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T CELL DEVELOPMENT

1. T CELLS DEVELOP IN THE THYMUS
2. THREE TYPES OF THYMIC EPITHELIAL CELL HAVE IMPORTANT ROLES IN T CELL PRODUCTION
3. STEM CELL MIGRATION TO THE THYMUS INITIATES T CELL DEVELOPMENT
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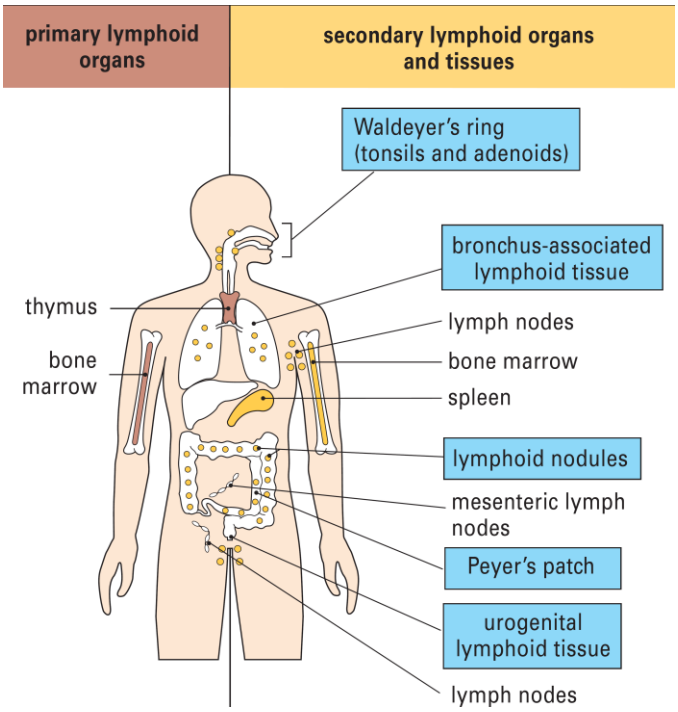
15. HOW REGULATORY T CELLS INACTIVATE TRADITIONAL T CELLS.

REFERENCE

1. ROITT'S ESSENTIAL IMMUNOLOGY
2. KUBY IMMUNOLOGY
3. CELLULAR AND MOLECULAR IMMUNOLGY ABBAS
4. IMMUNOLGY MALE AND BROSTOFF
5. IMMUNOLOGY KHAN
6. CELL BRUCE ALBERTS



1. Thymus and bone marrow are the primary (central) lymphoid organs. They are the sites of maturation for T and B cells, respectively.
2. Cellular and humoral immune responses occur in the secondary (peripheral) lymphoid organs and tissues.
3. Secondary lymphoid organs can be classified according to the body regions they defend.
   a. The spleen responds predominantly to blood-borne antigens. Lymph nodes mount immune responses to antigens circulating in the lymph, entering through the skin (subcutaneous lymph nodes) or through mucosal surfaces (visceral lymph nodes).
   b. Tonsils, Peyer's patches, and other mucosa-associated lymphoid tissues (MALT) (blue boxes) react to antigens that have entered via the surface mucosal barriers.
   c. Note that the bone marrow is both a primary and a secondary lymphoid organ because it gives rise to B and NK cells, but is also the site of B cell terminal differentiation (long-lived plasma cells).

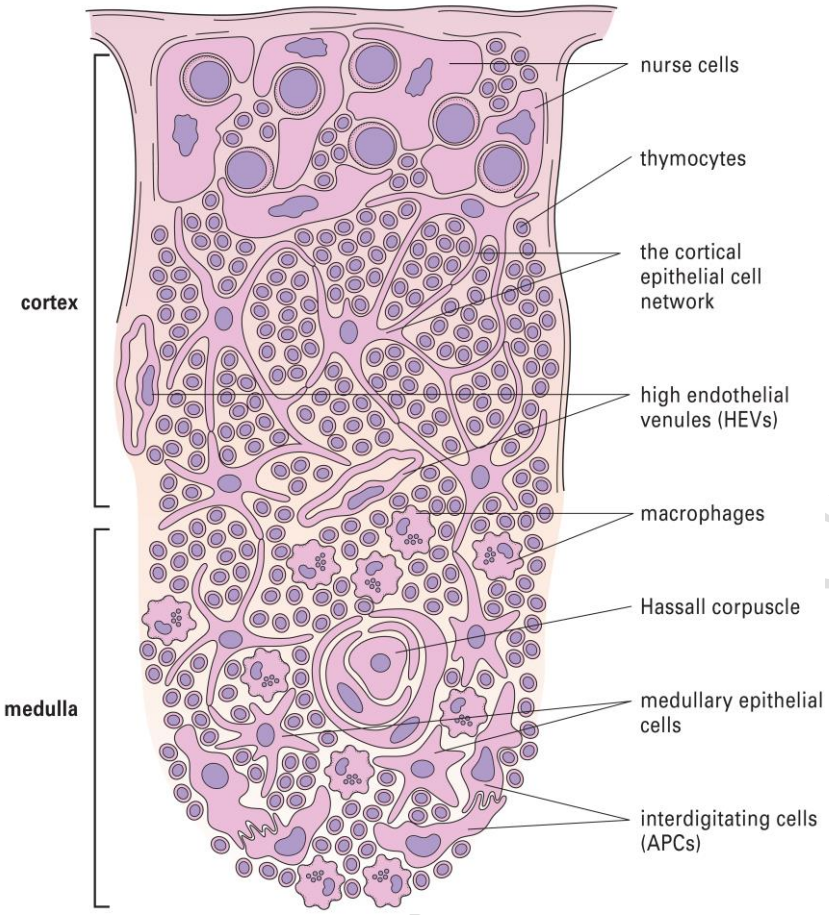
1. T CELLS DEVELOP IN THE THYMUS

1. The thymus in mammals is a bilobed organ in the thoracic cavity overlying the heart and major blood vessels. Each lobe is organized into lobules separated from each other by connective tissue trabeculae.
2. Within each lobule, the lymphoid cells (thymocytes) are arranged into:
   - an outer tightly packed cortex, which contains the majority of relatively immature proliferating thymocytes; and
   - an inner medulla containing more mature cells, implying a differentiation gradient from cortex to medulla
3. The main blood vessels that regulate cell traffic in the thymus are ***high endothelial venules*** (at the corticomedullary junction of thymic lobules. It is through these veins that T cell progenitors formed in the ***fetal liver and bone marrow*** enter the **epithelial anlage** and migrate towards the cortex.
4. In the cortex of the thymus the T cell progenitors undergo proliferation and differentiation processes that lead to the generation of mature T cells through a corticomedullary gradient of migration.

