Janeway's Immunobiology, 9th Edition
Chapter 8: Development and Survival of Lymphocytes

Development of B Lymphocytes

8-1 Lymphocytes derive from hematopoietic stem cells in the bone marrow

8.1 **True/False:** B and T lymphocytes develop from multipotent hematopoietic stem cells in the bone marrow. This process entails a continuum of development in which cells show progressive loss of multipotent potential, eventually becoming committed to a single lineage.

8-2 B-cell development begins by rearrangement of the heavy chain locus

8.2 **Multiple choice:** A key step in the development of B cells is the expression of the RAG-1 and RAG-2 recombinase proteins. The up-regulation of RAG-1 and RAG-2 in early pro-B cells is induced by:

A. The cytokine IL-7, which is made by bone marrow stromal cells
B. The chemokine CXCL12, which is made by bone marrow stromal cells
C. The B cell-specific transcription factors E2A and EBF
D. Signaling through the Igα subunit of the B-cell receptor complex
E. Signaling through the FLT3 receptor tyrosine kinase binding to membrane-bound FLT3

8.3 **Multiple choice:** B cell development in the bone marrow is an inherently wasteful process. Nearly half of the pro-B cells produced will die without progressing on to the next stage of B cell development. This massive loss of pro-B cells is due to:

A. The failure of many pro-B cells to up-regulate Pax5 and become committed to the B cell lineage.
B. The inability of many pro-B cells to proceed with rearranging a V_H to their rearranged DJ_H sequence.
C. Large insertions of untemplated nucleotides into the rearranged gene by TdT.
D. Detrimental DJ_H rearrangements on both alleles of the immunoglobulin heavy chain locus.
E. The failure of the pro-B cell to make a complete immunoglobulin heavy chain protein.

8-3 The pre-B-cell receptor tests for successful production of a complete heavy chain and signals for the transition from the pro-B cell to the pre-B cell stage

8.4 **Multiple choice:** The pre-B-cell receptor provides an important signal that induces transition of pro-B cells to pre-B cells. An important characteristic of this receptor is that:

A. It signals without binding to an extracellular ligand.
B. It is composed of immunoglobulin heavy chains and the VJ region of a rearranged λ light chain.
C. It is expressed at very high levels on the surface of the pro-B cell.

D. It signals without requiring association with B-cell receptor signaling subunits, Igα and Igβ.
E. It signals without requiring the B-cell receptor signaling kinase, BTK.

8-4 Pre-B-cell receptor signaling inhibits further heavy-chain locus rearrangement and enforces allelic exclusion

8.5 **Short answer:** A wild-type mouse that is heterozygous for two immunoglobulin heavy chain alleles ($IgH^{a/b}$) generates the population of B cells shown on the left of **Figure Q8.5**. A mouse strain, also $IgH^{a/b}$, carries an inactivating mutation in the VpreB gene. In addition to producing fewer mature B cells than the wild-type mice, the VpreB-deficient mice generate B cells as shown on the right.

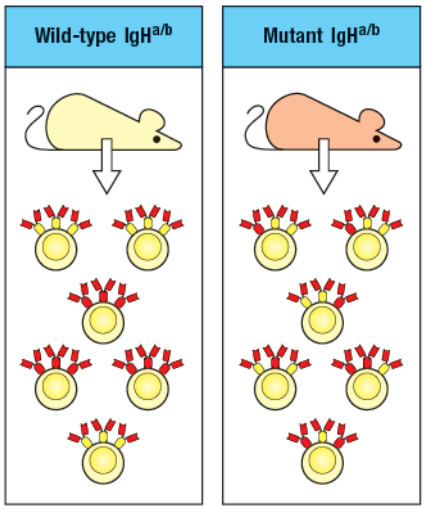

Figure Q8.5

What is the explanation of the difference seen between the wild-type and the VpreB-mutant B cells?

8-5 Pre-B cells rearrange the light-chain locus and express cell surface immunoglobulin

8.6 **Multiple choice:** In different mammalian species, the ratio of B cells expressing κ versus λ light chain-containing antibodies is about 65%:35%. In other species, such as mice, this ratio is vastly different, at 95%:5%. If a routine blood test performed on an individual revealed that their κ-expressing versus λ-expressing B cells were seen at a ratio of 95%:5%, this would likely indicate that the individual had:

A. An increased number of functional Vκ gene segments compared to the average human in the population
B. A defect in allelic exclusion of antibody light chain genes
C. A defect in isotypic exclusion of antibody light chains
D. A lymphoproliferative disorder
E. A genetic defect in one of their two λ light chain alleles

8-6 Immature B cells are tested for autoreactivity before they leave the bone marrow

8.7 **True/False:** Immature B cells expressing sIgM receptor emigrate from the bone marrow into the circulation. This is a passive process of cell diffusion, requiring no active signaling by the B cell.

8.8 **Multiple choice:** Autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are characterized by high levels of circulating autoreactive antibodies in the patient's circulation. An analysis of developing B cells in the bone marrow of these individuals might reveal that in some cases:

A. Developing B cells become anergic in response to strong B-cell receptor cross-linking by multivalent self-antigens.
B. Developing B cells are excluded from the B-cell follicles in the bone marrow and undergo rapid cell death.
C. sIgM-positive developing B cells show premature down-regulation of RAG recombinase proteins prior to the onset of receptor editing.
D. Hyperactive B-cell receptor signaling leads to rapid turnover of self-reactive developing B cells.
E. Developing B cells have defects in allelic exclusion and express two different B-cell receptors on their surface.

8-7 Lymphocytes that encounter sufficient quantities of self antigens for the first time in the periphery are eliminated or inactivated

8.9 **Multiple choice:** Self-reactive B cells can be eliminated from the repertoire at several stages of B cell maturation, including immature B cells that have already emigrated from the bone marrow into the circulation. This latter stage of tolerance induction is critical because:

A. Not all self-antigens are expressed or present in the bone marrow during B cell development.
B. Immature circulating B cells are more sensitive to antigen stimulation than the developing B cells in the bone marrow.
C. Receptor editing is not a perfect process and some self-reactive B cells may fail to be eliminated in the bone marrow.
D. Circulating immature B cells do not encounter tissue-specific antigens in peripheral organs and tissues.
E. Immature B cells are trapped in the bone marrow by strong B-cell receptor cross-linking.

8-8 Immature B cells arriving in the spleen turn over rapidly and require cytokines and positive signals through the B-cell receptor for maturation and long-term survival

8.10 **Multiple choice:** Individuals that overexpress the cytokine BAFF show increased susceptibility to autoimmune diseases such as Sjögren's syndrome, a disease that targets the exocrine glands that produce saliva, tears, and other bodily secretions. If one examined the circulating antibodies in these patients, one would expect to find:

A. Increased development of B cells in the bone marrow
B. A failure of receptor editing of immunoglobulin light chain genes in the bone marrow
C. An increased rate of immature B cell export from the bone marrow

D. Reduced B-cell receptor signaling following strong cross-linking of the receptor
E. An increased number of circulating mature autoreactive B cells

8.11 **Short answer:** Marginal zone B cells are thought to represent a lineage of cells important in rapid responses to blood-borne antigens. What are the two characteristics of these cells that indicate this function?

8-9 B-1 B cells are an innate lymphocyte subset that arises early in development

8.12 **Multiple choice:** B-1 B cells are considered a component of the innate rather than the adaptive immune response. The antibodies produced by B-1 B cells generally recognize capsular polysaccharide antigens found on many bacteria and viruses. These antibodies are considered part of the innate immune response because:
A. They recognize pathogens rather than innocuous harmless antigens.
B. They are produced prior to the exposure to the pathogen.
C. They are specific for carbohydrate rather than protein antigens.
D. They are secreted by B-1 B cells starting at 48 hour post-infection.
E. They are not generated by the process of VD-J recombination of immunoglobulin genes.

