BHARATHIDASAN UNIVERSITY

Tiruchirappalli – 620 024,
Tamil Nadu, India

Programme: M.Sc., Biotechnology (Marine)

Course Title : Immunology

Course Code : 21 CC7

Unit: IV
Immuno tolerance

Dr. K. ANBARASU
Professor
Department of Marine Biotechnology

- **Immunity**- a condition of being able to resist a particular disease especially through preventing development of a pathogenic microorganism or its products.
- **Antigen**

  Recognized by immune system

  Substance that induces immune response in a body

  Can be foreign or self
- **Antigen-presenting cells** - Antigen-presenting cells (APCs) are a heterogeneous group of immune cells that mediate the cellular immune response.
- Classical APCs include **dendritic cells, macrophages, Langerhans cells and B cells.**

Historical background

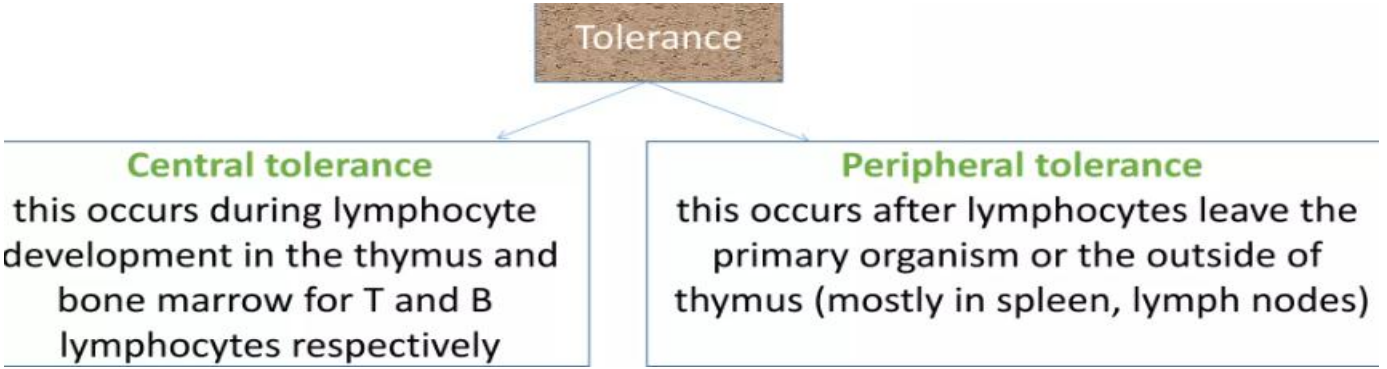
- **The phenomenon** of immune tolerance was first described by Ray D. Owens in 1945.
- He noted that dizygotic twin cattle sharing a common placenta
- They are also shared a stable mixture of each other's red blood cells and retained that mixture throughout life.
- Although Owens did not use the term immune tolerance, his study showed the body could be tolerant of these foreign tissues.
- Burnet and Medawar were ultimately credited for "the discovery of acquired immune tolerance" and shared the Nobel Prize in Physiology or Medicine in 1960.

Definition

- **Immunological tolerance** is also called **Immune tolerance or immuno tolerance**
- Immunological tolerance is a state of **unresponsiveness** of the immune system to substances or tissue that have the capacity to elicit an immune response in a given organism.
- **Tolerogens/Tolerogenic antigens :** antigens that induced tolerance
- **Self-tolerance :** Tolerance to self antigens
- **Autoimmunity :** Failure of self-tolerance
- In general, antigens that are present **during embryonic life** are considered **“self ”** and **do not stimulate an immunologic response.**

Classification

- **Tolerance** is classified into central or peripheral tolerance **depending on where the state is originally induced**



- The recognition of what is "foreign" and what belongs to ones "own" body is a fundamental property of the immune system.

- The achieving of this self-tolerance occurs mainly during the T cell maturation in the thymus.

- Surprisingly, 95% of all the cells formed there die before they are ready to mature and emigrate into the peripheral lymphatic organs.
- T cells, possessing receptors which strongly bind onto one of the body's own peptides bound to an endogenous MHC molecule, are triggered to undergo apoptosis.
- The repertoire of T cells thus consists of a combination of positive and negative selection processes.

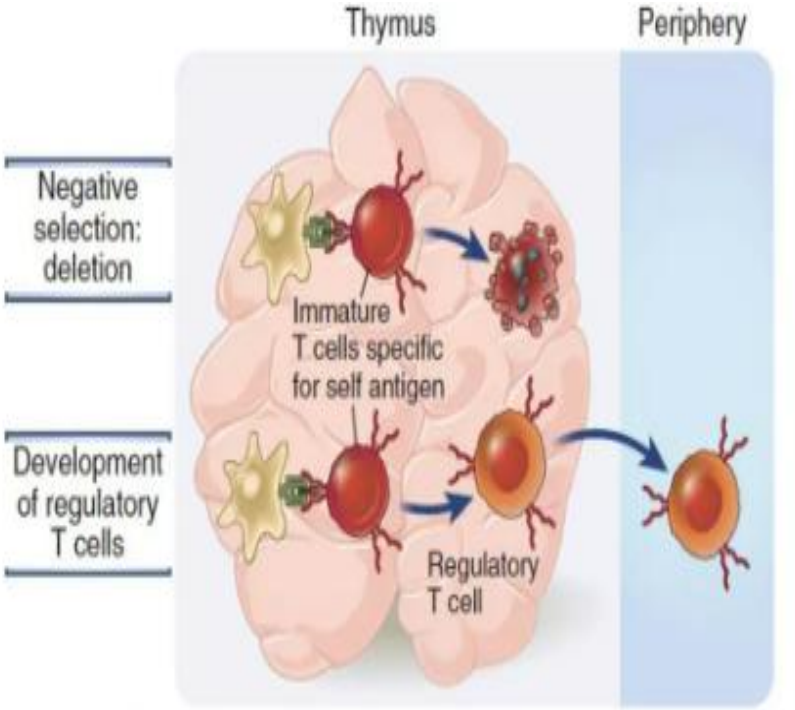
T Lymphocyte Tolerance

- Central T cell Tolerance
- Peripheral T cell Tolerance
- Factor that Determine the Tolerogenicity