2. THREE TYPES OF THYMIC EPITHELIAL CELL HAVE IMPORTANT ROLES IN T CELL PRODUCTION

1. At least three types of epithelial cell can be distinguished in the thymic lobules according to distribution, structure, function, and phenotype:
   1. the ***epithelial nurse cells*** are in the outer cortex; sustain the proliferation of progenitor T cells, mainly through cytokine production (e.g. IL-7);
   2. the ***cortical thymic epithelial cells (TECs)*** form an epithelial network responsible for the positive selection of maturing thymocytes, allowing survival of cells that recognize MHC class I and II molecules with associated peptides via TCRs of intermediate affinity
   3. the ***medullary TECs*** are mostly organized into clusters display a large variety of **organ-specific self-peptides through transcription factors such as AIRE (autoimmune regulator).**
4. ***Hassall's corpuscles*** are found in the thymic medulla. Their function is unknown, but they appear to contain degenerating epithelial cells rich in high molecular weight ***cytokeratins.***

5. The mammalian thymus involutes with age. In humans, atrophy begins at puberty and continues throughout life. Thymic involution begins within the cortex and this region may disappear completely, whereas medullary remnants persist.
6. Cortical atrophy is related to a sensitivity of the cortical thymocytes to corticosteroid, and all conditions associated with an acute increase in corticosteroids (e.g. pregnancy and stress) promote thymic atrophy.



A schematic representation of the cell types found in a fully developed thymic lobule.

1. Subcapsular epithelial cells that produce IL-7 (nurse cells) sustain T lymphoblast proliferation in the outer cortex.
2. Developing T cells interact with the cortical epithelial network where they are positively selected. Apoptotic cells are phagocytosed by macrophages present in the deep cortex and in the medulla.
3. TCRβ thymocytes co-expressing CD4 and CD8 undergo the process of negative selection by interacting with a variety of antigen-presenting cells
4. (APCs), such as dendritic cells, interdigitating cells, macrophages, and epithelial cells.
5. T cells that have survived the selection processes are exported from the thymus via high endothelial venules (HEVs) and lymphatic vessels.

3. STEM CELL MIGRATION TO THE THYMUS INITIATES T CELL DEVELOPMENT

1. The thymus develops from the endoderm of the ***third pharyngeal pouch*** as an epithelial rudiment that becomes seeded with blood-borne stem cells. Once in the thymus, the stem cells begin to differentiate into ***thymic lymphocytes (called thymocytes)***, under the influence of the epithelial microenvironment.
2. ***Notch-1 receptor*** has proved to be essential for T cell development, and is involved in T versus B cell fate determination through interaction with thymic epithelial cells expressing Notch ligands.
3. ***Epithelial cells, macrophages***, and ***bone marrow-derived IDCs***, molecules rich in ***MHC class II***, are important for the differentiation of T cells from this multipotent stem cell. For example, specialized epithelial cells in the peripheral areas of the cortex (the ***thymic nurse cells***, see above) contain thymocytes within pockets in their cytoplasm. The nurse cells support lymphocyte proliferation by producing the ***cytokine IL-7.***
4. The ***subcapsular region*** of the thymus is the only site where thymocyte proliferation occurs.
5. Thymocytes develop into ***large, actively proliferating, self-renewing lymphoblasts***, which generate the thymocyte population. Most mature ***T cells leave the thymus via HEVs*** at the corticomedullary junction, though other routes of exit may exist, including lymphatic vessels.

4. T CELLS CHANGE THEIR PHENOTYPE DURING MATURATION

As with the development of granulocytes and monocytes, 'differentiation' markers of functional significance appear or are lost during the progression from stem cell to mature T cell.

A. STAGE I THYMOCYTES ARE $CD4^-$, $CD8^-$

1. There are two phases of stage I (early) thymocytes.
2. ***In the first phase,*** *the* TCR genes are in the germline configuration and the cells:
    i. express CD44 and CD25;
    ii. are $CD4^-$, $CD8^-$ (i.e. double negative cells).
    a. In this early first phase, cells entering the thymus via the HEVs in the corticomedullary junction express CD44, which allows them to migrate towards the outermost cortex, the zone of thymocyte proliferation. These cells are **not fully committed to the T cell lineage**, because outside the thymic environment they can give rise to other hematopoietic lineages. Surface expression of **CD44** is downregulated once the cells are in the external cortex.
3. ***In the second phase the cells:***
    a. become $CD44^-$;
    b. are $CD25^+$
    c. remain double negative for CD4 and CD8;
    d. rearrange the β chain of the TCR;
    e. express cytoplasmic but not surface TCR-associatedCD3;
    f. are irreversibly committed to become T cells with continuous expression of Notch1;
    g. continue to express CD7 together with CD2 and CD5.
4. Proliferation markers such as the transferrin receptor (CD71) and CD38 (a marker common to all early hematopoietic precursors) are also expressed at this stage.

B. STAGE II THYMOCYTES BECOME $CD4^+$, $CD8^+$

1. Stage II (intermediate or common) thymocyte cells account for around 80% of thymocytes in the fully developed thymus.
2. Characteristically they:
    a. are CD1, $CD44^-$, $CD25^-$;
    b. become $CD4^+$, $CD8^+$ (double positives).
3. Genes encoding the TCR a chain are rearranged in these intermediate thymocytes; both chains of the TCR are expressed at low density on the cell surface in association with polypeptides of the CD3/antigen receptor complex.

