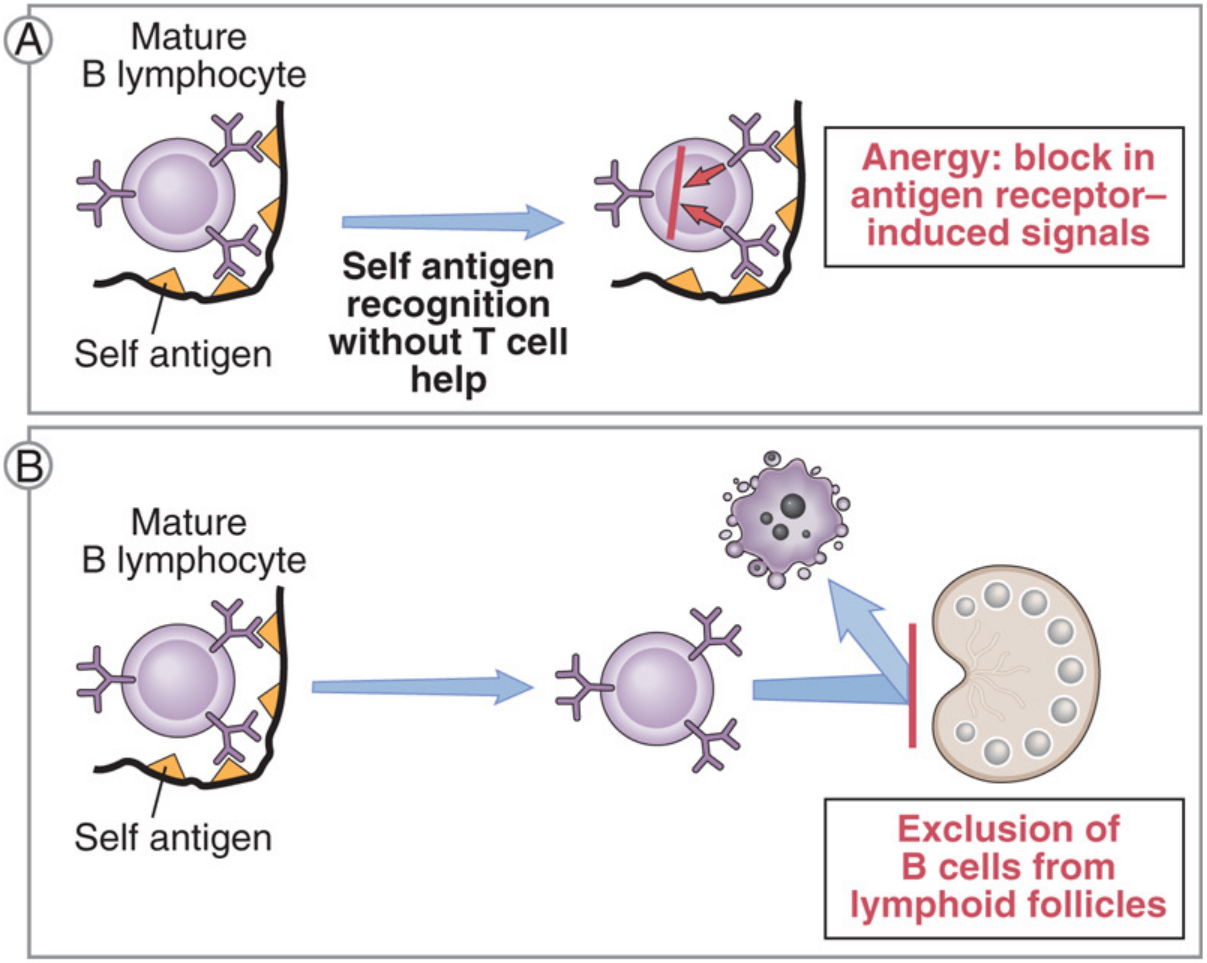


If editing fails, negative selection occurs the same as in T cells.