Central T Cell Tolerance

- During their maturation in thymus, many immature T cells that recognize antigens with high avidity die, and some of surviving cells in the CD4+ lineage develop into Tregs

- Thymus has special mechanism for expressing many protein antigens expressed in different peripheral tissues, produced in medullary thymic epithelial cells (MTECs) under the control of the autoimmune regulator (AIRE) protein, immature T cells specific for these antigens can be deleted



- Mutations in the AIRE gene are the cause of a multi-organ autoimmune disease, autoimmune polyendocrine syndrome type 1 (APS1) characterized by antibody- and lymphocyte-mediated injury to multiple endocrine organs; parathyroids, adrenals, and pancreatic islets
- In the absence of functional AIRE, these antigens are not displayed in the thymus, and T cells specific for the antigens escape deletion, mature, and enter the periphery → attack target tissues
- Patients with AIRE mutations also make neutralizing autoantibodies against their own IL-17
- Deficiency of IL-17 → patients susceptible to mucocutaneous candidiasis Abul K. Abbas, et al. Cellular and Molecular Immunology.

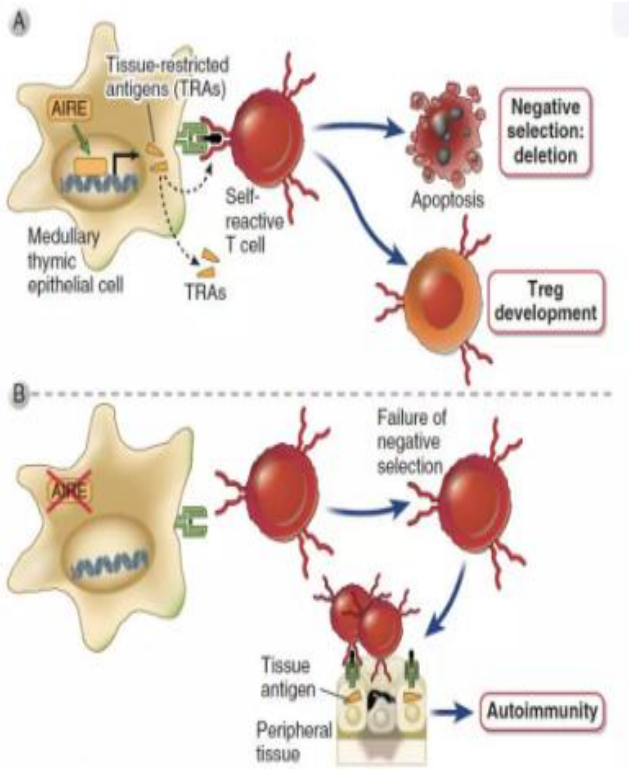
- Regulatory cells leave the thymus and inhibit responses against self antigens in the periphery