Development of T lymphocytes

8-10 T-cell progenitors originate in the bone marrow, but all the important events in their development occur in the thymus

8.13 **Multiple choice:** Genetically inherited immunodeficiency diseases can result from defects in nearly any component of the immune response. The most severe forms of immunodeficiency occur when T cells are absent or non-functional. An individual with normal B cells, but an absence of T cells might have a defect in:
A. RAG-1 or RAG-2 recombinase proteins
B. Terminal deoxynucleotidyl transferase (TdT)
C. Hematopoietic stem cells
D. Bone marrow stromal cells
E. Thymic stromal cells

8-11 Commitment to the T-cell lineage occurs in the thymus following Notch signaling

8.14 **True/False:** Progenitor cells that migrate from the bone marrow to the thymus are not yet committed to the T cell lineage. T cell lineage commitment occurs as a result of signals received by the progenitor cell from thymic epithelial cells.

8-12 T-cell precursors proliferate extensively in the thymus, but most die there

8.15 **Multiple choice:** The thymic cortex has a substantial population of macrophages in addition to the developing T cells (i.e., thymocytes). These macrophages are extremely useful in:
A. Eliminating bacterial infections in the thymus

B. Producing cytokines that promote T cell maturation
C. Engulfing apoptotic thymocytes
D. Maintaining the structural integrity of the thymic organ
E. Inducing inflammatory signals to increase blood flow to the thymus

8-13 Successive stages in the development of thymocytes are marked by changes in cell-surface molecules

8-14 Thymocytes at different developmental stages are found in distinct parts of the thymus

Questions 8.16 and 8.17 use the following information and Figure Q8.16. These questions may be used independently or as a group.

The mouse thymus normally contains about 1–2 × 10^8 thymocytes, the vast majority of which are $CD4^+CD8^+$ (double-positive) cells. When thymocytes from mice with a gene deficiency in the TCRα locus are compared with those from TCRβ-deficient mice, a striking difference between the two different knockout lines is observed, as shown in **Figure Q8.16** in a simplified version of flow cytometry data. The numbers of thymocytes in each thymus is indicated below the plots.

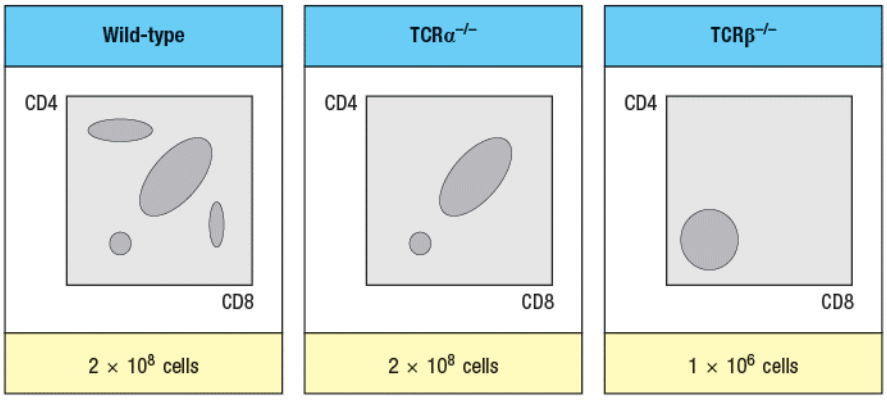

Figure Q8.16

8.16 **Short answer:** What is the explanation for the difference in thymocyte subsets and cell numbers observed when comparing $TCR\alpha^{-/-}$ to $TCR\beta^{-/-}$ thymocytes?

8.17 **Short answer:** Which region of the thymus organ would show a dearth of developing thymocytes in the $TCR\alpha^{-/-}$ thymus? Which region in the $TCR\beta^{-/-}$ thymus?

8-15 T cells with α:β or γ:δ receptors arise from a common progenitor

8.18 **Multiple choice:** Two distinct lineages of T cells can be identified based on their expression of α:β versus γ:δ T-cell receptors. A deficiency in the signaling receptor Notch1 would result in:
A. A loss of α:β but not γ:δ T cells
B. A loss of both of α:β and γ:δ T cells
C. A loss of α:β T cells and a massive expansion of γ:δ T cells

D. An increased number of both of α:β and γ:δ T cells
E. A normal number of both of α:β and γ:δ T cells

8-16 T cells expressing γ:δ TCRs arise in two distinct phases during development

8.19 Multiple choice: Unlike α:β T cells, γ:δ T cells are considered to be components of the innate immune system. One feature of γ:δ T cells that leads to their classification as innate cells is:
A. That they migrate from the thymus directly to barrier surfaces such as mucosa and epithelia
B. Their ability to produce pro-inflammatory cytokines
C. That their T-cell receptors are germline encoded rather than a product of VD-J recombination
D. Their rapid turnover in the tissue, with an average survival time of 3–4 days
E. Their ability to make growth factors that act on epithelial cells rather than other hematopoietic cells

8.20 True/False: One feature of γ:δ T cells that identifies them as innate, rather than adaptive, lymphocytes is their ability to produce effector cytokines within hours of initial activation.

8.21 Multiple choice: Like the γ:δ T cells in other specific mucosal surfaces, the γ:δ T cells that reside in the epithelial surface of the skin:
A. Are slow to respond to activation signals, requiring several days of priming and differentiation
B. Are able to produce cytokines that simultaneously induce type I, type II, and type III immune responses
C. Are primarily responsible for recruiting macrophages and dendritic cells to the tissue
D. Are characterized by the homogeneous expression of a single specific Vγ and Vδ in their T-cell receptors
E. Are unlikely to play any role in immunity to infection, but are likely important for tissue repair

8-17 Successful synthesis of a rearranged β chain allows the production of a pre-T-cell receptor that triggers cell proliferation and blocks further β-chain gene rearrangement

8.22 Short answer: If one swapped the regulatory elements of the TCRα and TCRβ loci, so that rearrangement and expression of the TCRα locus occurred first, in double-negative thymocytes, and TCRβ rearrangement and expression were delayed until the double-positive stage, would T cell development proceed normally to generate mature T cells? Why or why not?

8-18 T-cell α-chain genes undergo successive rearrangements until positive selection or cell death intervenes

8.23 Multiple choice: Approximately one in every three α:β T cells expresses two different rearranged TCRα chain proteins. Yet T cells are still considered to have ‘clonal

specificity' for recognizing antigen. The reason for asserting that each T cell has a single functional specificity for recognizing antigen is that:

A. Only one of the two TCRα chains expressed by a T cell will pair with its TCRβ chain.
B. T cells expressing two different TCRα chains will die by apoptosis when they are activated.
C. Only one T-cell receptor expressed by each T cell will recognize peptide presented by self-MHC molecules.
D. The majority of T cells in an individual will never encounter their specific peptide-MHC ligand and so will not be part of an immune response.
E. The majority of T cells are self-reactive and therefore eliminated during their development in the thymus.