Central B-cell tolerance

- B-cell tolerance can be to protein and non-protein antigens.
- Such antigens are usually abundant and expressed widely
- Although receptor editing and negative selection are well documented, no autoimmune diseases are known to result from loss of B-cell central tolerance

Peripheral B-cell Tolerance



Two mechanisms:

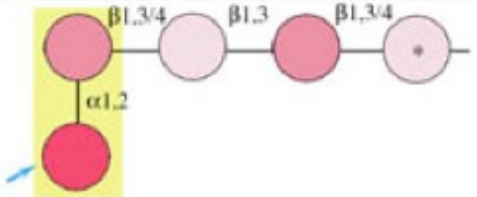
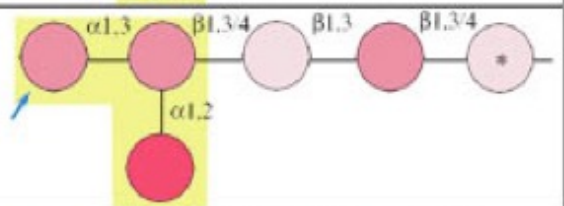
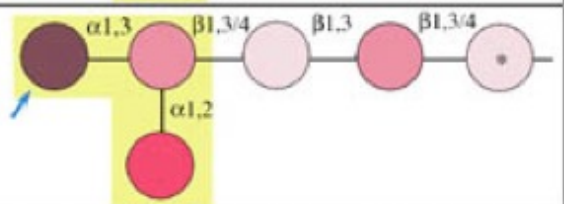
A. Anergy

B. Apoptosis

Anergy occurs due to a lack of T-cell help

B-cell tolerance example: ABO blood system

A person can have a severe immune response to a mismatched blood transfusion even if they have never received blood before, but matched blood is safe. Why?

Antigen	Structure	Minimal determinant structure
H		Fuc–α1→2–Gal–β1–R
B		Gal–α1→3 Gal–β1–R Fuc–α1→2
A		GalNAc–α1→3 Gal–β1–R Fuc–α1→2

Tolerance example: ABO blood system

- The ABO antigens happen to be identical to carbohydrates expressed on commensal bacteria in the gut.
- The bacterial antigens induce specific serum IgM Ab, which during a mismatched blood transfusion, bind to the foreign RBC and activate complement.
- *However, thanks to deletion of self-reactive B-cells in the bone marrow,* Type A individuals are tolerant to the A Ag, Type B individuals are tolerant to the B Ag, and everyone is tolerant to the H Ag, allowing matched transfusions.

Outline

- Characteristics of self-tolerance
- Mechanisms that induce and maintain self-tolerance
- Failure of self-tolerance = autoimmunity
- Risk factors and triggers for autoimmune disease
- Immune privilege vs. immune tolerance
- Therapy for autoimmune diseases