- Determination of deletion OR development of Tregs

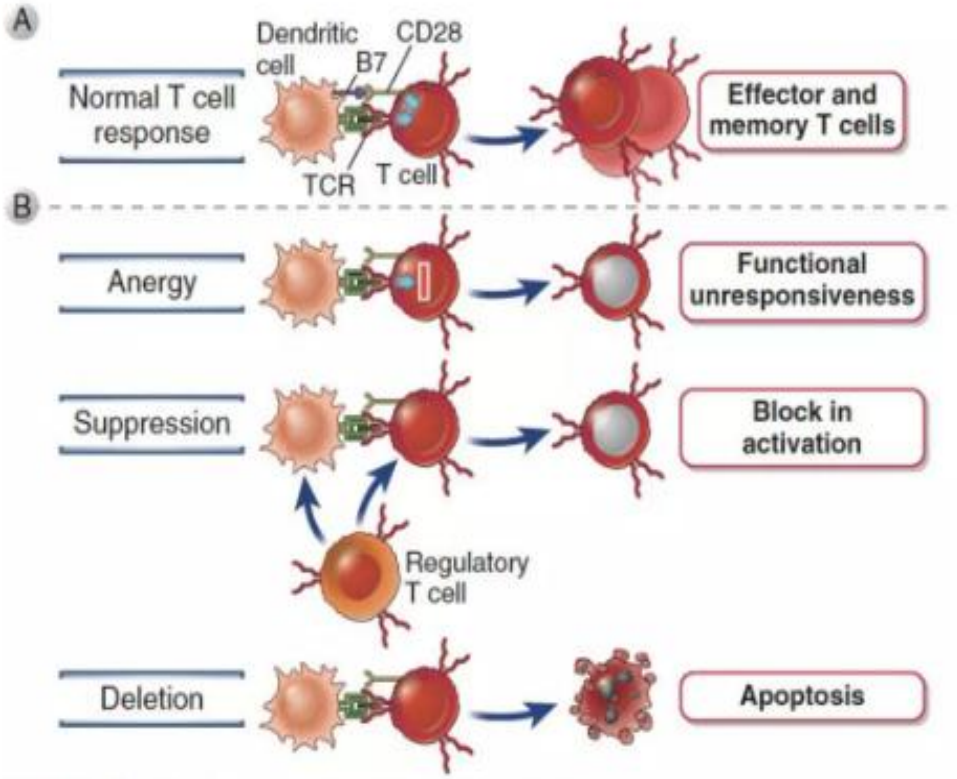
- Affinity of antigen recognition

- Types of antigen presenting cells (APCs) presenting antigen

- Availability of cytokines in thymus

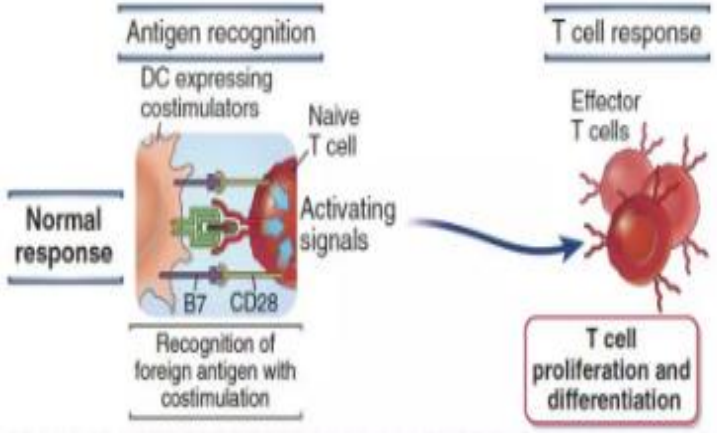


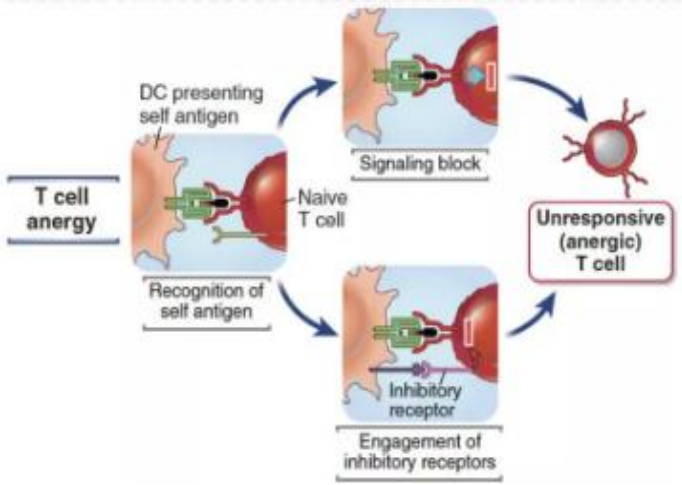
Peripheral T Cell Tolerance



Source: https://quizlet.com/88022014/chapter-14-abbas-flash-cards/

- Exposure of mature CD4+ T cells to antigen in absence of co-stimulation or innate immunity may make the cells incapable of responding to that antigen
- Mechanisms induce and maintain anergic state
- TCR-induced signal transduction is blocked in anergic cells
- Self antigen recognition may activate cellular ubiquitin ligases → ubiquitinate TCR-associated proteins and target them for proteolytic degradation in proteasomes or lysosomes
- T cells recognize self antigens, they engage inhibitory receptors of CD28 family, whose function is to terminate T cell responses





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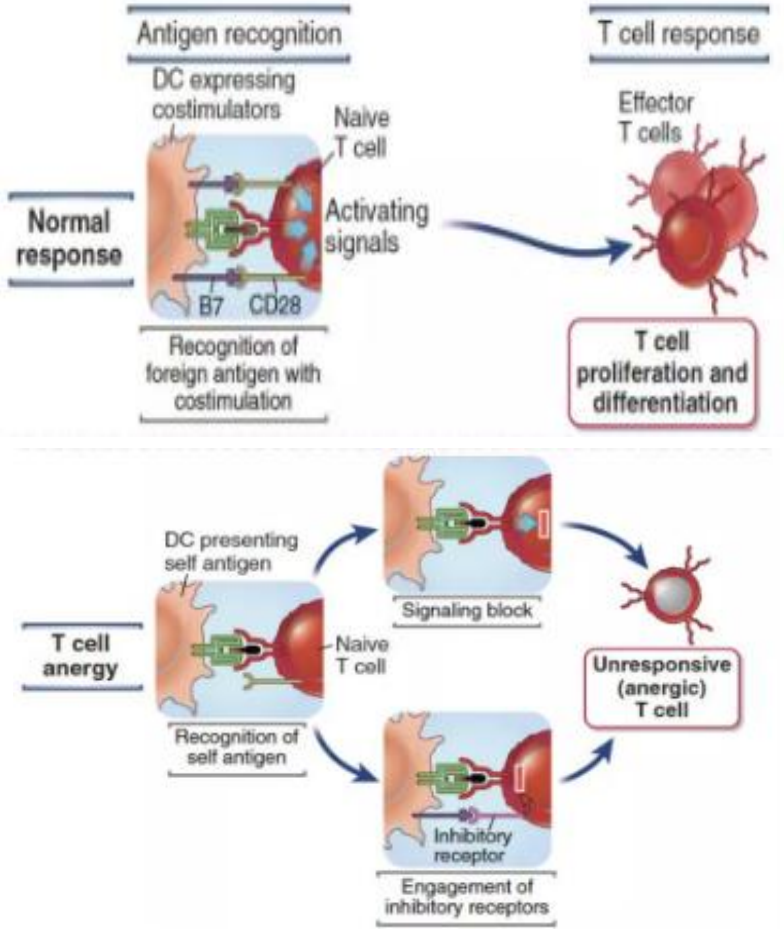