Positive and negative selection of T cells

8-19 Only thymocytes whose receptors interact with self-peptide:self-MHC complexes can survive and mature

8.24 **Multiple choice:** Experiments performed with T-cell receptor transgenic mice identified the fate of developing thymocytes that failed positive selection. Based on these findings, examination of thymocytes in MHC class I-MHC class II-deficient mice (lacking all MHC class I and class II expression in the thymus) would show:

A. A 100-fold decrease in total thymocytes numbers
B. A block in T cell development at the $CD4^-CD8^-$ double-negative stage
C. Normal numbers and subsets of thymocytes and peripheral T cells
D. Normal numbers of thymocytes, but no peripheral T cells
E. A block in T cell development at the $CD4^+CD8^+$ double-positive stage

8-20 Positive selection acts on a repertoire of T-cell receptors with inherent specificity for MHC molecules

8.25 **True/False:** The repertoire of T-cell receptors, like that of antibodies, is formed by the random rearrangement of multiple gene segments that combine to generate the variable domain of each receptor subunit. The bias of T-cell receptors for binding to peptide:MHC complexes, rather than to all possible antigenic structures like antibodies, is simply the result of positive selection in the thymus.

8-21 Positive selection coordinates the expression of CD4 or CD8 with the specificity of the T-cell receptor and the potential effector functions of the T cell

Questions 8.26 and 8.27 use the following information and Figure Q8.26. These questions may be used independently or as a group.

Two mutant lines of mice have been identified, each of which has a defect in T cell development. The subsets of thymocytes found in each mutant mouse line are shown in **Figure Q8.26A**.

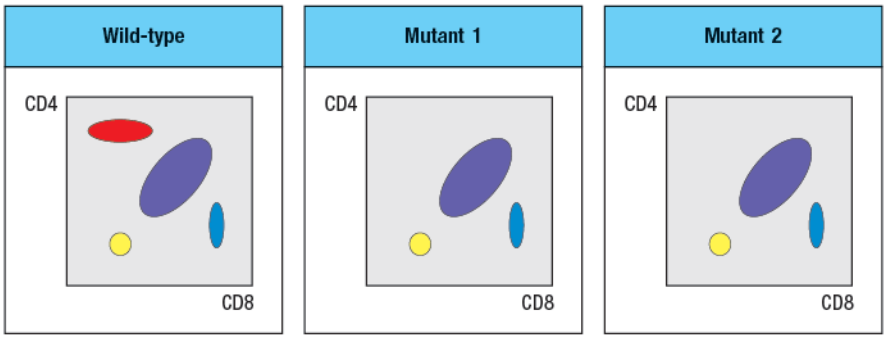

Figure Q8.26A

To narrow down the possible defects in each mutant line, a series of bone marrow chimeras are made in which bone marrow from one strain of mice (the 'donor' strain) is used to reconstitute a second strain (the 'recipient' strain), immediately following the irradiation of the recipient strain to eliminate its own hematopoietic cells. In this procedure, the resulting chimeras have hematopoietic cells that are 100% derived from the donor strain, and all other cells and tissues are derived from the recipient strain. The thymocyte profiles of the series of bone marrow chimeras is shown in **Figure 8.26B** in each case the label at the top of each FACS plot refers to 'donor bone marrow → recipient':

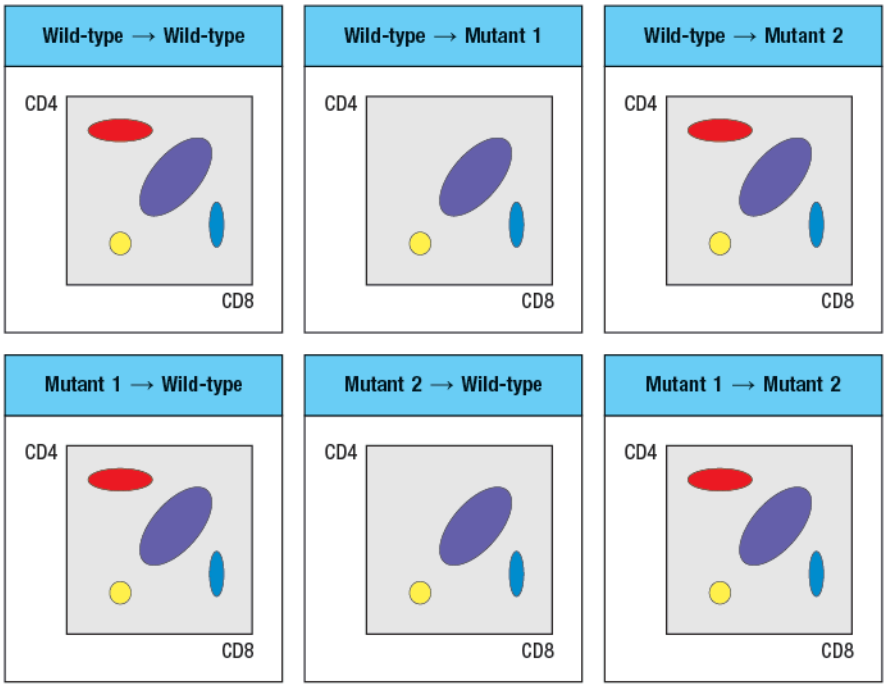

Figure Q8.26B

8.26 **Multiple choice:** A potential candidate molecule for the gene that is defective in Mutant-1 is:

A. CD4
B. MHC class II
C. Th-POK

D. MHC class I
E. Runx3

8.27 Multiple choice: A potential candidate molecule for the gene that is defective in Mutant-2 is:
A. CD4
B. MHC class II
C. Th-POK
D. MHC class I
E. Runx3

8-22 Thymic cortical epithelial cells mediate positive selection of developing thymocytes

8.28 Multiple choice: MHC class II molecules expressed on the surface of thymic cortical epithelial cells normally have a wide repertoire of different peptides bound to them. By engineering a construct that fuses the MHC class II protein to a single peptide sequence, and expressing this construct in thymic cortical epithelial cells that have their endogenous MHC class II genes knocked out, it is possible to generate a mouse line where all MHC class II proteins expressed on all thymic cortical epithelial cells are bound to the same peptide. These mice are often referred to as 'single-peptide' mice. Examination of the T cell developing in these single peptide mice would likely show:
A. A significant reduction in the numbers of mature CD4 T cells
B. No change in the numbers of mature CD4 T cells
C. A block in T cell development at the $CD4^+CD8^+$ double-positive stage
D. A repertoire of T-cell receptors on mature CD4 T cells restricted to a single Vβ
E. A block in T cell development at the $CD4^-CD8^-$ double-negative stage

8-23 T cells that react strongly with ubiquitous self antigens are deleted in the thymus

8.29 Multiple choice: Proteins found in the circulation travel throughout the body, including the thymus. One example is serum albumin. Developing T cells with T-cell receptors specific for peptides of human serum albumin bound to MHC class II molecules would likely be:
A. Positively selected and would mature into CD4 T cells
B. Positively selected and would mature into CD8 T cells
C. Negatively selected in the thymus and deleted from the mature repertoire
D. Targeted for peripheral mechanisms of self-tolerance after emigrating from the thymus
E. Excluded from the T cell zones of the spleen after emigrating from the thymus

8-24 Negative selection is driven most efficiently by bone marrow-derived antigen-presenting cells

8.30 Multiple choice: While many cell types in the thymus are able to induce negative selection of developing self-reactive thymocytes, bone marrow-derived antigen-presenting cells, such as macrophages and dendritic cells, appear to be the most important for this process. One likely reason for the prominent role of bone

marrow-derived antigen-presenting cells in inducing negative selection of developing thymocytes is:

A. Bone marrow-derived antigen-presenting cells are the most abundant stromal cells in the thymus.
B. Bone marrow-derived antigen-presenting cells are very good at inducing mature T cell activation.
C. Bone marrow-derived antigen-presenting cells are highly phagocytic and have specialized mechanisms for presenting peptides on both MHC class I and class II.
D. Bone marrow-derived antigen-presenting cells are concentrated in the thymic medulla where negative selection is most prominent.
E. Bone marrow-derived antigen-presenting cells are hematopoietic in origin, so share the same genetic make-up as the developing thymocytes.