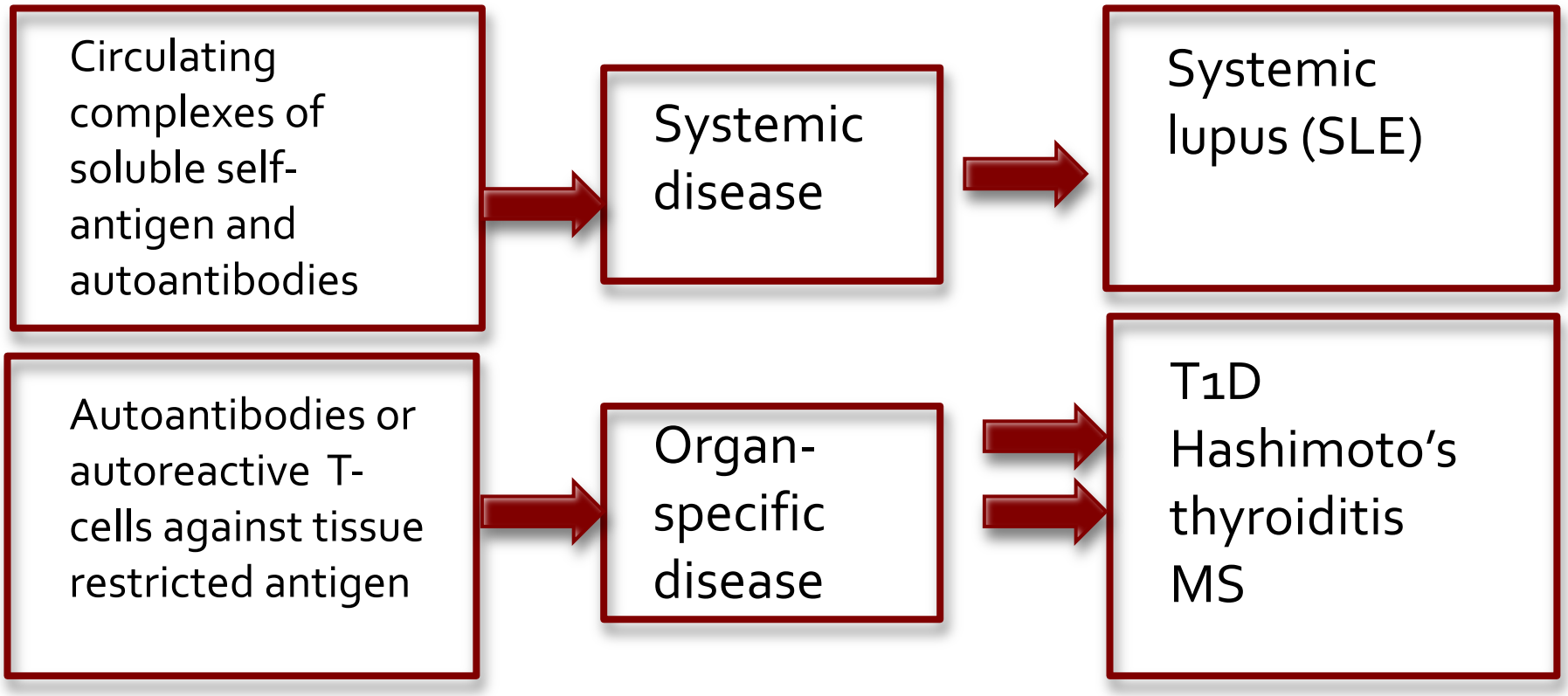
Autoimmunity

Autoimmunity

- Definition: an immune response against self antigens (although the antigen is not known in all cases)
- Affects 2-5% of Americans (females>>males)
- Major risk factors
  - Genetic susceptibility
  - Exposure to environmental triggers
- Multifactorial, difficult to identify triggers because of long lag time

Systemic or organ specific?

- Autoimmune diseases can be systemic or organ specific



Autoimmune pathogenesis is varied

- Mechanisms
  - Immune complex
  - Circulating autoantibodies
  - Autoreactive T-cells

Discussed in detail with disease manifestations and in the Hypersensitivity lecture

- Autoreactivity to a single antigen can lead to tissue injury and subsequent immune responses to additional antigens (epitope spreading)

MHC/HLA genes are *the* most important genetic factor in autoimmune susceptibility

Evidence	Examples		
	Disease	MHC allele	Relative risk
"Relative risk" of developing an autoimmune disease in individuals who inherit particular HLA allele(s) compared with individuals lacking these alleles	Ankylosing spondylitis	HLA-B27	90
	Rheumatoid arthritis	HLA-DR4	4
	Type 1 diabetes mellitus	HLA-DR3/DR4	25
	Pemphigus vulgaris	HLA-DR4	14

However, MHC is not sufficient. Most human autoimmune diseases are polygenic *AND* require an environmental trigger.