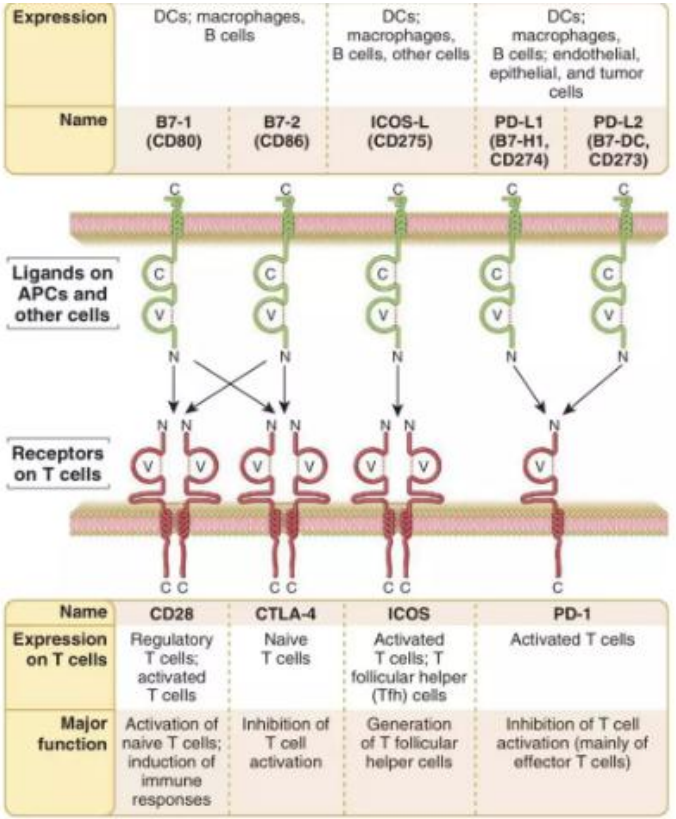
Peripheral T Cell Tolerance

- Regulation of T Cell Responses by Inhibitory Receptors

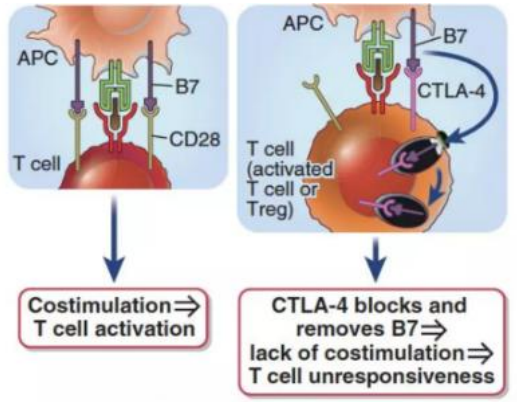
- Inhibitory receptors: CTLA-4 and PD-1
- CTLA-4 (cytotoxic T lymphocyte antigen-4): member of CD28 receptor family, like the activating receptor CD28, it binds to B7 molecules

- People with mutations in CTLA4 gene develop inflammatory lesions containing activated T cells and macrophages affecting multiple organs

- Polymorphisms in CTLA4 gene are associated with several autoimmune diseases in humans, including type 1 diabetes and Graves' disease

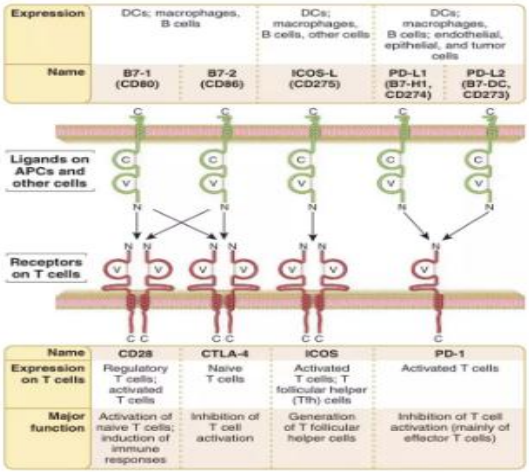


- CTLA-4 inhibits T cell activation in 2 different ways:
- Cell-intrinsic mechanism, upon activation, responding T cells begin to express CTLA-4 and shuts off further activation → terminate response
- Cell-extrinsic pathway, Tregs express high CTLA-4 and prevent activation of responding cells
- CTLA-4 functions as competitive inhibitor of CD28 and reduces the availability of B7 for the CD28 receptor
- CTLA-4 has 10- to 20-fold higher affinity for B7 than does CD28



PD-1 (programmed death-1)

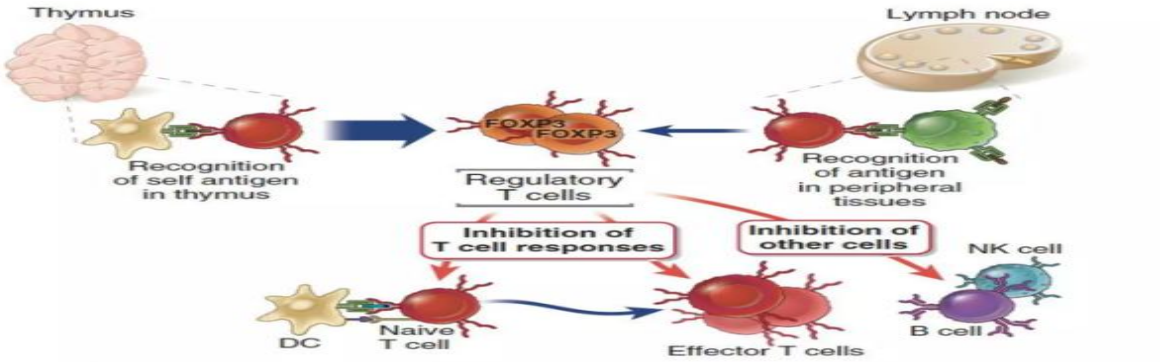
- Recognizes 2 ligands: PD-L1 (expressed on APCs and many other tissue cells) and PD-L2 (expressed mainly on APCs)
- Receptor PD-1 expressed on antigen-activated T cells



	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	$CD4^+$ and $CD8^+$	$CD8^+ > CD4^+$
Cellular expression	Tregs, activated T cells	Activated T cells
Main signals inhibited	Competitive inhibitor of CD28 costimulation (by binding to B7 with high affinity and removing B7 from APCs)	Inhibits kinase-dependent signals from CD28 and TCR (by recruiting and activating phosphatase following binding to its ligands PDL-1 or PDL-2)
Role in Treg-mediated suppression of immune responses	Yes	Probably no

Suppression by Regulatory T Cells

- Regulatory T cells are a subset of CD4+ T cells - suppress immune responses and maintain self-tolerance - Most of CD4+ Tregs express high levels of interleukin-2 (IL-2) receptor $\boldsymbol{\alpha}$ chain (CD25) and transcription factor called FoxP3 - FoxP3 - member of forkhead family of transcription factors, critical for the development and function of most Tregs



- Autoimmune disease called IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome caused by mutations in FOXP3 gene and associated with deficiency of Tregs

Phenotypic Markers and heterogeneity of Regulatory T Cells

- Regulatory role best established is CD4+ FoxP3+ CD25high
- FoxP3 and CD25 essential for generation, maintenance, and function
- Use IL-2 as their growth and survival factor
- FoxP3+ Tregs typically express high levels of CTLA-4