8-25 The specificity and/or the strength of signals for negative and positive selection must differ

8.31 **Short answer:** Current evidence indicates that >95% of the $CD4^+CD8^+$ double-positive thymocytes generated will die in the thymus, and will never develop into mature CD4 or CD8 T cells. While a small proportion of these double-positive thymocytes may fail to produce a functional α:β T-cell receptor, the majority of them do express a T-cell receptor complex on their surface. For any given double-positive thymocyte undergoing cell death in the thymus, what are the two possible explanations for its failure to mature?

8-26 Self-recognizing regulatory T cells and innate T cells develop in the thymus

8.32 **True/False:** Some specialized subsets of α:β T cells complete their development in the thymus and avoid negative selection, in spite of having T-cell receptors with high affinity for self-MHC complexes.

8-27 The final stage of T-cell maturation occurs in the thymic medulla

8.33 **Multiple choice:** The final stages of T cell development occur in the thymic medulla, after the developing cells become CD4 or CD8 single-positive. One important change that occurs during this final maturation is:

A. The down-regulation of the pre-T-cell receptor α (pre-Tα) chain protein
B. The up-regulation of genes encoding effector cytokines and cytolytic effector proteins
C. The increased susceptibility to T-cell receptor-induced apoptosis
D. The loss of susceptibility to T-cell receptor-induced apoptosis
E. The up-regulation of signaling proteins required for T cell activation

8.34 **True/False:** T cell development in the thymus shares some similarities to a pipeline. As new progenitor cells enter the thymus, the most mature thymocytes are pushed out of the thymus to enter the circulation by a passive process.

8-28 T cells that encounter sufficient quantities of self antigens for the first time in the periphery are eliminated or inactivated

8.35 **Multiple choice:** Experimental mouse models have been developed to study the mechanisms leading to the breakdown of self-tolerance and the onset of autoimmunity. One strategy is to express a foreign antigen, such as a viral protein, in a single defined cell type in a peripheral organ. For instance, the lymphocytic choriomeningitis virus (LCMV) glycoprotein has been expressed in β-islet cells of the pancreas by making a line of mice that is transgenic for a construct linking the LCMV-glycoprotein gene to the insulin promoter. In these transgenic mice, the LCMV protein is expressed only in pancreatic β-islet cells. Thymocytes with T-cell receptors specific for a peptide of LCMV-glycoprotein bound to MHC class I develop normally in the thymus, and do not undergo negative selection. The fate of these T cells once they emigrate from the thymus would likely be:

A. They would be activated in the periphery and attach and kill the pancreatic β-islet cells.
B. They would either be deleted in the periphery or would become unresponsive.
C. They would induce an inflammatory response in the pancreas that would up-regulate co-stimulatory molecules on antigen-presenting cells.
D. They would secrete cytokines that promote T cell proliferation.
E. They would differentiate into virus-specific memory T cells that would protect mice upon infection with LCMV.

8.36 **Synthesis question:** An infant is admitted to the hospital with a history of recurrent and persistent bacterial infections. His physician suspects he has an immunodeficiency disease, and obtains a sample of the patient's peripheral blood. The white blood cells are analyzed by antibody staining followed by flow cytometry, and the results are shown in **Figure Q8.36A**.

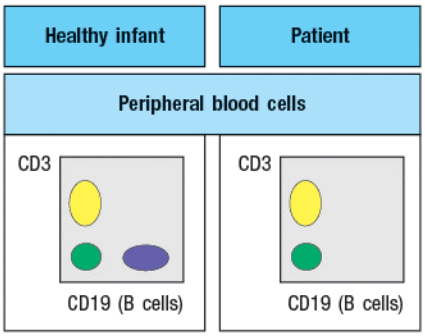

Figure Q8.36A

To determine the origin of the peripheral blood cell defect, a bone marrow biopsy is taken from the patient and compared to a healthy control, as shown in **Figure Q8.36B**.

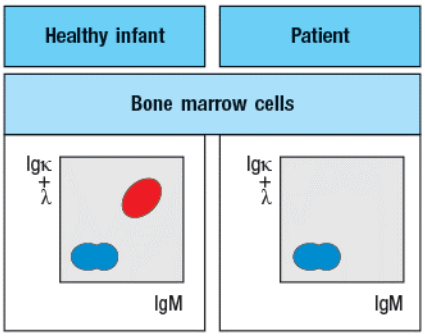

Figure Q8.36B

To obtain additional information, bone marrow cells are treated with a chemical that permeabilizes the cell membrane, allowing antibodies to enter the cells and bind to their target antigens within the cells, a technique known as 'intracellular staining'. The results of this analysis are shown in **Figure Q8.36C.**

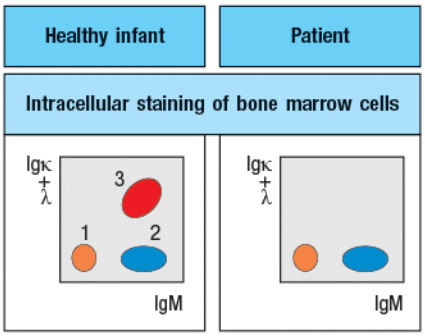

Figure Q8.36C

a) What are populations 1, 2, and 3 in **Figure Q8.36C**?

b) In the analysis of cell surface expression (non-permeabilized bone marrow cells), what are the IgM^{lo} $Ig\kappa^{+}\lambda^{neg}$ cells as indicated by the arrow in **Figure Q8.36D**?

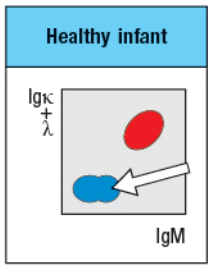

Figure Q8.36D

c) What is the most likely defect causing the patient's immunodeficiency?

The results shown above indicate a specific defect in B cell development. To help identify the defective or missing protein in the patient's developing B cells, bone marrow cells are

isolated and protein lysates are prepared for immunoblotting. A series of antibodies are tested and the results are shown in **Figure Q8.36E**.

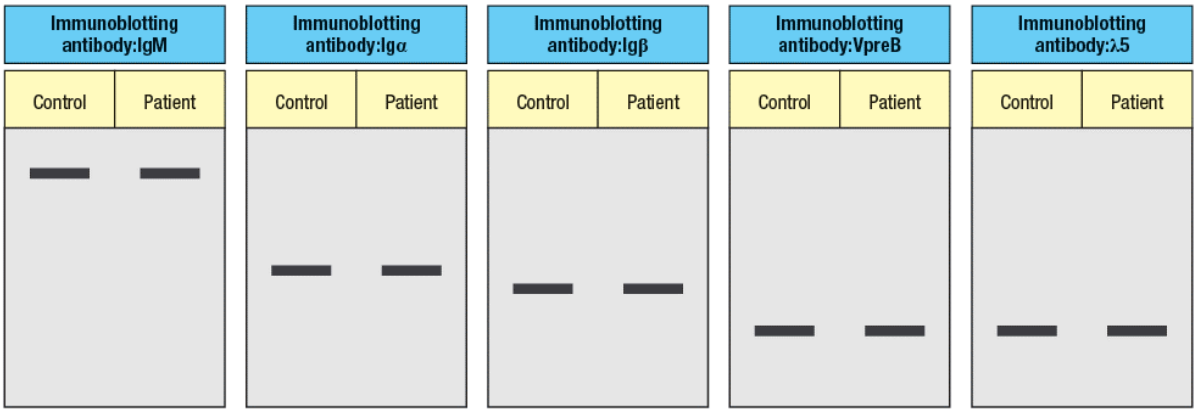

Figure Q8.36E

d) Given the results from the immunoblotting experiments, what is the most likely candidate molecule (or type of molecule) responsible for the patient's immunodeficiency disease?