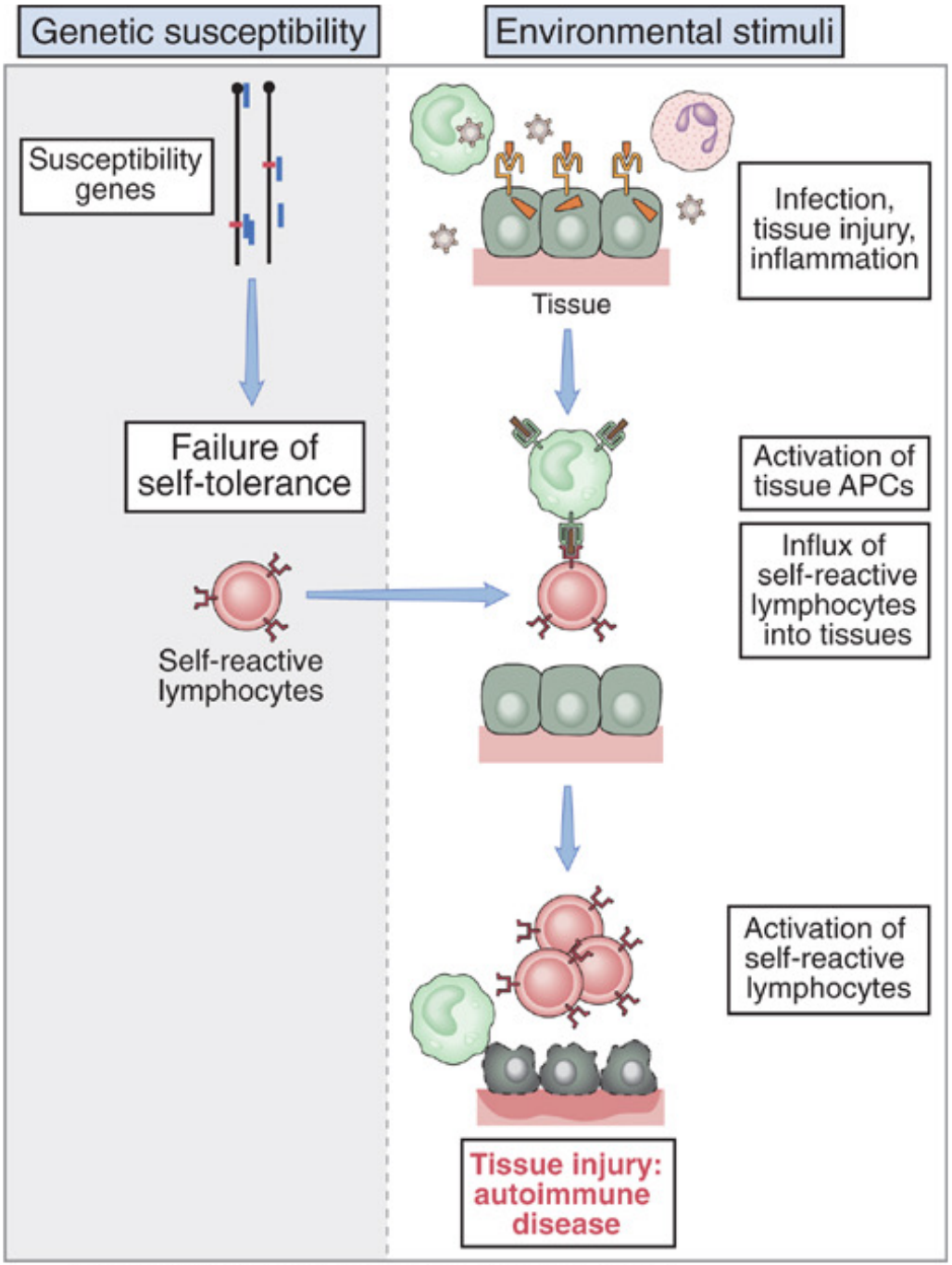
Non-MHC genes associated with polygenic autoimmune diseases

gene	disease
Complement C2, C4	Lupus-like disease
FcγRIIb	Lupus-like disease (defective feedback of B cell activation)
NOD-2	Crohn's disease, Blau syndrome
PTPN22	Several diseases (abnormal tyrosine phosphatase signaling)
IL-2, IL-2Rα/β	Multiple Sclerosis (MS), Type 1 diabetes (T1D)

Exceptions: Human autoimmune diseases requiring only one defective gene

Some non-MHC genes associated with autoimmune diseases were already mentioned:

gene	**disease**
AIRE	Autoimmune polyendocrine syndrome
Fas, FasL	Autoimmune Lympho-proliferative syndrome (ALPS)
Foxp3	X-linked polyendocrinopathy and enteropathy (IPEX)