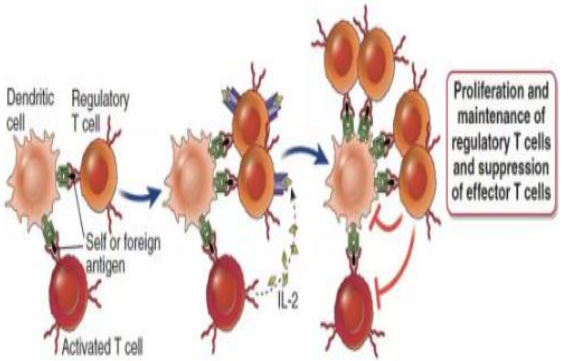
TABLE 4.1 Subsets of Treg Cells	
Treg Cells	**Suppressor Mechanism(s)**
Tr1, IL-10-Treg	IL-10, TGF-β, CTLA-4, PD-1
Th3	TGF-β, IL-10
$CD4^+$ $CD25^+$ Treg	Membrane TGF-β, CTLA-4, PD-1, GITR, IL-10
$CD8^+$ $CD25^+$ $CD28^-$ Treg	Same as $CD4^+$ $CD25^+$
$CD4^-$ $CD8^-$ Treg	Induction of apoptosis
TCR γδ Treg	IL-10, TGF-β

Forkhead Winged Transcription Factor, Foxp3

- Mutations diminishing the function of Foxp3 result in loss of the naturally occurring Treg compartment. Human subjects affected by X-linked autoimmune and allergic dysregulation syndrome (XLAAD) have significant skewing of patient T lymphocytes toward the Th2 phenotype
- In nTreg cells, FOXP3 can directly interact with Runt-related transcription factor 1 (RUNX1), which impairs expression of IL-2 and IFN-gamma and exerts suppressive activity
- Induction of RUNX1 and RUNX3 by TGF-b plays an essential role in generation and suppressive function of induced Treg cells
- RUNX1 and RUNX3 bind to FOXP3 promoter and activate induction of FOXP3-expressing functional Treg cells

Generation and Maintenance of Treg Cells

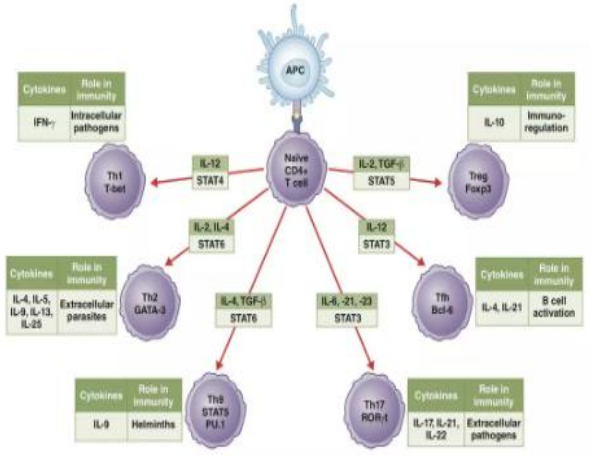
- **Tregs are generated mainly by self antigen recognition in thymus and by recognition of self and foreign antigens in peripheral lymphoid organs**
- Thymic regulatory T cells , natural Tregs
- In peripheral lymphoid organs, antigen recognition in absence of strong innate immune responses favors generation of regulatory cells from naive CD4+ T lymphocytes



- Peripheral regulatory T cells (pTreg), adaptive or inducible Tregs
- Generation of some Tregs requires cytokine transforming growth factor (TGF)-Β TGF-Β stimulates expression of FoxP3
- Survival and functional competence of Tregs dependent on cytokine IL-2
- IL-2 promotes differentiation of T cells into regulatory subset and also required for maintenance of Treg
- FoxP3+ Tregs do not produce IL-2, this growth factor is provided by conventional T cells responding to self or foreign antigens

Generation and Maintenance of Treg Cells

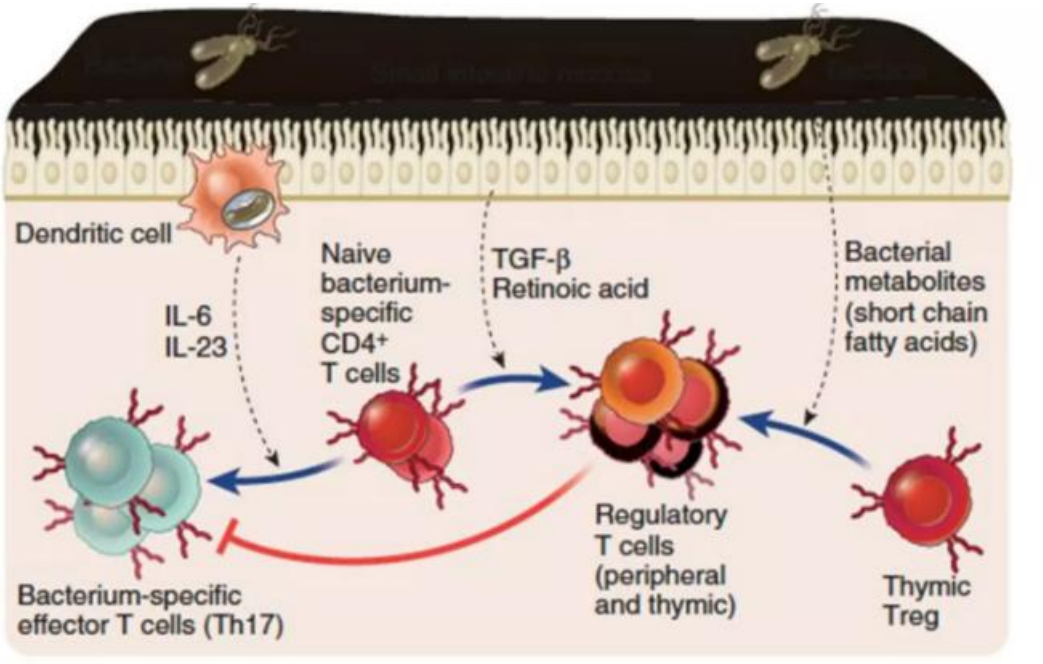
- IL-2 activates transcription factor STAT5, which enhance expression of FoxP3 as well as other genes that are involved in the function of Tregs
- Particular subsets of dendritic cells may be especially important for stimulating development of Tregs in peripheral tissues
- Dendritic cells exposed to retinoic acid, vitamin A analogue, inducers of Tregs, especially in mucosal lymphoid tissues



