Janeway's Immunobiology, 9th Edition
Chapter 11: Integrated Dynamics of Innate and Adaptive Immunity

Integration of innate and adaptive immunity in response to specific types of pathogens

11-1 The course of an infection can be divided into several distinct phases

11.1 **Multiple choice:** While innate immune responses to all types of infections induce local inflammatory responses due to activation of blood vessel endothelial cells, some components of the innate response differ depending on the nature of the pathogen. In the case of intracellular bacterial or protozoan infections, tissue-resident dendritic cells and macrophages produce a cytokine that stimulates ILC cells to produce:

A. IL-13
B. TNF-α
C. IL17
D. IL22
E. IFN-γ

11-2 The effector mechanisms that are recruited to clear an infection depend on the infectious agent

11.2 **Multiple choice:** IL-23 is a cytokine made by macrophages and dendritic cells in response to extracellular bacterial and fungal infections. Mice with a genetic defect in the production of IL-23 are highly susceptible to the gastrointestinal bacterial pathogen, *Citrobacter rodentium*. Thus, unlike wild-type mice which clear the infection, mice that fail to produce IL-23 succumb to the bacteria and die 1–2 weeks post-infection. Yet, this cytokine does not directly act on the bacteria nor does it function to recruit the granulocytes that are needed to eliminate the pathogen. Instead, IL-23:

A. Functions as a chemoattractant for eosinophils and basophils
B. Stimulates IL-17 and IL-22 production by ILC3 cells
C. Activates tissue-resident ILC2 cells to produce IL-5 and IL-13
D. Induces the differentiation of naive CD8 T cells into cytotoxic T cells
E. Stimulates gastrointestinal epithelial cells to produce antimicrobial peptides

11.3 **Short answer:** In addition to producing distinct innate responses locally at the site of infection, the different cytokines produced during type I, type 2, or type 3 immune responses also induce distinct adaptive immune responses that are tailored to the eradication of the three different classes of pathogens. One example is the production of different classes of antibodies during type I, type 2, or type 3 responses. Which step during the induction of the adaptive immune response is the key to generating and coordinating the three different immune modules?

Effector T cells augment the effector functions of innate immune cells

11-3 Effector T cells are guided to specific tissues and sites of infection by changes in their expression of adhesion molecules and chemokine receptors

11.4 Multiple choice: In response to an intracellular bacterial or viral infection, effector T_H1 cells, macrophages, NK cells, and CD8 cytotoxic effector cells are all recruited to the site of infection. The coordinated recruitment of all of these cell types is orchestrated by:

A. The secretion of the inflammatory cytokine IFN-γ in the tissue
B. The action of TNF-α on the endothelial cells, leading to fluid leakage into the tissue
C. The up-regulation of integrin ligands such as VLA-4 on the blood vessel endothelial cells
D. The shared expression of chemokine receptors on these different cell types
E. The shared expression of S1PR1 on these cells, recruiting them out of lymphoid tissues

11-4 Pathogen-specific effector T cells are enriched at sites of infection as adaptive immunity progresses

11.5 Multiple choice: Initially after an infection, the majority of the T cells present in the tissue at a site of infection are not specific for the infecting pathogen, but over the course of several days, this changes and antigen-specific T cells become enriched at this site. This is because:

A. T cells do not use their T-cell receptors during extravasation from blood into tissues.
B. Early after infection, there are few antigen-specific T cells in the host.
C. Naive T cells do not express the homing receptors to extravasate into sites of inflammation.
D. T cells up-regulate CD69 early after activation and are retained in the lymphoid organs.
E. T cells require several days to down-regulate CCR7.