C. STAGE III THYMOCYTES BECOME EITHER $CD4^+$ OR $CD8^+$

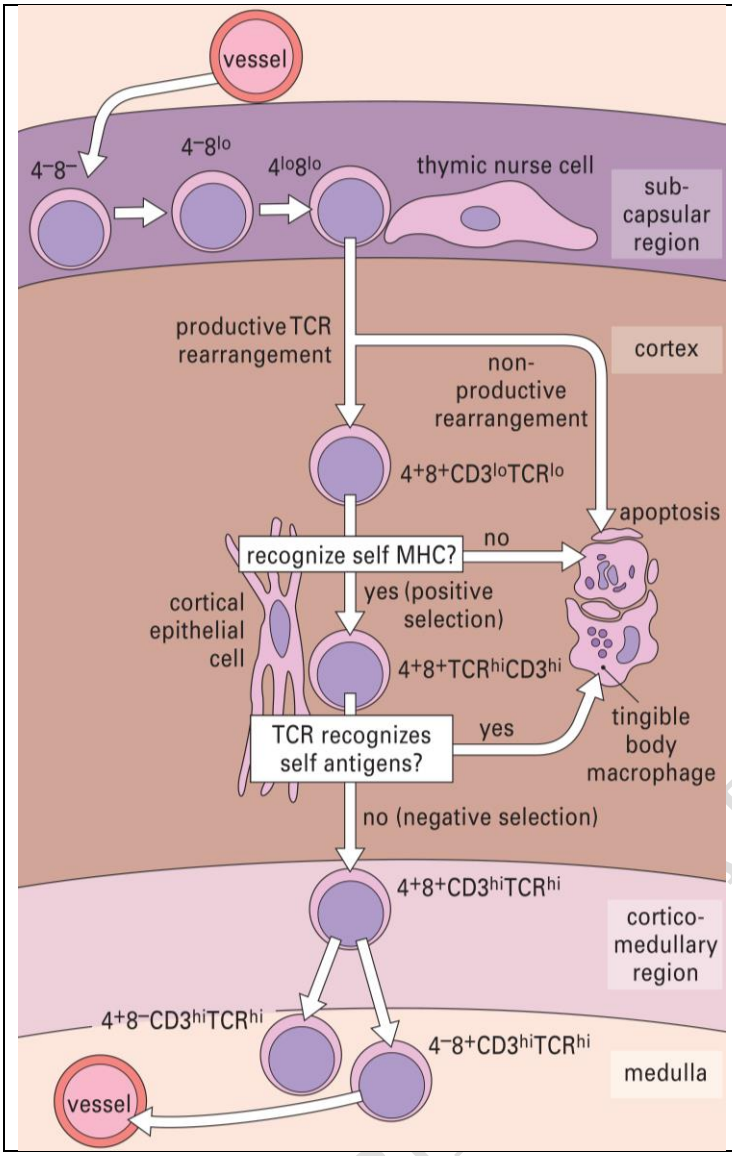
1. Stage III (mature) thymocytes show major phenotypic changes, namely:
    i. loss of CD1;
    ii. cell surface CD3 associated with the ab TCR expressed at a higher density;
    iii. the distinction of two subsets of cells expressing either CD4 or CD8 (i.e. single positives).
2. Most stage III thymocytes:
    i. lack CD38 and the transferrin receptor;
    ii. are virtually indistinguishable from mature, circulating T cells.
3. All stage III cells re-express the ***receptor CD44,*** which is thought to be involved in migration and homing to peripheral lymphoid tissues. ***L-selectin (CD62L)*** is also expressed at this time. The T cell receptor is generated during development in the thymus.

5. THE T CELL RECEPTOR IS GENERATED DURING DEVELOPMENT IN THE THYMUS

TCR gene recombination takes place within the subcapsular and outer cortex of the thymus, where there is active cell proliferation.

Through a random assortment of different gene segments, a large number of different TCRs are made and thymocytes that fail to make a functional receptor die. The TCRs associate with peptides of the CD3 complex, which transduces activating signals to the cell.

6. POSITIVE AND NEGATIVE SELECTION OF DEVELOPING T CELLS TAKES PLACE IN THE THYMUS



In this model, pre-thymic T cells are attracted to and enter the thymic rudiment at the corticomedullary junction.

1. They reach the subcapsular region where they proliferate as large lymphoblasts, which give rise to a pool of cells entering the differentiation pathway.
2. Many of these cells are associated with epithelial thymic nurse cells.
3. Cells in this region first acquire CD8 and then CD4 at low density. They also rearrange their T cell receptor (TCR) genes and may express the products of these genes at low density on the cell surface.
4. Maturing cells move deeper into the cortex and adhere to cortical epithelial cells. These epithelial cells are elongated and branched, and thus provide a large surface area for contact with thymocytes.
5. The TCRs on the thymocytes are exposed to epithelial MHC molecules through these contacts. This leads to positive selection. Those cells that are not selected undergo apoptosis and are phagocytosed by macrophages.
6. There is an increased expression of CD3, TCR, CD4, and CD8 during thymocyte migration from the subcapsular region to the deeper cortex.
7. Those TCRs with self-reactivity are now deleted through contact with autoantigens presented by medullary thymic epithelial cells, interdigitating cells, and macrophages at the corticomedullary junction – a process called negative selection.
8. Following this stage, cells expressing either CD4 or CD8 appear and exit to the periphery via specialized vessels at the corticomedullary junction.

1. Positive selection ensures only TCRs with an intermediate affinity for self MHC develop further.
2. T cells:
    a. recognize antigenic peptides only when presented by self MHC molecules on APCs; and
    b. show 'dual recognition' of both the antigenic peptides and the polymorphic part of the MHC molecules.
3. **Positive selection (the first stage of thymic education)** ensures that only those TCRs with an intermediate affinity for self MHC are allowed to develop further. There is evidence that positive selection is mediated by TECs acting as APCs.
    a. T cells displaying very high or very low receptor affinities for self MHC undergo apoptosis and die in the cortex.
    b. T cells with TCRs that have intermediate affinities are rescued from apoptosis, survive, and continue along their
    c. pathway of maturation. *A possible exception is provided by some T cells equipped with γδ receptors, which (like B cells) recognize native antigenic conformations with no need for APCs.*
4. **Negative selection** ensures that only T cells that fail to recognize self-antigen proceed in their development. Some of the positively selected T cells may have TCRs that recognize self-components other than self MHC.

a. These cells are deleted by a 'negative selection' process, which occurs:
    1. in the deeper cortex;
    2. at the corticomedullary junction; and
    3. in the medulla.
b. T cells interact with antigen presented by interdigitating cells, macrophages, and medullary TECs.
c. The role of medullary TECs for negative selection has been emphasized recently by the finding that these cells express genes for virtually all tissue antigens in the body, and that these genes are activated by certain transcription factors (TF) to express ***these antigens (e.g. AIRE).***
d. Only T cells that fail to recognize self-antigen are allowed to proceed in their development. The rest undergo apoptosis and are destroyed. These, and all the other apoptotic cells generated in the thymus, are phagocytosed by (tangible body) macrophages in the deep cortex.
e. T cells at this stage of maturation ($CD4^+$ $CD8^+$ TCR^{lo}) go on to express TCR at high density and lose either CD4 or CD8 to become 'single positive' mature T cells.
f. The separate subsets of CD4 and CD8 cells possess specialized homing receptors (e.g. CD44), and exit to the T cell areas of the peripheral (secondary) lymphoid tissues where they function as mature 'helper' and 'cytotoxic' T cells, respectively.