Molecular Mechanisms of Treg Cell Generation

- TGF-b contributes to generation of both Th17 and Treg cells
- TGF-b directs peripheral conversion of effector T cells into FOXP3+ Treg cells
- In the presence of IL-6, TGF-b promotes generation of Th17 from naïve T cells

- Retinoic acid also plays a key role in the balance of inflammatory Th17 cells and suppressive Treg cells by inhibiting the formation of Th17 cells and enhancing the expression of FOXP3 through STAT3/STAT5 independent signaling pathway



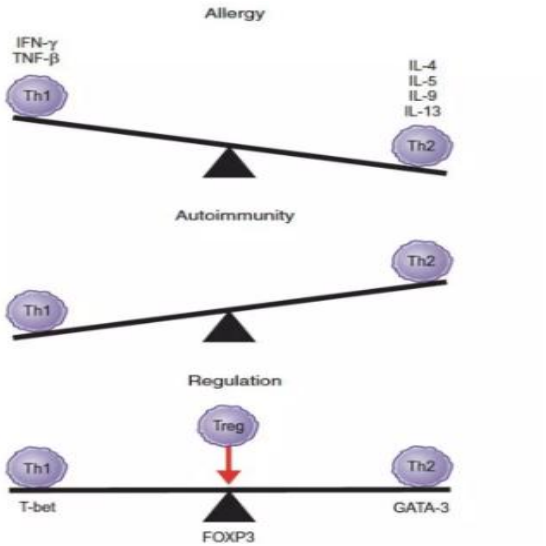
Regulation by Regulatory T Cells

- Regulatory T cells abundant in GALT and prevent inflammatory reactions against intestinal commensal microbes
- FOXP3+ Tregs induced in response to antigens encountered locally
- Generation of these peripheral Tregs include local production of retinoic acid and TGF-beta by CD103+ DCs and lamina propria macrophages -> promote FoxP3 expression and inhibit generation of Th1 and Th2 cells
- Fermentation metabolites, such as short-chain fatty acid butyrate produced by intestinal commensal bacteria, especially Clostridia species, stimulate peripheral expansion of thymic Tregs
- Tregs - suppress immune responses by several mechanisms, dominant mechanism in the gut seems to be production of immunosuppressive cytokine IL-10
- TGF-beta, IL-10, and IL-2 play crucial roles in maintaining homeostasis in gut immune system
- Deficiencies in these cytokines or their receptors result in pathologic bowel inflammation
- Mice with engineered deficiencies in TGF-beta, IL-10, IL-10 receptor, IL-2, and the IL-2 receptor is uncontrolled bowel inflammation

- Inflammation does not occur in mice raised in germ-free conditions
- Macrophages are another important source of IL-10
- Target cells express receptors for and regulated by TGF-& and IL-10 include DCs, effector T cells, innate effector cells such as macrophages, and epithelial cells
- Tregs require IL-2 for their maintenance

Mechanisms of Action of Regulatory T Cells

- Suppress immune responses at induction of T cell activation in lymphoid organs and the effector phase of these responses in tissues
- Directly suppress B cell activation
- Inhibit the proliferation and differentiation of natural killer (NK) cells



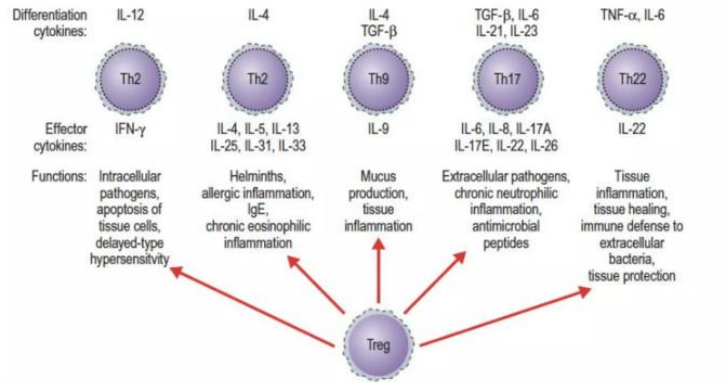


Fig. 4.6 Antigen presentation by dendrite cells to naïve T cells and other factors (innate immune response substances, vitamins, cytokines in the environment) induce the T cells to produce interleukins and differentiate into Th1, Th2, Th9, Th17, or Th22 cells.

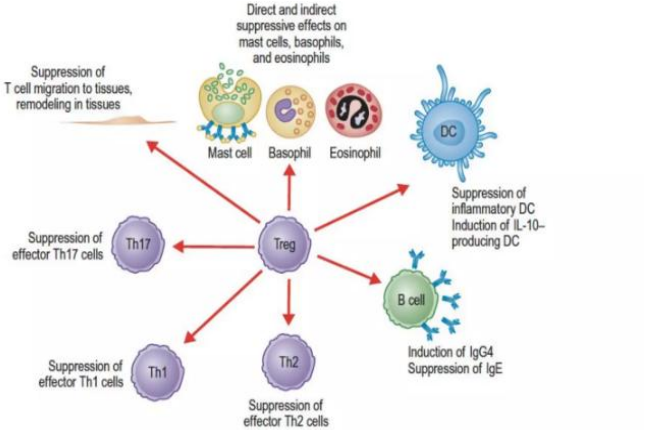


Fig. 4.7 Immune deviation toward Treg cell response is an essential step in allergen immunotherapy and natural allergen exposure of nonallergic individuals.