11-5 T_H1 cells coordinate and amplify the host response to intracellular pathogens through classical activation of macrophages

11.6 Multiple choice: Individuals with the HIV-induced immunodeficiency disease AIDS have a progressive loss in the number of CD4 T cells in their bodies. These patients have a greatly increased rate of life-threatening disease caused by the inability of their immune system to control infections of the intracellular bacterium, *Mycobacterium tuberculosis* (Mtb). Mtb infects macrophages and then replicates in the cell's phagosomes. The most important immune mechanism lacking in these patients that leads to their increased susceptibility to Mtb is a defect in:

A. CD4 T cell help for cytotoxic effector CD8 T cells
B. The activation of macrophages by T_H1 effector cells
C. The production of opsonizing antibodies that requires T_{FH} cell help for B cells
D. The production of TNF-α by the infected macrophages
E. The recruitment of neutrophils to the site of infection

11.7 Multiple choice: Infections of intracellular pathogens (e.g., mycobacteria, listeria, toxoplasma, viruses, etc.) cause a rise in the numbers of monocytes in the blood, a symptom known as monocytosis. In the cases of these infections, monocytosis is likely caused by:

A. Increased production of monocytes in the bone marrow induced by T_H1 cytokines
B. Loss of monocytes into tissues due to inflammation, leading to increased production in the bone marrow
C. Differentiation of blood monocytes into macrophages, inducing bone marrow production of new monocytes
D. Sticking of blood monocytes to vessel walls due to integrin binding, reducing the numbers of monocytes in the circulation
E. Apoptosis of monocytes caused by the toxic effects of the infecting pathogen

11-6 Activation of macrophages by T_H1 cells must be tightly regulated to avoid tissue damage

11.8 True/False: Nitric oxide and superoxide radicals are toxic compounds that induce substantial DNA damage. When released by activated M1 macrophages, these compounds cause damage to microbial pathogens and may also cause damage to host cells in the vicinity.

11-7 Chronic activation of macrophages by T_H1 cells mediates the formation of granulomas to contain intracellular pathogens that cannot be cleared

11.9 Multiple choice: Leprosy is a disease caused by the intracellular bacterium *Mycobacterium leprae*, which infects macrophages and replicates in their phagosomes. Human patients with leprosy have a persistent infection of the mycobacteria, as their immune systems are unable to complete eradicate the pathogen. Furthermore, two different forms of the disease have been identified. Some patients have many skin lesions containing a large number of bacteria with little inflammatory response. This is the very disfiguring form of the disease, and is known as lepromatous leprosy. In other patients, few skin lesions and only occasional bacteria are observed, and the skin lesions are accompanied by a robust inflammatory response. These patients have the form of the disease known as tuberculoid leprosy. If one examined a skin biopsy from a patient with tuberculoid leprosy, one would expect to see:

A. A large influx of neutrophils and other granulocytes
B. A widespread occurrence of tissue necrosis
C. A substantial number of granulomas
D. Evidence of large numbers of dead or dying mycobacteria
E. A large number of skin epithelial cells with intracellular bacteria

11-8 Defects in type 1 immunity reveal its important role in the elimination of intracellular pathogens

11.10 Multiple choice: *Toxoplasma gondii* is a single-celled parasitic protozoan that infects and replicates in macrophages. It is common in the environment, and is transmitted to humans by the ingestion of undercooked meat or by accidental ingestion of the parasite's oocytes from contaminated water or cat litter. Infected individuals with healthy

immune systems are generally asymptomatic, and rapidly clear the infection. However, in AIDS patients, infections of *Toxoplasma gondii* can lead to severe disease and even death. To investigate the immune mechanisms important in controlling *Toxoplasma gondii*, a mouse model of the infection was developed. Mice were infected with the protozoa at a dose where the majority of the mice survive the infection, and at the same time, were injected with a neutralizing antibody to a cytokine made by T cells (anti-'X' IgG). A second group of mice received the protozoa plus a control IgG antibody, as shown in **Figure Q11.10**.

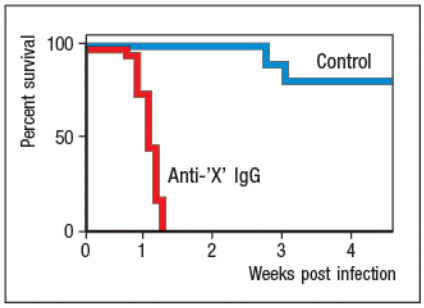

Figure Q11.10

The most likely candidate for cytokine 'X' is:

A. IFN-γ
B. IL-2
C. IL-4
D. IL-17
E. GM-CSF

11-9 T_H2 cells coordinate type 2 responses to expel intestinal helminths and repair tissue injury

11.11 Short answer: The immune response to helminthic worm infections in the gastrointestinal tract requires specialized mechanisms due to the fact that helminths are too large to be ingested and destroyed by phagocytes. For example, cytokines made by T_H2 cells elicit responses from multiple non-hematopoietic cell types that aid in parasite expulsion. Name two of these cell types and for each of them, their response to T_H2-produced cytokines.