8.37 **Synthesis question:** To investigate how T-cell receptor signaling regulates T cell development in the thymus, a mutant mouse is generated with a deficiency in the T cell tyrosine kinase, LCK ($Lck^{-/-}$ mice). Analysis of thymocytes from these mice, stained with antibodies to CD4 and CD8, is shown in **Figure Q8.37A.**

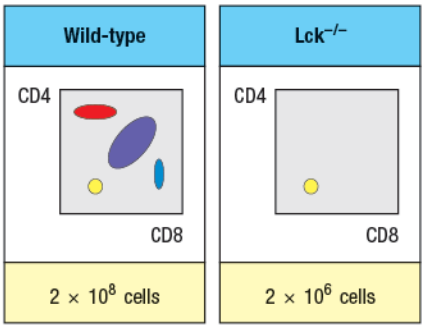

Figure Q8.37A

a) What is the explanation for the altered number and subsets of thymocytes in $Lck^{-/-}$ mice?

Due to the defect observed in the germline Lck-deficient mice, it is not possible to use these mice to examine any potential role for this T-cell receptor signaling protein at later stages of thymocyte development. To circumvent this problem, conditional Lck-deficient mice are generated by crossing mice with a homozygous 'floxed' allele of Lck ($Lck^{fl/fl}$) to mice that express the cre recombinase in $CD4^{+}CD8^{+}$ double-positive thymocytes (i.e., CD4-cre). When cre is expressed at the double-positive stage, the Lck gene is deleted, and thymocytes become Lck-deficient at that time. The thymocyte profile of these $Lck^{fl/fl}$ x CD4-cre mice is shown in **Figure Q8.37B.**

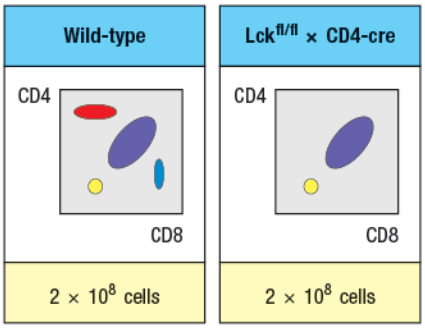

Figure Q8.37B

b) What is the explanation for the altered thymocyte profile seen in $Lck^{fl/fl}$ x CD4-cre mice?

To further assess the impact of altered T-cell receptor signaling on thymocyte development, another line of mice is generated that express a super-active version of the Lck kinase (called 'Lck-super') under control of the CD4 promoter. This super-active Lck is expressed starting at the double-positive stage in the thymus. Lck-super is activated by T-cell receptor signaling, just like wild-type Lck, but when activated has an approximately tenfold increased kinase activity. Surprisingly, mice expressing Lck-super do not develop increased numbers of mature T cells, but instead, show the following (**Figure Q8.37C)**.

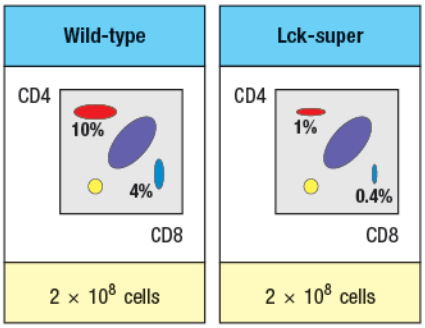

Figure Q8.37C

c) What is the most likely explanation for the altered profile of thymocytes seen in the Lck-super mice?

Struck by the findings in the Lck-super mice, an investigator performs one further experiment. This researcher clones the rearranged T-cell receptor α and β chain genes from two CD4 single-positive thymocytes: one that is isolated from a WT thymus, and the second, isolated from an Lck-super thymus. Each T-cell receptor α:β pair is used to generate a T-cell receptor transgenic line, so that nearly 100% of all double-positive thymocytes in each transgenic line express only the transgenic T-cell receptor. The two T cell receptor transgenics are named based on which mouse line the receptor chains were originally isolated from. The one from the wild-type line is known as $TCR\text{-}tg^{wt}$, and the one originally isolated from the Lck-super line is known as $TCR\text{-}tg^{super}$. In each case, thymocytes from the T-cell receptor transgenic lines are analyzed on a wild-type background, or after crossing to the Lck-super line. The results are shown in **Figure Q8.37D**.

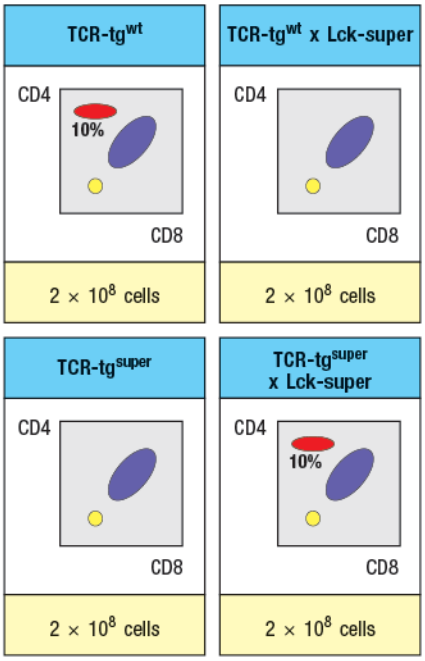

Figure Q8.37D

d) Explain the results observed in this experiment.

ANSWERS

8.1: True.
B and T lymphocytes arise from hematopoietic stem cells (HSC) that are self-renewing and can give rise to all blood cell lineages. These HSC give rise to multipotent progenitors (MPP), which is no longer self-renewing. From the MPP, one branch gives rise to the progenitor of granulocytes, megakaryocytes and erythrocytes; the other branch gives rise to common lymphoid progenitors (CLP). At this point, the CLP has lost the ability to generate myeloid and erythroid lineages, but is not committed to making a single specific lymphocyte lineage. Progenitor cell transfer and lineage repopulation experiments have shown that the CLP population is actually heterogeneous and represents a continuum of cells with decreasing multipotent potential. A subset of CLP cells with the broadest potential is able to generate B cells, T cells, and NK cells. A second subset of CLPs is able to generate only B cells and T cells, and a third subset of CLPs is committed exclusively to the B-cell lineage. B-cell committed CLPs give rise to pro-B cells.

8.2: C.
The stages of B cell development are generally defined by the ordered rearrangement of the immunoglobulin heavy and light chain genes. Heavy chain rearrangements occur first, and begin in early pro-B cells. Rearrangement of the heavy-chain locus is initiated in the pro-B cell when E2A and EBF induce the expression of several key proteins that enable gene rearrangement to occur, including the RAG-1 and RAG-2 components of the V(D)J recombinase. Only one gene locus is rearranged at a time, in a fixed sequence. The first rearrangement to take place is the joining of a D gene segment to a J segment at the immunoglobulin heavy-chain (IgH) locus. D to JH rearrangement takes place mostly in the early pro-B-cell stage, but can be seen as early as the common lymphoid progenitor. In the absence of E2A or EBF this initial rearrangement event fails to occur.