7. ADHESION OF MATURING THYMOCYTES TO EPITHELIAL AND ACCESSORY CELLS IS CRUCIAL FOR T CELL DEVELOPMENT

1. Adhesion of maturing thymocytes to epithelial and other accessory cells is mediated by the interaction of complementary adhesion molecules, such as:
    a. CD2 with LFA-3 (CD58); and
    b. LFA-1 (CD11a, CD18) with ICAM-1 (CD54).
2. These interactions induce the production of the cytokines IL-1, IL-3, IL-6, IL-7, and GM-CSF, which are required for T cell proliferation and maturation in the thymus.
3. Early thymocytes also express receptors for IL-2, which together with IL-7 sustains cell proliferation.

8. NEGATIVE SELECTION MAY ALSO OCCUR OUTSIDE THE THYMUS IN PERIPHERAL LYMPHOID TISSUES

1. Not all self-reactive T cells are eliminated during intra-thymic development, probably because not all self antigens can be presented in the thymus. The thymic epithelial barrier that surrounds blood vessels may also limit access of some circulating antigens.
2. Given the survival of some self-reacting T cells, a separate mechanism is required to prevent them attacking the body.
3. Experiments with transgenic mice have suggested that peripheral inactivation of self-reactive T cells (peripheral tolerance) could occur via several mechanisms as follows:
    a. downregulation of the TCR and CD8 (in cytotoxic cells) so that the cells are unable to interact with target autoantigens;
    b. anergy, due to the lack of crucial co-stimulatory signals provided by the target cells, followed by induction of apoptosis after interaction with autoantigen;
    c. regulatory T cells (Tregs).

9. REGULATORY T CELLS ARE INVOLVED IN PERIPHERAL TOLERANCE

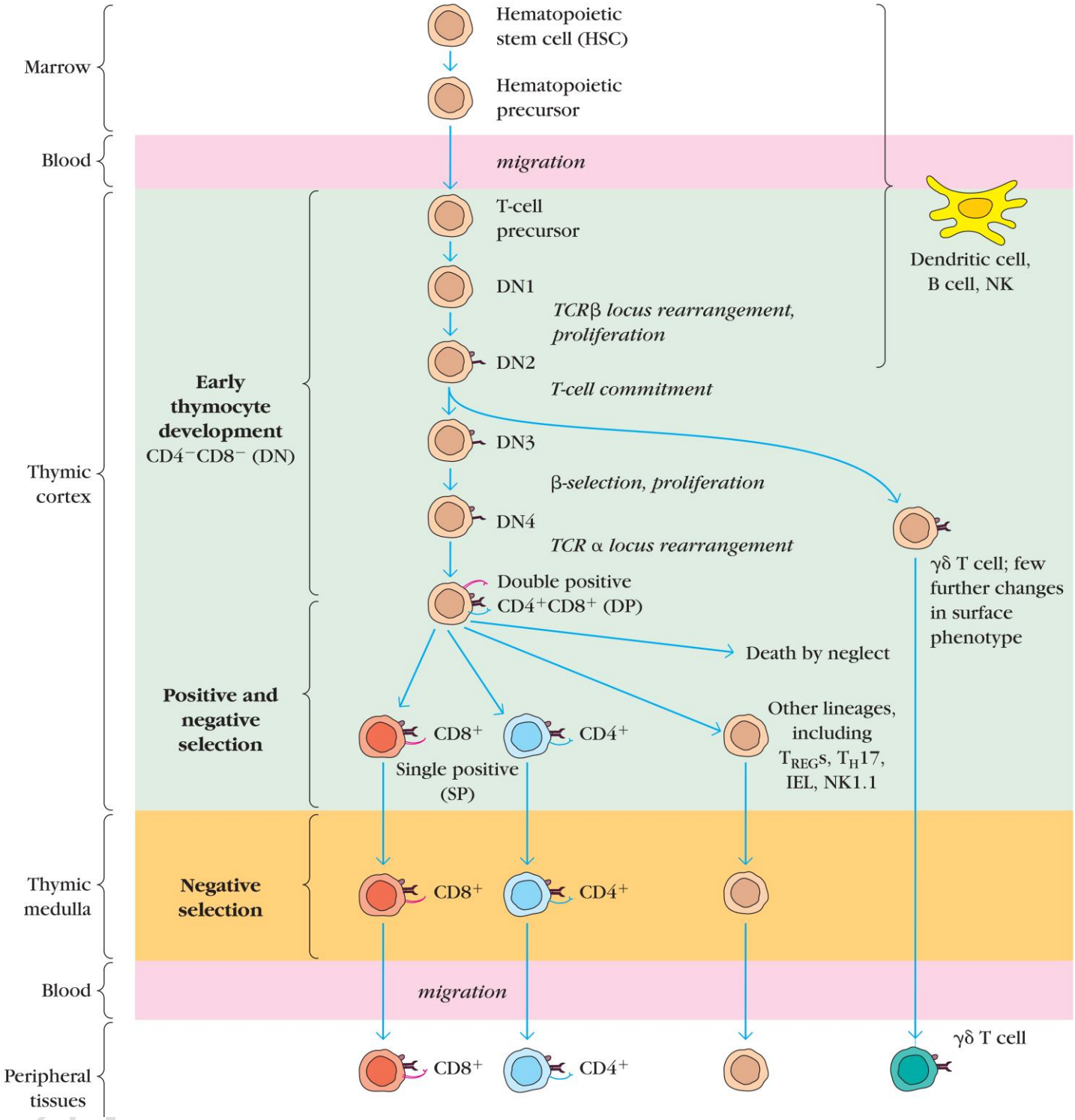
1. Tregs have been the subject of intensive research over the past few years, especially in the areas of autoimmunity and vaccine development.
2. In addition to NKT cells and γδ T cells regulating immune responses, there is now substantial evidence that separate $CD4^+$ subsets also have this function. The general consensus is that there are two main types of Treg – naturally occurring, and inducible following activation by specific antigen.
3. Naturally occurring Tregs:
    a. constitutively express CD25 (the a chain of the receptor for IL-2);

   b. constitute about 5–10% of the peripheral CD4+ T cells;
   c. express the unique transcription factor FoxP3;
   d. constitutively express the marker CTLA4;
   e. do not proliferate in response to antigenic challenge;
   f. are thought to produce their suppressive effects through cell contact (e.g. with APCs, TH1 or TH2 cells).
4. Antigen-induced Tregs:
   a. also express CD25;
   b. can develop from CD25–, CD4
   c. are believed to exert their suppressive effects through IL-10.

10. THERE IS SOME EVIDENCE FOR EXTRATHYMIC DEVELOPMENT OF T CELLS

1. The vast majority of T cells require a functioning thymus for differentiation, but small numbers of cells carrying T cell markers that are often oligoclonal in nature have been found in athymic ('nude') mice.
2. The importance of extrathymic development in animals that are euthymic (i.e. that have a normal thymus) is at present unclear.