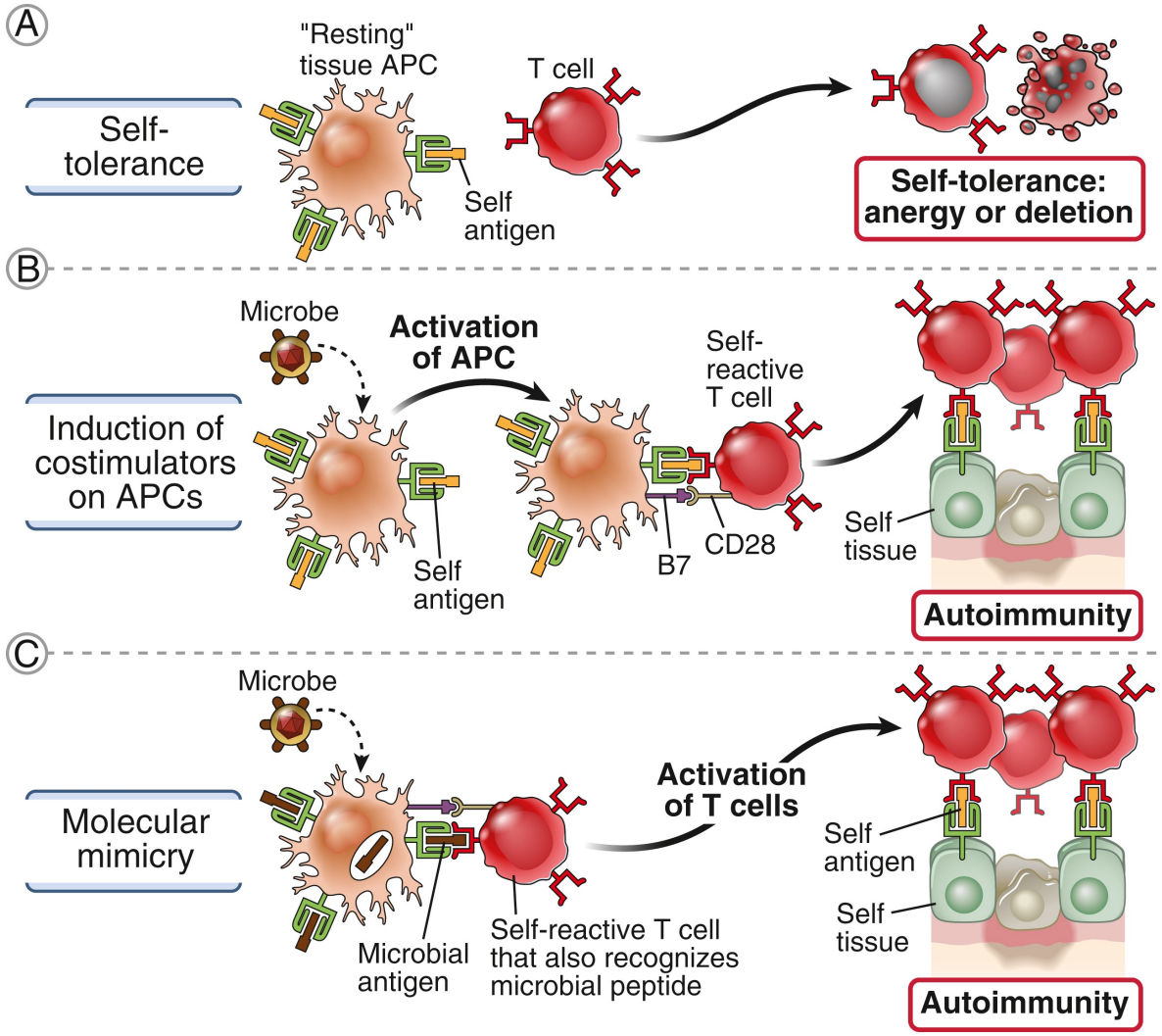
Environmental triggers of Autoimmune Disease

- Chemical exposure
- Infections
- Diet
- Trauma
- Being female

Chemical triggers of autoimmune disease

- **Certain drugs:** Procainamide, hydrolyzine and quinidine can produce lupus-like symptoms in genetically susceptible individuals
- **Some metals:** mercury, gold and silver can induce lymphocyte proliferation and autoimmune symptoms
- **Some solvents:** Trichloroethylene is associated with increased risk of lupus and scleroderma

Mechanisms of infection-induced autoimmunity



1. Induction of costimulatory molecules on APC presenting self-antigens
2. Molecular mimicry

Diet and autoimmunity

- Vitamin D decreases Th1 cell responses and increases Treg responses
  - May explain observed lower incidence of autoimmune diseases in sunnier climates. Vitamin D supplementation reduces autoimmunity incidence (Hahn et al BMJ 2022)
- Celiac disease: autoimmune disease triggered by gluten in genetically susceptible individuals (HLA-DQ2/8), IgA autoantibodies to transglutaminase 2
- Iodine: excess dietary iodine causes increased iodination of thyroglobulin. Highly iodinated thyroglobulin is more immunogenic.