8.3: E.
To produce a complete immunoglobulin heavy chain, the late pro-B cell now proceeds with a rearrangement of a V_H gene segment to a DJ_H sequence. In contrast to D to J_H rearrangement, V_H to DJ_H rearrangement occurs first on only one chromosome. A successful rearrangement leads to the production of intact μ heavy chains, after which V_H to DJ_H rearrangement ceases and the cell becomes a pre-B cell. Pro-B cells that do not produce a μ chain are eliminated, as they fail to receive an important survival signal mediated by the pre-B-cell receptor. At least 45% of pro-B cells are lost at this stage.

8.4: A.
Formation of the pre-B-cell receptor and signaling through this receptor provide an important checkpoint that mediates the transition between the pro-B cell and the pre-B cell. In mice that either lack λ5 or have mutant heavy-chain genes that cannot produce the transmembrane domain, the pre-B-cell receptor cannot be formed and B-cell development is blocked after heavy-chain gene rearrangement. In normal B-cell development, the pre-B-cell receptor complex is expressed transiently, perhaps because the production of λ5 mRNA stops as soon as pre-B-cell receptors begin to be formed. Although present at only low levels on the cell surface, the pre-B-cell receptor generates signals required for the transition from pro-B cell to pre-B cell. No antigen or other external ligand seems to be involved in signaling by the receptor. Instead, pre-B-cell receptors are thought to interact with each other, forming dimers or oligomers

that generate signals. Dimerization involves ‘unique’ regions in the amino termini of λ5 and VpreB proteins that are not present in other immunoglobulin-like domains and which mediate the cross-linking of adjacent pre-B-cell receptors on the cell surface. This signal requires B-cell receptor signaling subunits, Igα and Igβ, as well as the tyrosine kinase, BTK.

8.5: Failure of allelic exclusion.
VpreB is a necessary component of the pre-B-cell receptor; in its absence pre-B-cell receptor signaling will not occur. The signaling generated by pre-B-cell receptor clustering halts further rearrangement of the heavy-chain locus and allows the pro-B cell to become sensitive to IL-7. This induces cell proliferation, initiating the transition to the large pre-B cell. Successful rearrangements at both heavy-chain alleles could result in a B cell producing two receptors of different antigen specificities. To prevent this, signaling by the pre-B-cell receptor enforces allelic exclusion, the state in which only one of the two alleles of a gene is expressed in a diploid cell.

8.6: D.
As well as allelic exclusion, light chains also display isotypic exclusion, that is, the expression of only one type of light chain—κ or λ—by an individual B cell. The ratios of κ-expressing versus λ-expressing mature B cells vary from one extreme to the other in different species. In mice and rats it is 95% κ to 5% λ, in humans it is typically 65%:35%, and in cats it is 5%:95%, the opposite of that in mice. These ratios correlate most strongly with the number of functional Vκ and Vλ gene segments in the genome of the species. They also reflect the kinetics and efficiency of gene segment rearrangements. The κ:λ ratio in the mature lymphocyte population is useful in clinical diagnostics, because an aberrant κ :λ ratio indicates the dominance of one clone and the presence of a lymphoproliferative disorder.

8.7: False.
sIgM associates with Igα and Igβ to form a functional B-cell receptor complex, and the fate of an immature B cell in the bone marrow depends on signals delivered from this receptor complex when it interacts with ligands in the environment. Igα signaling is particularly important in dictating the emigration of B cells from the bone marrow and/or their survival in the periphery: mice that express Igα with a truncated cytoplasmic domain that cannot signal show a fourfold reduction in the number of immature B cells in the marrow, and a 100-fold reduction in the number of peripheral B cells. The release of immature B cells from the bone marrow into the circulation is also dependent on their expression of S1P1, a G-protein-coupled receptor that binds to the lipid ligand S1P and promotes cell migration toward the high concentrations of S1P that exist in the blood.

8.8: C.
Immature B cells that express an autoreactive receptor recognizing a multivalent self antigen can be rescued by further gene rearrangements that replace the autoreactive receptor with a new receptor that is not self-reactive. This mechanism is termed receptor editing. When an immature B cell first produces sIgM, RAG proteins are still being made. If the receptor is not self-reactive, the absence of sIgM cross-linking allows gene rearrangement to cease and B-cell development continues, with RAG proteins eventually disappearing. For an autoreactive receptor, however, an encounter with the self antigen results in strong cross-linking of sIgM; RAG expression continues, and light-chain gene rearrangement can continue. These secondary rearrangements can rescue immature self-reactive B cells by deleting the self-reactive light-chain gene and replacing it with another sequence. If the new light chain is not

autoreactive, the B cell continues normal development. The importance of receptor editing as a mechanism of tolerance is well established, as defects in this process contribute to the human autoimmune diseases systemic lupus erythematosus and rheumatoid arthritis, two diseases characterized by high levels of autoreactive antibodies.

8.9: A.
While large numbers of autoreactive B cells are purged from the population of new lymphocytes in the bone marrow, only lymphocytes specific for autoantigens that are expressed in or can reach this organ are affected. Some antigens, like the thyroid product thyroglobulin, are highly tissue specific, or are compartmentalized so that little if any is available in the circulation. Therefore, newly emigrated self-reactive B cells that encounter their specific autoantigen for the first time in the periphery must be eliminated or inactivated also.

8.10: E.
The follicle provides signals necessary for B-cell survival. In particular, the TNF-family member BAFF (for B-cell activating factor belonging to the TNF family) is made by several cell types, but is produced abundantly by the follicular dendritic cells (FDCs). Weak B-cell receptor signals together with the BAFF-R signals are essential to promote the final stages of B-cell maturation in the periphery. Disregulation of the appropriate balance between B-cell receptor and BAFF-R signaling occurs in individuals who overexpress BAFF, and has been linked to the development of autoimmune diseases, such as Sjögren's syndrome, that result from a failure to purge autoreactive B cells.

8.11:
1. Their presence in the marginal zones of the spleen that lie at the white pulp/red pulp junction.
2. Their expression of high levels of cell-surface complement receptors.

Marginal zone B cells represent a minor population of B cells found in the spleen. They are named for their predominance at the marginal zones that lie at the white pulp/red pulp junctions. Marginal zone B cells can be identified by their expression of very high levels of the complement receptor CD21. Due to their location and expression of complement receptors, marginal zone B cells are poised to make rapid responses to antigens or pathogens filtered from the blood. Therefore, it is thought that marginal zone B cells represent an early line of defense for blood-borne pathogens.

8.12: B.
B-1 B cells are considered to be part of the innate immune system. These cells are present only in low numbers in secondary lymphoid organs, and are found in large numbers in the peritoneal and pleural cavities instead. B-1 B cells are the major source of 'natural' antibodies, which are constitutively produced circulating antibodies that are secreted by these B cells prior to any infections. Most antibodies made by B-1 B cells recognize capsular polysaccharide antigens, and B-1 B cells are important in controlling infections of pathogenic viruses and bacteria.

8.13: E.
Experiments in mice first showed that surgical removal of the thymus (thymectomy) at birth resulted in immunodeficient mice. Much evidence, including observations in immunodeficient children, has since confirmed the importance of the thymus in T-cell development. In DiGeorge

syndrome in humans and in mice with the nude mutation, the thymus does not form and the affected individual produces B lymphocytes but few T lymphocytes. DiGeorge syndrome is a complex combination of cardiac, facial, endocrine, and immune defects associated with deletions of chromosome 22q11. The nude mutation in mice is due to a defect in the gene for Foxn1, a transcription factor required for terminal epithelial cell differentiation. Rare cases of a defect in the human FOXN1 gene (which is on chromosome 17) have been associated with T-cell immunodeficiency, and the absence of a thymus. All other choices above would lead to a deficiency in B cells as well as T cells, with the exception of TdT, which is not required for either B cell or T cell development.