Mechanisms of Action of Regulatory T Cells

- Production of immunosuppressive cytokines IL-10 and TGF- B
- Reduced ability of APCs to stimulate T cells by binding of CTLA-4 on Tregs to B7 molecules on APCs, resulting in competitive inhibition of CD28-mediated costimulation
- **Consumption of IL-2** High level of expression of IL-2 receptor, these cells absorb IL-2 and deprive other cell populations of this growth factor, resulting in reduced proliferation and differentiation of other IL-2–dependent cells

Inhibitory Cytokines Produced by Treg Cells

TGF-b and IL-10 involved in generation and functions of Tregs

Transforming Growth Factor-b

- TGF-b1 produced by CD4+ Tregs, activated macrophages, and many other cell types
- TGF-b has many important and diverse roles in immune system
- TGF-b inhibits proliferation and effector functions of T cells and activation of macrophages TGF-b also suppresses the activation of other cells, such as neutrophils and endothelial cells
- TGF-b regulates differentiation of functionally distinct subsets of T cells

    - TGF-b stimulates development of peripheral FoxP3+ Tregs

    - In combination with IL-1 and IL-6, TGF-b promotes development of Th17 subset of CD4+ T cells (opposing actions depending on context in which it is produced)

    - TGF-b can also inhibit development of Th1 and Th2 subsets
- TGF-b stimulates production of immunoglobulin A (IgA) antibodies by inducing B cells to switch to this isotype
- TGF-b promotes tissue repair after local immune and inflammatory reactions subside - Stimulate collagen synthesis and matrix-modifying enzyme production by macrophages and fibroblasts and by promoting angiogenesis -> fibrosis and sclerosis

Interleukin-10

- IL-10 - inhibitor of activated macrophages and dendritic cells and involved in control of innate immune reactions and cell-mediated immunity
- IL-10 is produced by many immune cell populations, including activated macrophages and dendritic cells, Tregs, and Th1 and Th2 cells, some B lymphocytes (regulatory B cells
- IL-10 inhibits production of IL-12 by activated dendritic cells and macrophages, IL-10 inhibit IFN-gamma production
- IL-10 inhibits expression of costimulators and class II MHC molecules on dendritic cells and macrophages

TABLE 4.2 Functions of IL-10 and Transforming Growth Factor-β

Cell Type	IL-10	Transforming Growth Factor-β
Dendritic cells	Inhibits DC maturation, leading to reduced MHC class II and costimulatory ligand expression Inhibits proinflammatory cytokine secretion Inhibits APC function for induction of T cell proliferation and cytokine production (Th1 and Th2)	Promotes Langerhans cell development Inhibits DC maturation and antigen presentation Downregulates FcεRI expression on Langerhans cells
T cells	Suppresses allergen-specific Th1 and Th2 cells Blocks B7/CD28 costimulatory pathway on T cells	Promotes T cell survival Inhibits proliferation, differentiation and effector function, including allergen-specific Th1 and Th2 cells Promotes the Th17 lineage
B cells and Ig	Enhances survival Promotes Ig production, including IgG4	Inhibits proliferation Induces apoptosis of immature or naïve B cells Inhibits most Ig class switching Switch factor for IgA
Breg cells	All functions shown by Treg cells are possible and induction of IgG4	All functions shown by Treg cells are possible
IgE	Suppresses allergen-specific IgE	Suppresses allergen-specific IgE
$CD25^+$Treg	Indirect effect on the generation	Upregulates Foxp3 Promotes generation in the periphery Potential effects on homeostasis
IL-10-Treg	Promotes IL-10-Treg induction	Can promote IL-10 synthesis
Monocytes/ macrophages	Inhibits proinflammatory cytokine production and antigen presentation	Inhibits scavenger and effector functions including proinflammatory cytokine production and antigen presentation Promotes chemotaxis
Eosinophils	Inhibits survival and cytokine production	Chemoattractant
Mast cells	Inhibits mast cell activation, including cytokine production	Promotes chemotaxis Variable effects on other functions; may inhibit expression of FcεR
Neutrophils	Inhibits chemokine and proinflammatory cytokine production	Potent chemoattractant

Loss of Suppressive Capacity of Treg Cells During Inflammatory Responses

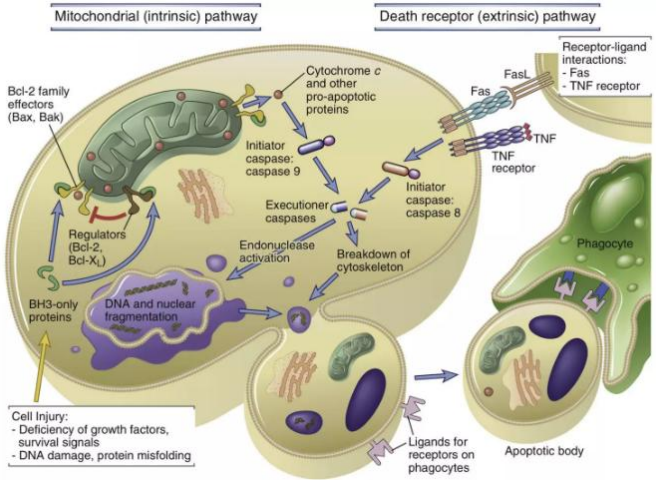
- Strong proinflammatory signals lead to loss of Treg function
- Activation of DC through TLR leads to production of signals, including IL-6, which block the suppressive effect of CD4+CD25+ Treg cells
- IL-6 can promote disease via 2 mechanisms: directly enhancing Th2 responses and overcoming suppressive function of CD4+CD25+ Treg cells
- TNF-alpha as well as IL-7 and IL-15 also overcome regulatory activity

Deletion of T Cells by Apoptotic Cell Death

- T lymphocytes that recognize self antigens with high affinity or are repeatedly stimulated by antigens may die by apoptosis
- 2 major pathways of apoptosis, peripheral deletion of mature T cells:
    - Mitochondrial (or intrinsic) pathway
    - Death receptor (or extrinsic) pathway

These 2 death pathways may function in different ways to maintain self-tolerance

- T cells that recognize self antigens in absence of costimulation may activate Bim, resulting in apoptosis by mitochondrial pathway
- In normal immune responses, responding lymphocytes receive signals from TCR, costimulators, and growth factors
- These signals stimulate expression of because of relative lack of costimulation and growth factors, anti-apoptotic members of Bcl-2 family, Bcl-2 and Bcl-XL, are expressed at low levels, and actions of Bim, Bax, and Bak are thus not counteracted