11. DEVELOPMENT OF T CELLS IN MOUSE



1. T-cell precursors from the bone marrow travel to the thymus via the bloodstream, undergo development to mature T cells, and are exported to the periphery, where they can undergo antigen induced activation and differentiation into effector cells and memory cells.

2. Each stage of development occurs in a specific microenvironment and is characterized by specific intracellular events and distinctive cell-surface markers.

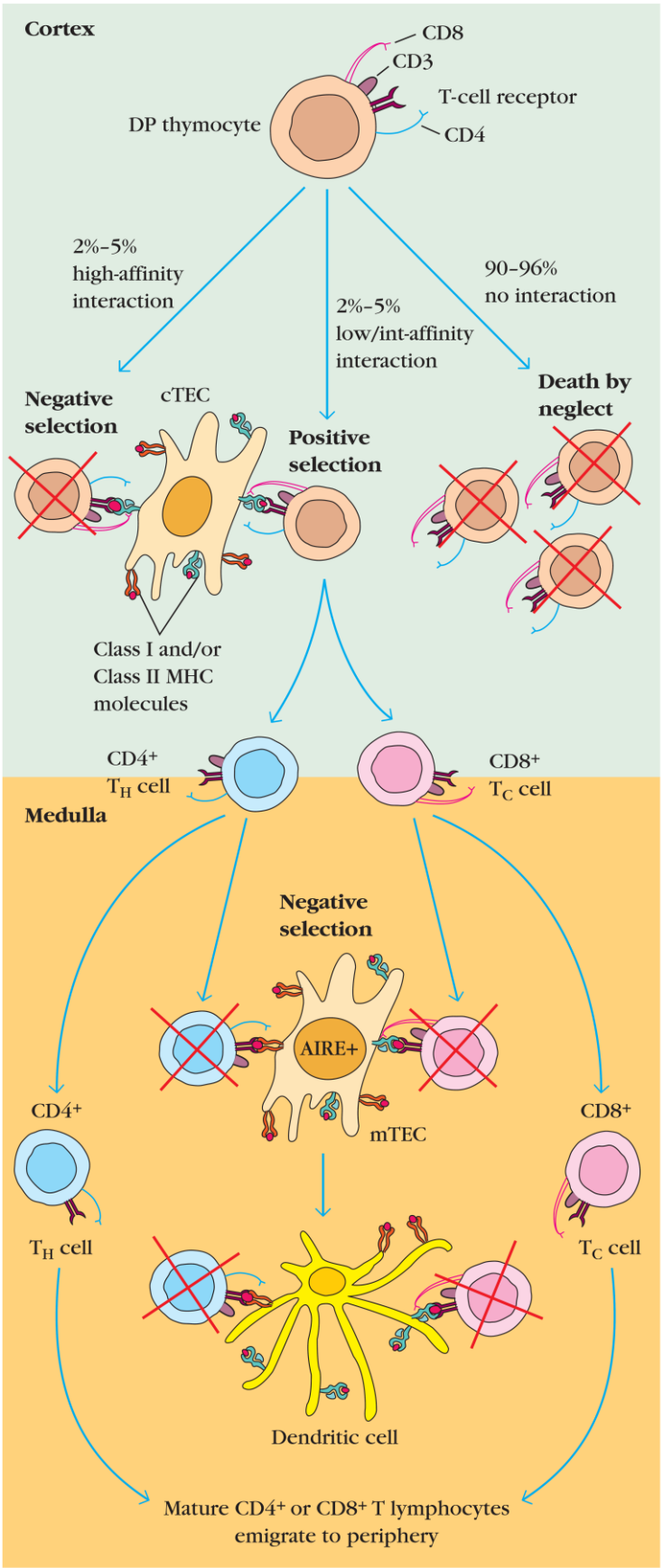
3. The most immature thymocytes are $CD4^-CD8^-$(double negative, DN) and pass through several stages (DN1-DN4) during which they commit to the T-cell lineage and begin to rearrange their T-cell receptor (TCR) gene loci. Those that successfully rearrange their TCRβ chain proliferate, initiate rearrangement of their TCRα chains, and become $CD4^+CD8^+$(double positive, DP) thymocytes, which dominate the thymus. DP thymocytes undergo negative and positive selection in the thymic cortex.

4. Positively selected thymocytes continue to mature and migrate to the medulla, where they are subject to another round of negative selection to self-antigens that include tissue-specific proteins.

5. Mature T cells express either CD4 or CD8 (single positive, SP) and leave the thymus with the potential to initiate an immune response. Although most thymocytes develop into conventional **TCR αβ** $CD4^+$or $CD8^+$ T cells, some DN and DP thymocyte cells develop into other cell lineages, including lymphoid dendritic cells, **TCR γδ** T cells, natural killer T cells (NKT), regulatory T cells (TREG), and intraepithelial lymphocytes (IELs), each of which has a distinct function.