8.14: True.
T lymphocytes develop from lymphoid progenitors in the bone marrow that also give rise to B lymphocytes. Some of these progenitors leave the bone marrow and migrate to the thymus. In the thymus, the progenitor cell receives a signal from thymic epithelial cells that is transduced through a receptor called Notch1 to switch on specific genes. Notch signaling is widely used in animal development to specify tissue differentiation; in lymphocyte development, the Notch signal instructs the precursor to commit to the T-cell lineage rather than the B-cell lineage.

8.15: C.
Approximately 98% of the thymocytes that develop in the thymus are destined to die in the thymus by apoptosis. Cells undergoing apoptosis are recognized and ingested by macrophages, and apoptotic bodies, which are the residual condensed chromatin of apoptotic cells, are seen inside macrophages throughout the thymic cortex.

8.16: The TCRβ chain, but not the TCRα chain is required for the pre-TCR to assemble at the $CD4^-CD8^-$ (double-negative) stage of thymocyte development. The pre-TCR signal is critical for inducing double-negative thymocytes to differentiate into $CD4^+CD8^+$ double-positive thymocytes. This signal is also essential to induce multiple rounds of cell division as thymocytes progress from double-negative to double-positive. This proliferation leads to ~100-fold increase in thymocyte numbers. As a consequence, double-negative thymocytes lacking TCRβ will fail to proliferate and will fail to differentiate into double-positive cells.

8.17: For the $TCR\alpha^{-/-}$, the thymic medulla would show a dearth of thymocytes, as these mice lack CD4 and CD8 single-positive thymocytes. For the $TCR\beta^{-/-}$, the thymic cortex would show a dearth of thymocytes, as these mice, as shown, lack double-positive thymocytes. The $TCR\beta^{-/-}$ mice would also likely lack thymic medullary structures.

8.18: B.
α:β and γ:δ T cells arise from a common progenitor, and both subsets develop in the thymus. A progenitor cell that migrates from the bone marrow to the thymus requires signaling through Notch1 for commitment to the T cell lineage. This T cell commitment is required for the development of both α:β and γ:δ T cells; hence in the absence of Notch1 signaling, both lineages of T cells would be absent.

8.19: A.
Cells of the adaptive immune system are found circulating between the blood, the spleen, and the secondary lymphoid organs, waiting for their antigen to enter the body and activate them to respond. In contrast, the majority of γ:δ T cells do not recirculate in the blood and secondary

lymphoid organs, but instead, take up residence in barrier surfaces such as mucosa and epithelia that line the body surfaces. Each subset of γ:δ T cells is programmed during its development in the thymus to home to a particular tissues or small set of tissues.

8.20: True.
Unlike most α:β T cells that require a long period of initial T cell activation, proliferation, and differentiation prior to acquiring the ability to produce effector molecules, γ:δ T cells complete their maturation with a pre-programmed commitment to produce specific effector cytokines. Each subset of γ:δ T cells residing in distinct anatomical locations are programmed to produce a specific array of effector cytokines. For α:β T cells, this programming occurs during the process of initial T cell activation, and is determined by the nature of the pathogenic infection.

8.21: D.
In mice, the majority of γ:δ T cells in the body arise during embryonic development and the early neonatal period. In the fetal thymus, the first T cells to develop are γ:δ T cells that all express TCRs assembled from the same Vγ and Vδ regions. As a consequence, most of the γ:δ T cells produced in each of these early waves share the same specificity, although the antigen recognized in each case is not known. The Vγ5-bearing cells become established selectively in the epidermis; they are programmed to secrete keratinocyte growth factor and inflammatory cytokines and chemokines. In contrast, Vγ6-bearing cells become established in the lung, the dermis of the skin, and the epithelium of the reproductive tract, and are programmed to secrete IL-17.

8.22: No.
The rearrangement and expression of the TCRβ locus must occur first in double-negative thymocytes. This is because the TCRβ chain protein, but not the TCRα chain protein can pair with the pre-Tα chain protein to form the pre-TCR. In the absence of signaling through the pre-TCR, double-negative thymocytes do not continue to develop, and the thymus generally lacks double-positive thymocytes. Therefore, no subsequent rearrangement of the TCRβ locus would occur, and thymocyte development would be halted at the double-negative stage if the TCRα locus rearranged first.

8.23: C.
T cells with dual specificity might be expected to give rise to inappropriate immune responses if the cell is activated through one receptor yet can act upon target cells recognized by the second receptor. However, only one of the two receptors is likely to be able to recognize peptide presented by a self MHC molecule, and so the T cell will have only a single functional specificity. This is because once a thymocyte has been positively selected by self-peptide:self-MHC recognition, α-chain gene rearrangement ceases. Thus, the existence of cells with two α-chain genes productively rearranged and two α chains expressed at the cell surface does not truly challenge the idea that a single functional specificity is expressed by each cell.

8.24: E.
When rearranged receptor genes from a mature T cell specific for a peptide presented by a particular MHC molecule were introduced into a recipient mouse (i.e., generating a T-cell receptor transgenic mouse), and that mouse lacked the MHC molecule recognized by the transgenic T-cell receptor, it was found that the thymocytes in the mouse never progressed further than the double-positive stage. Instead all of the thymocytes in this mouse were found to

die in the thymus within 3 or 4 days of their last division. Generalizing this finding to all of the T cells developing in a non-T-cell receptor transgenic mouse leads to the prediction that each individual thymocyte requires an interaction with self-peptide:self-MHC complexes to progress past the double-positive stage of development. Hence, in a mouse lacking all MHC class I and class II proteins, no developing T cells would develop past the double-positive stage.

8.25: False.
Positive selection acts on a repertoire of T-cell receptors whose specificity is determined by randomly generated combinations of V, D, and J gene segments. Despite this, T-cell receptors exhibit a bias toward recognition of MHC molecules even before positive selection. An inherent specificity of T-cell receptors for MHC molecules has been detected by examining mature CD4 T cells that represent the unselected receptor repertoire. When such T cells are examined, roughly 5% can respond to any one MHC class II genotype. Because these cells developed without selection by MHC molecules, this reactivity must reflect an inherent MHC-specificity encoded in the germline V gene segments. This specificity should significantly increase the proportion of receptors that can be positively selected by any individual's MHC molecules.

8.26: B.

8.27: C.
Both Mutant-1 and Mutant-2 mouse strains lack CD4 T cells. In the case of Mutant-1, this defect is not intrinsic to the developing T cells, as normal CD4 T cell development is restored when Mutant-1 bone marrow is used to reconstitute WT recipient mice. In contrast, the defect in Mutant-2 mice is intrinsic to the developing T cells, as no CD4 T cells develop when Mutant-2 bone marrow is used to reconstitute WT recipients. Since the defect in Mutant-1 is not in the developing T cells, it must be a defect in the thymic environment. The only choice is MHC class II, as a defect in MHC class I would not lead to an absence of CD4 T cells. For Mutant-2, the defect is in the developing T cells. It therefore could be in CD4 or in Th-POK; however, since the Mutant-2 mice have normal CD4 expression on their double-positive cells, the defect cannot be in the CD4 gene, but must be in Th-POK. Runx3 is required for CD8 T cell development, but is not required for CD4 T cell development.