Trauma and autoimmunity

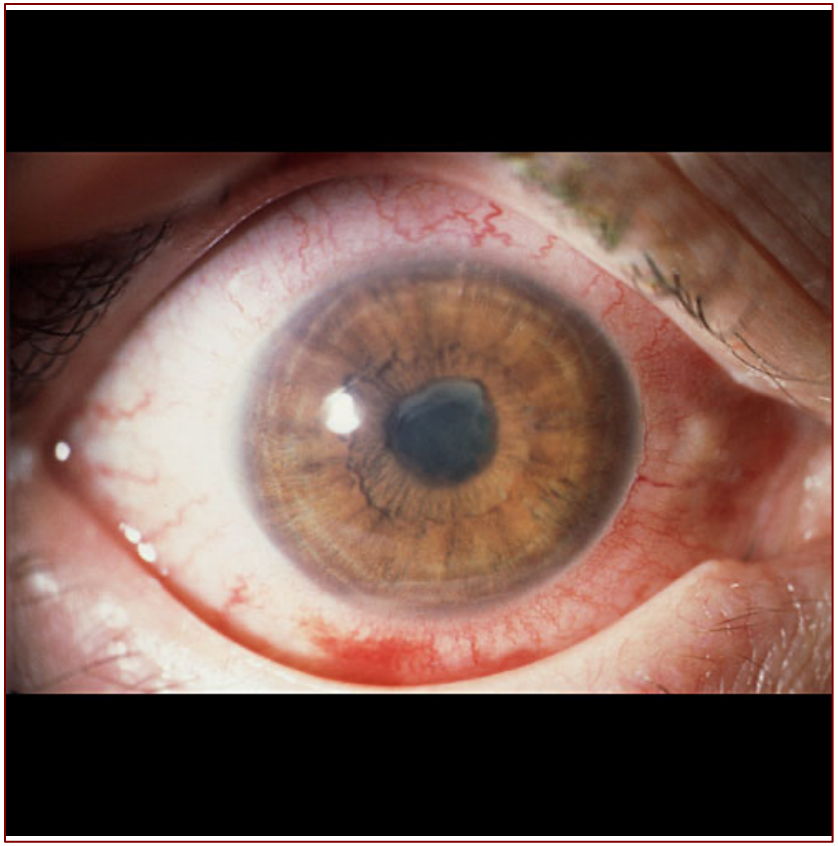
- Trauma activates the innate immune system and results in recruitment and activation of leukocytes at the trauma site
  - e.g. Gastroenteritis may be a trigger for various inflammatory bowel conditions
- Self antigens not normally exposed to the immune system may be released – especially in the case of “immune-privileged” sites

Immune privileged sites
(a tissue-specific type of immune suppression)

- Places in the body in which immune responses to foreign antigens are inhibited
- Both active and passive mechanisms are involved
- Not antigen specific
- Does NOT require exposure to antigen for induction

- Brain
- Eyes
- Testes
- Placenta and fetus

Passive mechanisms of immune privilege

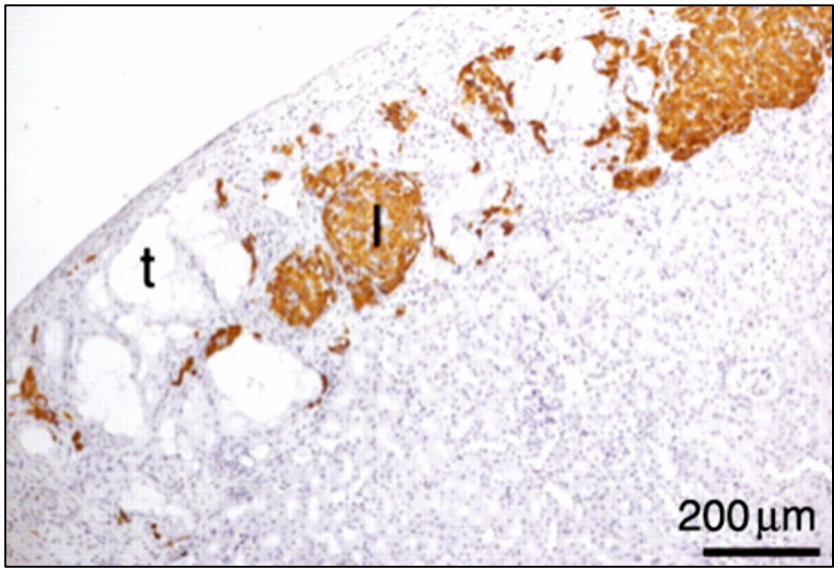


Sequestration and separation from immune system

- Examples are intraocular proteins and sperm
- Trauma to eye or testes can result in autoimmune uveitis or orchitis
- Traumatic brain injury can result in anti-pituitary antibodies