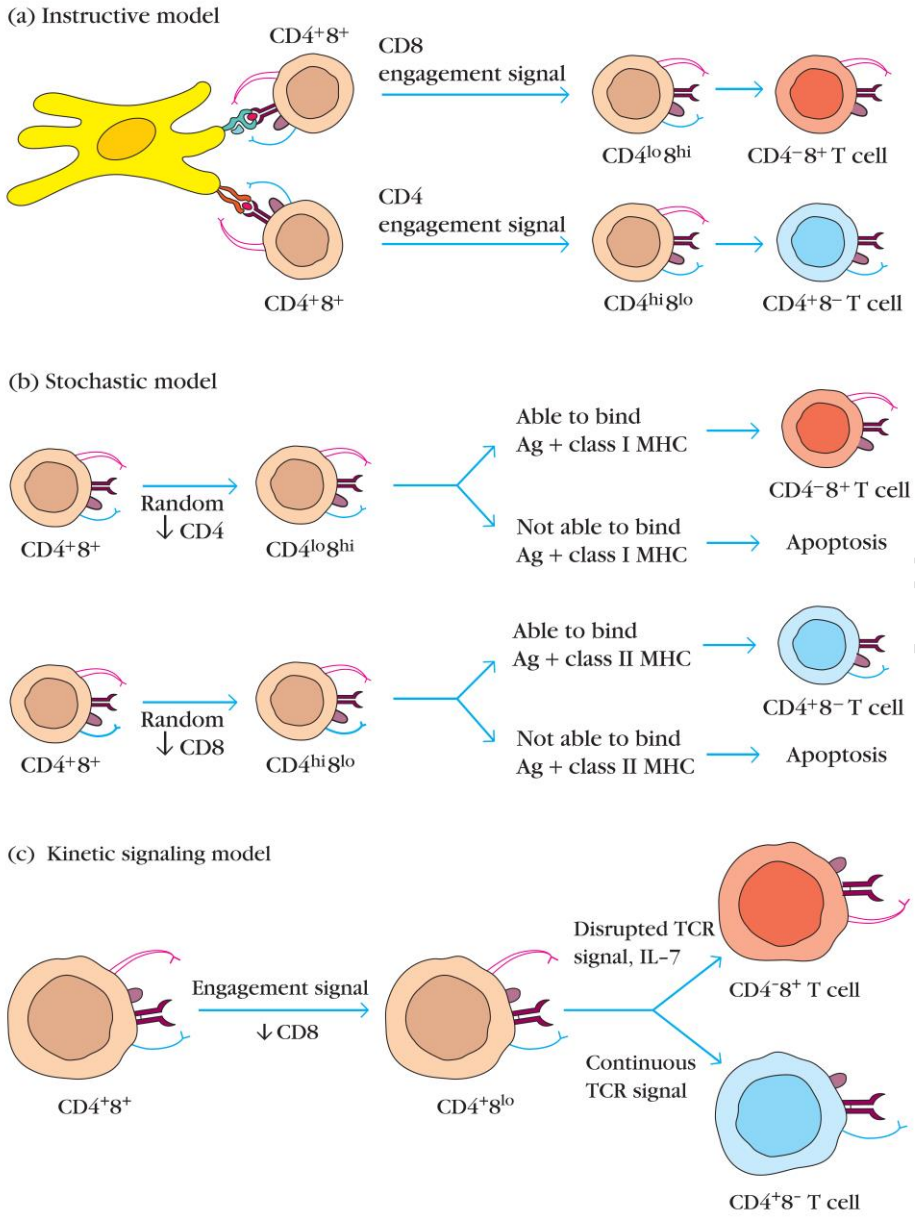
	Genotype	Location	Description
DN1	c-kit $(CD117)^{++}$, $CD44^+$, $CD25^-$	Bone marrow to thymus	Migration to thymus
DN2	c-kit $(CD117)^{++}$, $CD44^+$, $CD25^+$	Subcapsular cortex	TCRγ, δ, and β chain rearrangement; T-cell lineage commitment
DN3	c-kit $(CD117)^+$, $CD44^-$, $CD25^+$	Subcapsular cortex	Expression of pre-TCR; β selection
DN4	c-kit $(CD117)^{low/-}$, $CD44^-$, $CD25^-$	Subcapsular cortex to cortex	Proliferation, allelic exclusion of β-chain locus; α-chain locus rearrangement begins; becomes DP thymocyte

12. POSITIVE AND NEGATIVE SELECTION



1. Thymic selection involves multiple interactions of DP and SP thymocytes with both the cortical and medullary thymic stromal cells, as well as dendritic cells and macrophages. Selection results in a mature
2. T-cell population that is both self-MHC restricted and self-tolerant. DP thymocytes that express new TCR__ dimers browse the MHC/peptide complexes expressed by the *c*ortical *t*hymic *e*pithelial *c*ells (cTECs). The large majority of DP thymocytes die in the cortex by neglect because of their failure to bind MHC/peptide combinations with sufficient affinity.
3. The small percentage whose TCRs bind MHC/peptide with high affinity die by clonal deletion (negative selection).
4. Those DP thymocytes whose receptors bind to MHC/peptide with intermediate affinity are positively selected and mature to single positive ($CD4^+$ or $CD8^+$) T lymphocytes.
5. These migrate to the medulla, where they are exposed to AIRE_ *m*edullary *t*hymic *e*pithelial *c*ells (mTECs), which express tissue-specific antigens and can mediate negative selection.
6. Medullary dendritic cells can acquire **mTEC** antigens by engulfing **mTEC**s, and mediate negative selection (particularly of MHC Class II restricted $9CD4^u$ thymocytes).

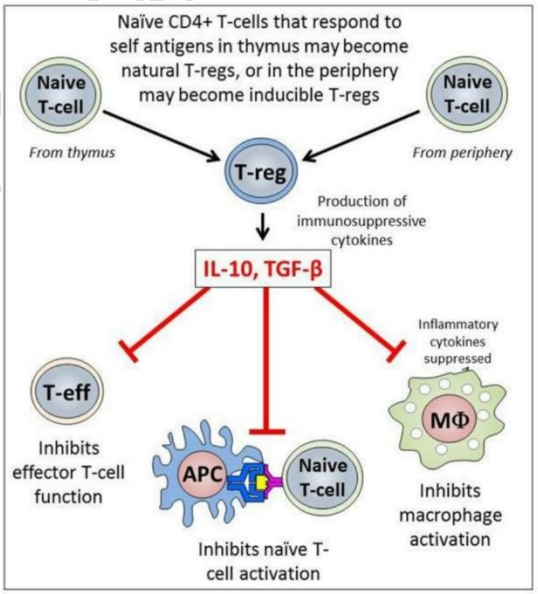
13. PROPOSED MODELS OF LINEAGE COMMITMENT, the decision of double-positive thymocytes to become helper CD4⁺ or cytotoxic CD8⁺ T cells.

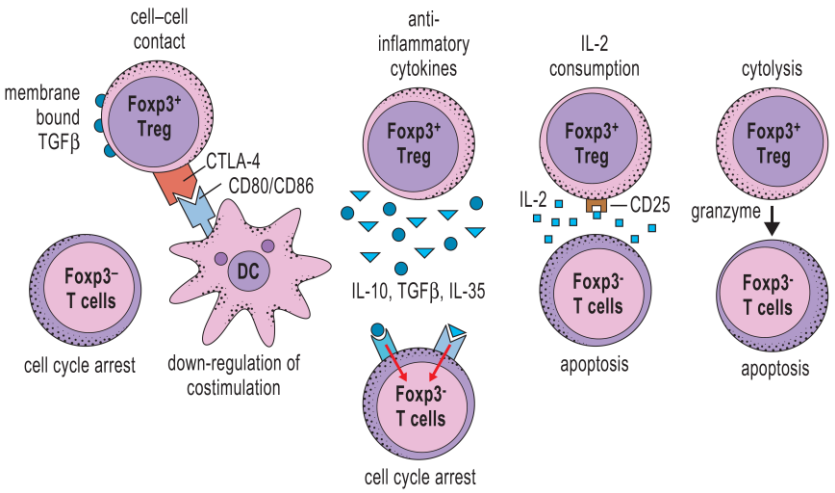


1. **According to the instructive model**, interaction of a coreceptor with the MHC molecule for which it is specific results in down-regulation of the other coreceptor.
2. **According to the stochastic model**, down-regulation of CD4 or CD8 is a random process.
3. **According to the kinetic signaling model**, the decision to commit to the CD4 or CD8 lineage is based on the continuity of the TCR signal that a thymocyte receives. **Positive selection** results in down-regulation of CD8 on all thymocytes. This will not alter the intensity of a TCR/CD4/MHC Class II signal, and cells receiving this signal will continue development to the CD4 SP lineage. However, down-regulation of CD8 diminishes (interrupts) a TCR/CD8/MHC Class I signal, an experience that sends a cell toward the CD8 lineage. IL-7 signals are required to "seal" CD8 lineage commitment.