8.28: A.
The critical role of the thymic cortical epithelium in positive selection raises the question whether there is anything distinctive about the antigen-presenting properties of these cells. Thymic epithelium differs from other tissues in the expression of key proteases that are involved in MHC class I and II antigen processing. Cortical epithelial cells express cathepsin L as opposed to the more widely expressed cathepsin S, and mice deficient in cathepsin L have severely impaired CD4 T-cell development. Thymic epithelial cells from mice lacking cathepsin L exhibit a relatively high proportion of MHC class II molecules on their surface that retain the class II invariant chain-associated peptide (CLIP). These data indicate that the peptide repertoire displayed by the MHC molecules on cortical epithelial cells is important in generating the broad diversity of T cells that develop, and that selection of CD4 cells on a single peptide:MHC class II complex would lead to a reduced number of CD4 T cells.

8.29: C.
Protein antigens that traffic to the thymus would be taken up and presented by thymic antigen-presenting cells. Developing T cells with high affinity T-cell receptors for peptides

derived from these antigens would undergo negative selection in the thymus, and therefore would be depleted from the mature peripheral T cell repertoire.

8.30: C.
Bone marrow-derived antigen-presenting cells such as macrophages and dendritic cells have efficient mechanisms for taking up antigens and presenting peptides derived from these antigens on both MHC class I and class II molecules. These cells are generally phagocytic, and have specialized pathways for presenting the broadest range of self-peptides to the developing T cells.

8.31: Based on the 'affinity hypothesis', a given double-positive thymocyte will follow one of several possible fates based on the strength of its T-cell receptor for binding self peptide:MHC complexes on stromal cells in the thymus. Thymocytes whose T-cell receptors fail to bind self peptide:MHC will die by neglect, due to a failure of positive selection. Thymocytes whose T-cell receptors have low affinity interactions are rescued from cell death, leading to positive selection. Finally, thymocytes whose T-cell receptors have high-affinity interactions with self peptide:MHC are induced to undergo apoptosis and thus negative selection. Thus a thymocyte that dies at the double-positive stage with a functional α:β T-cell receptor on its surface either fails positive selection and dies by neglect, or undergoes negative selection.

8.32: True.
One such subset is the CD4 T-regulatory (T_{reg}) cells. The repertoire of TCRs expressed on T_{reg} cells is thought to be comprised of receptors with high affinity for self-peptide:self-MHC complexes. Evidence supporting this conclusion comes from studies showing that some lines of TCR transgenic mice generate large numbers of T_{reg} cells when the mice also express the antigen for this TCR. In addition, studies using mice expressing a fluorescent reporter that monitors TCR signal strength have shown that T_{reg} cells express high levels of the fluorescent reporter, both during their development as well as after their export from the thymus, indicating that they likely express TCRs with high affinity for self. This process of positive selection following high-affinity TCR interactions with self-peptide:self-MHC complexes has been termed agonist selection—in other words, interactions of a T-cell receptor with a self-peptide:self-MHC that would normally activate a mature T cell expressing that TCR.

8.33: D.
After surviving positive and negative selection, thymocytes complete their maturation in the thymic medulla and then emigrate to peripheral lymphoid organs. Their final maturation results in changes to the T-cell receptor signaling machinery. Whereas an immature double-positive or single-positive thymocyte stimulated through the T-cell receptor will undergo apoptosis, a mature single-positive thymocyte responds by proliferating. This change in signaling machinery is critical for the production of mature T cells that will function in immune responses to infections.

8.34: False.
CD4 and CD8 single-positive thymocytes that have successfully survived positive and negative selection are found in the medulla but are not yet fully mature. At the termination of the maturation process, which takes 3–4 days, the CD4 and CD8 single-positive thymocytes up-regulate the sphingosine 1-phosphate (S1P) receptor, known as S1P1. S1P1 is a G-protein-coupled receptor that promotes chemotaxis of the cells toward the ligand S1P. Due to

the high levels of S1P in the blood, single-positive thymocytes are induced to leave the thymus by entering the blood, where they become part of the recirculating naive T-cell population.

8.35: B.
Many autoreactive T cells are purged during their development in the thymus. Nonetheless, not all self antigens are expressed in the thymus, and some autoreactive T cells complete their maturation and migrate to the periphery. Our understanding of the fates of autoreactive T cells in the periphery comes mainly from the study of mice transgenic for self-reactive T-cell receptors. In some cases, T cells reacting to self antigens in the periphery are eliminated. This usually follows a brief period of activation and cell division, and so is known as activation-induced cell death. In other cases, the self-reactive cells may be rendered anergic. When studied *in vitro*, these anergic T cells prove refractory to signals delivered through the T-cell receptor. In either case, self-tolerance would be maintained and no attack on the pancreas would ensue.

8.36:

a) Population 1 is pro-B cells that have not yet completed Ig heavy chain rearrangement, and therefore do not express any intracellular full-length IgM protein. Population 2 is pre-B cells that have completed Ig heavy chain rearrangement but not light chain rearrangement. These cells express intracellular IgM, but not Igκ or Igλ. Population 3 is immature B cells that have completed Ig heavy and light chain rearrangements and express both proteins, both intracellularly and on the cell surface.

b) Pre-B cells expressing the pre-B-cell receptor. This receptor is composed of IgM heavy chain protein plus the surrogate light chain proteins, VpreB and λ5, as well as the signaling subunits Igα and Igβ. As shown, this receptor is expressed at very low levels on the surface of pre-B cells.

c) A defect in B cell development due to a block at the pre-B cell stage. The most likely explanation is a failure in the pre-B-cell receptor complex or signaling. In the absence of this signal, maturation of pre-B cells to immature B cells will not occur.

d) A defect in signaling through the pre-B-cell receptor is likely responsible for this patient's immunodeficiency. A good candidate is the Btk tyrosine kinase, as this kinase is essential for pre-B-cell receptor signaling. In the absence of Btk, patients show a block in B cell development at the pre-B cell stage. If not Btk, another B cell signaling protein defect might also account for this immunodeficiency.

8.37:

a) Lck is required for pre-T-cell receptor signaling. In the absence of pre-T-cell receptor signaling, thymocyte development is arrested at the double-negative stage. Therefore there are no double-positive or CD4 or CD8 single-positive thymocytes in $Lck^{-/-}$ mice. In addition, without pre-T-cell receptor signaling, proliferative expansion of thymocytes as they transition from double-negative to double-positive also fails to occur. Consequently, there is ~100-fold reduction in total thymocyte numbers in $Lck^{-/-}$ mice.

b) Lck is required for positive selection. When the double-positive thymocytes become Lck-deficient, they lose their ability to signal through their T-cell receptors. Without T-cell receptor signaling, positive selection cannot occur, and all thymocytes die at the double-positive stage.

c) Enhanced negative selection.

When thymocytes express Lck-super, even weak T-cell receptor interactions will generate strong signals. Nearly all of the thymocytes whose T-cell receptors recognize self-peptide:MHC complexes will receive strong signals and undergo negative selection. There will be very few thymocytes whose T cell receptors would be able to induce the weak signals required for positive selection without pushing the cells into the negative selection signaling range.

d) The T-cell receptor isolated from a wild-type CD4 single-positive thymocyte (TCR-tg^{wt}) undergoes normal positive selection in a wild-type thymus, but when expressed in the Lck-super thymus, cells expressing this T-cell receptor undergo negative selection and are deleted. Therefore the TCR-tg^{wt} x Lck-super thymus has no mature CD4 cells.

The T-cell receptor isolated from the Lck-super CD4 single-positive thymocyte (TCR-tg^{super}) undergoes normal positive selection in the Lck-super thymus. However, when this receptor is expressed in a wild-type thymus, it fails to generate sufficient signaling for positive selection. As a result, double-positive thymocytes expressing TCR-tg^{super} die by neglect, as their T-cell receptors have insufficient affinity for positive selection in cell expressing the normally active Lck.