- Repeated stimulation of T cells results in coexpression of death receptor Fas and its ligand Fas-L, and engagement of Fas triggers apoptotic death
- When T cells repeatedly activated, FasL is expressed on cell surface, and it binds to surface Fas on the same or adjacent T cells → activates cascade of caspases, which ultimately cause apoptotic death

Factors Determine Tolerogenicity of Self Antigens

	Features That Favor Stimulation of Immune Responses	Features That Favor Tolerance
Persistence	Short-lived (eliminated by immune response)	Prolonged, leading to persistent antigen receptor engagement
Portal of entry; location	Subcutaneous, intradermal; absence from generative organs	Intravenous, mucosal; presence in generative organs
Presence of adjuvants	Antigens with adjuvants: stimulate helper T cells	Antigens without adjuvants: absence of costimulation
Properties of APCs	Mature dendritic cells: High levels of costimulators	Immature (resting) dendritic cells: Low levels of costimulators and cytokines

Deletion of T Cells by Apoptotic Cell Death

- Tissue dendritic cells - normally in a resting (immature) state and express low levels of costimulators; constantly presenting self antigens without providing strong costimulation, and T cells that recognize these antigens become anergic or differentiate into regulatory T lymphocytes instead of effector and memory lymphocytes
- By contrast, dendritic cells activated by microbes are the principal APCs for initiating T cell responses
- Local infections and inflammation may activate resident dendritic cells, leading to increased expression of costimulators, breakdown of tolerance, and autoimmune reactions

B Lymphocyte Tolerance

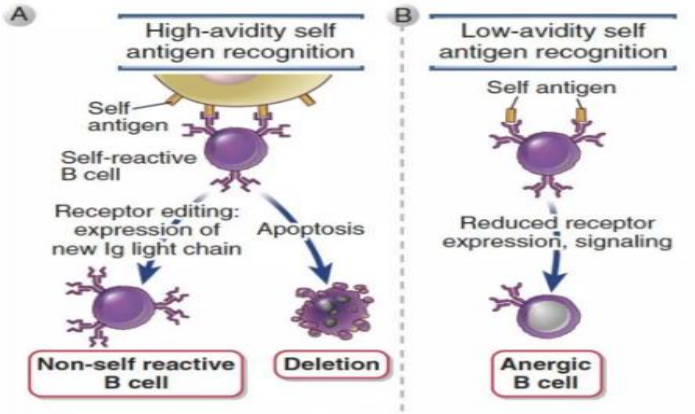
- Maintaining unresponsiveness to thymus-independent self antigens, such as polysaccharides and lipids
- Preventing antibody responses to protein antigens

Central B Cell Tolerance

Immature B lymphocytes that recognize self antigens in the bone marrow with high affinity change their specificity or are deleted Receptor editing

Receptor editing

- B cells reactivate their RAG1 and RAG2 genes and initiate new round of VJ recombination in the Ig kappa light chain gene locus
- Previously rearranged V kappa J kappa exon in self-reactive immature B cell is deleted, and new Ig light chain is expressed, thus creating BCR with new specificity



Deletion

- If editing fails, immature B cells may die by apoptosis

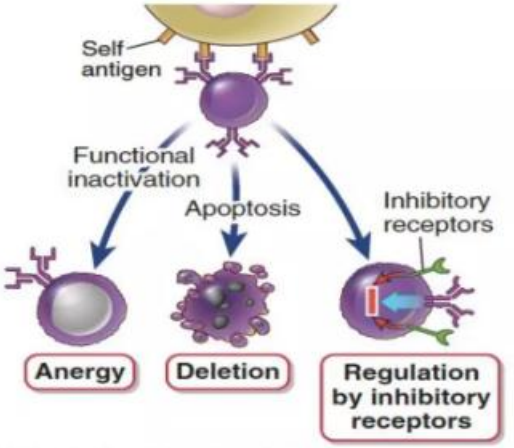
Anergy

- If developing B cells recognize self antigens weakly (e.g., if the antigen is soluble and does not cross-link many antigen receptors or if the BCRs recognize the antigen with low affinity), cells become functionally unresponsive (anergic) and exit bone marrow in unresponsive state
- Anergy is due to downregulation of antigen receptor expression and a block in antigen receptor signaling

Peripheral B Cell Tolerance

Mature B lymphocytes that recognize self antigens in peripheral tissues in absence of specific helper T cells may be rendered functionally unresponsive or die by apoptosis

- Signals from helper T cells may be absent if these T cells are deleted or anergic or if the self antigens are non-protein antigens
- As in T cells, antigen recognition without additional stimuli results in tolerance



Anergy and deletion

- Some self-reactive B cells that are repeatedly stimulated by self antigens become unresponsive
- Require higher than normal levels of the growth factor BAFF for survival
- Have shortened life span
- B cells that bind with high avidity to self antigens in periphery may also undergo apoptotic death by mitochondrial pathway

Signaling by inhibitory receptors

- Immunoreceptor tyrosine-based activation motifs (ITIMs) in cytoplasmic tail of CD22 are phosphorylated by Lyn, and this inhibitory receptor then recruits SHP-1, thus attenuating B cell receptor signaling

Tolerance to Commensal Microbes

- Commensal microbes - abundant in the gut, skin, and other tissues but do not elicit immune responses despite being foreign
- Many of these microbes cannot invade epithelial barriers and not accessible to adaptive immune system
- Commensal microbes elicit little or no innate immunity and thus fail to induce costimulators and other signals that are required for effective adaptive immune responses
- Also induce and activate Tregs

Tolerance to Other Foreign Antigens

- Protein antigens administered with adjuvants favor immunity
- Repeated doses of antigens administered without adjuvants tend to induce tolerance (adjuvants stimulate innate immune responses and expression of costimulators on APCs)
- Oral administration of protein antigen often leads to suppression of systemic humoral and cell-mediated immune responses to immunization with the same antigen = oral tolerance