14. LYMPHOCYTES; POLARIZING SIGNALS.

1. **There are three types of lymphoid cells: B cells, T cells, and natural killer (NK) cells.** B and T cells are members ofclonal populations distinguished by antigen receptors of unique specificity.
    a. **B cells synthesize and display membrane antibody, and**
    b. **T cells synthesize and display T-cell receptors (TCRs).**
    c. **NK cells do not synthesize antigen specific receptors;**
    d. **a small population of TCR expressing T cells have features of NK cells and are called NKT cells.**
2. T cells can be further subdivided into
    a. **helper T cells, which typically express CD4 and recognize pMHC class II, and**
    b. **cytotoxic T cells, which typically express CD8 and recognize pMHC class I.**
3. In broad terms, **T helper type 1 (T_H1) cells** and **T helper type 17 (T_H17) cells** (the latter so named because they secrete IL-17) regulate our response to intracellular pathogens, and
4. **T helper type 2 (T_H2) cells** and **T follicular helper (T_{FH}) cells** regulate our response to extracellular pathogens, such as bacteria and parasitic worms.
5. Each $CD4^+$ **T_H**-cell subtype produces a different set of cytokines that enable or "help" the activation of B cells, T cells, macrophages, and various other. **Which helper subtype dominates a response depends largely on what type of pathogen (intracellular versus extracellular, viral, bacterial, fungal, helminth) has infected an animal.**
6. Another type of $CD4^+$ T cell, the **regulatory T cell (T_{REG})**, has the unique capacity to inhibit immune responses.
7. These cells, called natural T cells, arise during maturation in the thymus from cells that bind self proteins with high affinity (autoreactive cells).
8. They can also can be induced at the site of an immune response in an antigen-dependent manner **(iT_{REG} cells).**
9. Regulatory T cells are identified by the presence of CD4 and CD25 on their surfaces, as well as by the expression of the internal transcription factor FoxP3.
10. **T_{REG}** cells quell autoreactive responses and play a role in limiting our normal T-cell responses to pathogens.

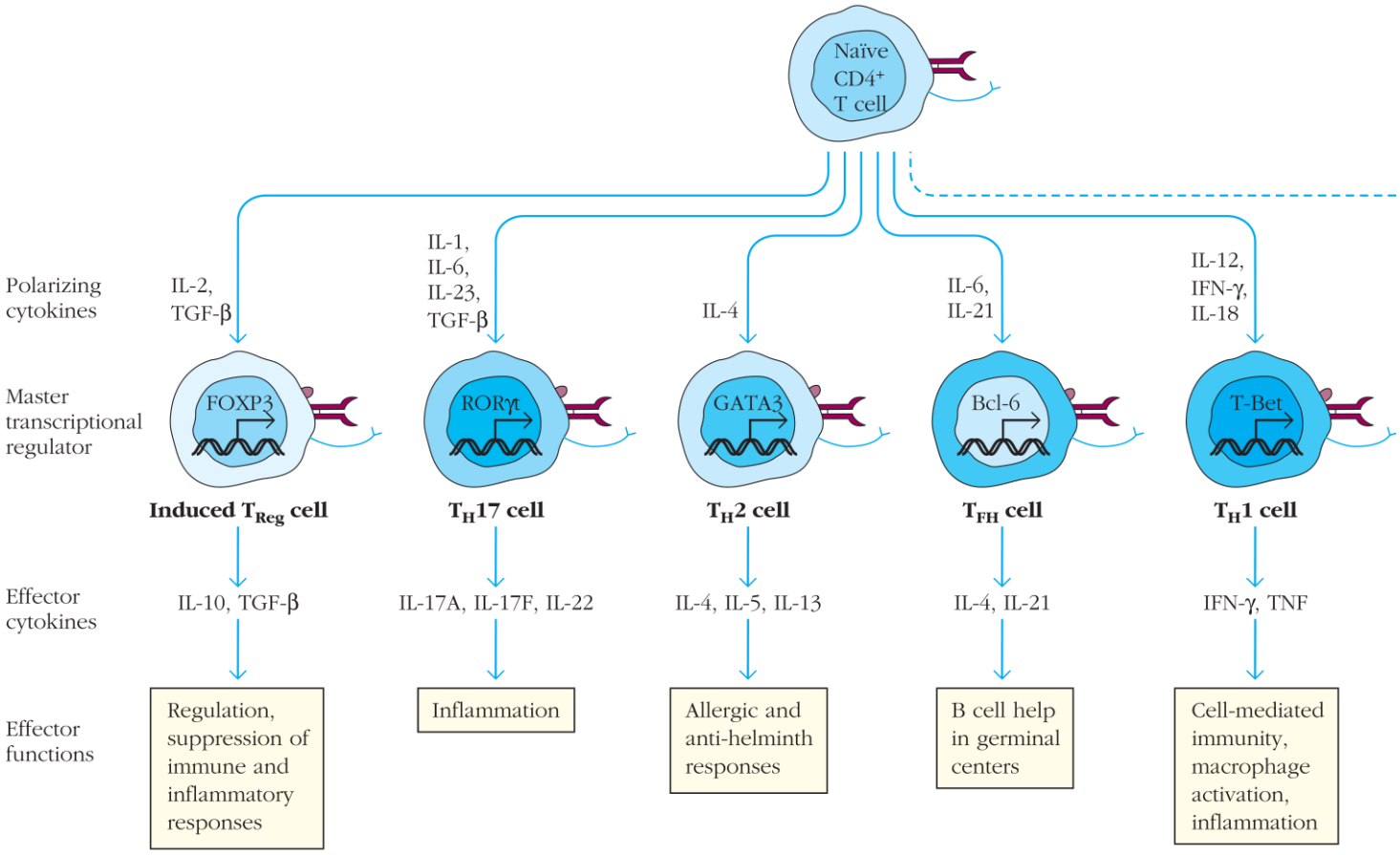




T_{REGS} MAY SUPPRESS BY A VARIETY OF MECHANISMS.