Testes: another example of active mechanism of immune privilege

- The Sertoli cells confer immune privilege
- Passive mechanism in testis: tight junctions create a physical barrier separating sperm from blood
- Active mechanism: secretion of factors that arrest lymphocyte proliferation by blocking IL-2
- Could Sertoli Cells help protect transplants?



Sex and autoimmunity

- Many autoimmune diseases are more common in females:
  - SLE, Hashimoto's, Systemic sclerosis 10:1
  - Sjogren's syndrome 5:1 and many others
  - Exceptions: Type 1 diabetes (in humans), ulcerative colitis, psoriasis are equally prevalent, ankylosing spondylitis 3x more prevalent in males
- Increased female to male ratio is reproduced in almost all animal models of autoimmune disease
  - Some can be induced in male animals by castration

Possible causes of sex differences

- Immune differences:
  - Females have a higher percentage of T-cells
  - T cells express androgen receptor, the activation of which contributes to T cell exhaustion (Kwon et al Sci Imm 2022).
  - Females have higher IgM titers after age 6
  - In lab rats, females fight infections better, reject transplants faster, and release more histamines and IFN-γ to the same stimuli
  - Women respond more vigorously to a variety of vaccines studied, at all ages, than men
  - Women suffer less post-traumatic immune suppression than men
- Environmental differences?

Therapies for autoimmune disease

- Anti-inflammatory drugs to block tissue injury
  - Corticosteroids
  - Anti-TNF and IL-1 antibodies / antagonists
  - Anti-integrin antibodies to block migration of leukocytes into tissues
- Costimulation blockade (anti-B7, soluble CTLA-4)
- B-cell depletion/inhibition (anti-CD20 or IVIg)
- Plasmapheresis
- T-cell immunosuppressive/ cytotoxic drugs (Cyclosporin A, methotrexate)

Vaccine to induce Tregs?

SHARE

RESEARCH ARTICLE

A noninflammatory mRNA vaccine for treatment of experimental autoimmune encephalomyelitis

Christina Krienke[1,2], Laura Kolb[1,*], Elif Diken[1,*], Michael Streuber[1], Sarah Kirchhoff[1], T…mas Bukur[1], Özl…
+ See all authors and affiliations

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Vol. 371, Issue 6525, pp. 145-153
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Article | Figures & Data | Info & Metrics | eLetters

Precision therapy for immune tolerance

Autoimmune diseases, such as multiple sclerosis (MS), result from a breach of immunological self-tolerance and tissue damage by autoreactive T lymphocytes. Current treatments can cause systemic immune suppression and side effects such as increased risk of infections. Krienke *et al.* designed a messenger RNA vaccine strategy that lacks adjuvant activity and delivers MS autoantigens into lymphoid dendritic cells. This approach expands a distinct type of antigen-specific effector regulatory T cell that suppresses autoreactivity against targeted autoantigens and promotes bystander suppression of autoreactive T cells against other myelin-specific autoantigens. In mouse models of MS, the vaccine delayed the onset and reduced the severity of established disease without showing overt symptoms of general immune suppression.

Science, this issue p. 145

Mouse model of Multiple Sclerosis

Uses mRNA vaccine without adjuvant to induce tolerance

Summary - Tolerance

- Immunological tolerance is specific unresponsiveness to an antigen induced by exposure of lymphocytes to that antigen
- Tolerance is induced in two stages of lymphocyte development:
  - central tolerance acts on immature lymphocytes
  - peripheral tolerance acts on mature lymphocytes
- Some tissues in the body are immune privileged

Summary - Autoimmunity

- Autoimmune diseases result from a failure of self-tolerance.
- Multiple factors contribute to autoimmunity, including the inheritance of susceptibility genes and environmental triggers such as infections.
- In humans and animal models there is a significantly higher prevalence of autoimmune diseases in females, which is likely multifactorial