1. Via cell-to-cell contact
2. (secreted or cell surface molecules such as CTLA-4 expression, or membrane bound TGFβ).
3. Release of suppressor cytokines such as IL-10, TGFb and IL-35.
4. IL-2 consumption (Tregs can express high levels of CD25, the IL-2 receptor).
5. Cytolysis, akin to CD8+ T cell killing.

T Helper Subset Differentiation



This figure synthesizes current information about the distinguishing features of T helper subset differentiation and activity. Polarizing cytokines, master transcriptional regulators, effector cytokines, and broad functions in health and disease are depicted for each of the major helper subsets. Neither cross-regulation nor the potential plasticity in differentiation among subsets is depicted, but both are described in the text. *[Adapted from S. L. Swain, K. K. McKinstry, and T. M. Strutt, Expanding roles for CD41 T cells in immunity to viruses,* Nature Reviews. Immunology 12:*136–148.]*

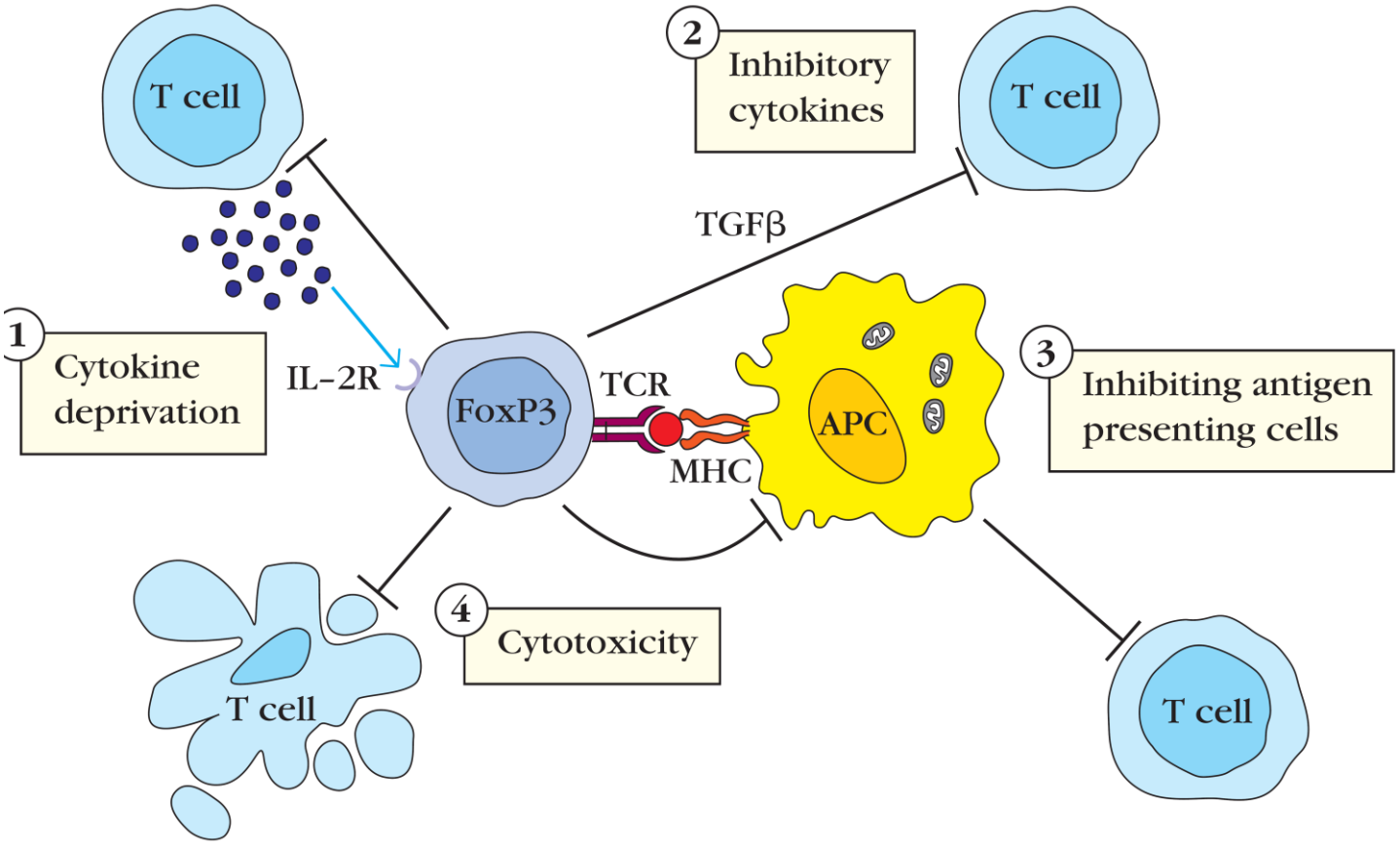
15. HOW REGULATORY T CELLS INACTIVATE TRADITIONAL T CELLS.

1. T_{REG} cells can develop in the thymus and appear to represent an alternative fate for autoreactive T cells. As we have seen, most thymocytes that express receptors with high affinity for self-antigen die via negative selection.
2. However, a small fraction appear to commit to the regulatory T-cell lineage and leave the thymus to patrol the body and thwart autoimmune reactions.

What determines whether a self-reactive thymocyte dies or differentiates into a T_{REG} cell is still an open question.

1. Investigators are currently trying to understand if the choice is made based on subtle differences in affinity for self or on differences in their maturation state when they receive a high-affinity signal.

2. Recent work suggests that T_{REGs} develop in a unique microenvironmental niche within the thymus, and that the available space for developing cells in this niche is limited.
3. These findings suggest that thymocytes that commit to the regulatory T-cell lineage are likely to receive unique stimulatory signals. These *natural* T_{REGs} share the periphery with *induced* TREGs that can develop from conventional mature T cells that are exposed to TGF-β and IL-10 cytokines



Some possible mechanisms of **T_{REG}** activity are illustrated in this schematic. These may all contribute to quelling immune responses in vivo.

1. ***Cytokine deprivation*. T_{REGs}** express relatively high levels of high-affinity IL-2 receptors and can compete for the cytokines that activated T cells need to survive and proliferate.
2. ***Cytokine inhibition***. **T_{REGs}** secrete several cytokines, including IL-10 and TGF-_, which bind receptors on activated T cells and reduce signaling activity.
3. ***Inhibition of antigen presenting cells***. T_{REGs} can interact directly with MHC Class II expressing antigen-presenting cells and inhibit their maturation, leaving them less able to activate T cells.
4. ***Cytotoxicity*. T_{REGs}** can also display cytotoxic function and kill cells by secreting perforin and granzyme.