11-10 T_H17 cells coordinate type 3 responses to enhance the clearance of extracellular bacteria and fungi

11.12 Multiple choice: *Helicobacter pylori* is a human gastrointestinal (GI) pathogen that can lead to a state of chronic GI inflammation in some individuals, and has been linked to gastric ulcers and other diseases. Studies have shown that human mucosal gastric biopsies of infected individuals have dendritic cells producing IL-23, and that human monocytes isolated and cultured from healthy individuals produce IL-23, but not IL-12, in response to stimulation with live *H. pylori*. Given these findings, which of the following responses would be enhanced in the GI tract of *H. pylori*-infected individuals compared to uninfected individuals?

A. Mucus production by goblet cells
B. Recruitment of neutrophils

C. Recruitment of eosinophils
D. Production of nitric oxide and superoxide
E. Production of IL-13

11-11 Differentiated effector T cells continue to respond to signals as they carry out their effector functions

11.13 Multiple choice: Inflammatory bowel disease (colitis) is a CD4 T-cell mediated disease that can be transferred to naive mice by administration of effector CD4 T cells that home to the gastrointestinal tract and induce inflammation. Simultaneous administration of neutralizing antibodies to IL-12p40 can prevent the disease, as can neutralizing antibodies to IL-23p19. Disease symptoms can be exacerbated by administration of IL-23, but not of IL-12. These data strongly suggest that:

A. Both T_H1 and T_H17 effector cells contribute to disease.
B. T_H1 cells producing INF-γ are the major causes of disease.
C. IL-12p35 is a critical component of disease induction.
D. The inducible IL-12Rβ subunit is essential for disease induction.
E. Neutralizing antibodies to IL-17 would prevent disease.

11-12 Effector T cells can be activated to release cytokines independently of antigen recognition

11.14 Multiple choice: Allergic airway inflammation can be induced in mice by immunizing them with an allergen that produces a T_H2 effector response, and then challenging the immunized mice with an inhaled form of that allergen. In this disease model, the T_H2 effector cells present in the lung respond to the inhaled allergen challenge by producing type 2 cytokines that recruit eosinophils and induce airway inflammation. In addition, a component of this T_H2 response is antigen-independent, as shown by the effects of administering a neutralizing antibody along with the allergen challenge. This neutralizing antibody (anti-'X' IgG) has the effects shown in **Figure Q11.14**.

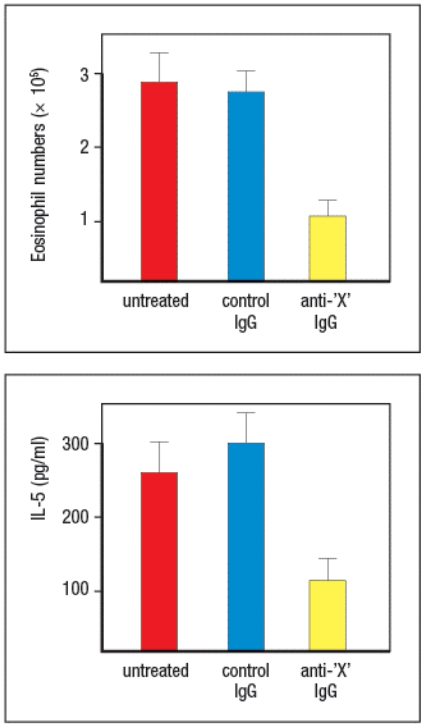

Figure Q11.14

In this experiment, the anti-'X' antibody was shown to inhibit the response of the T_H2 cells, and therefore is likely to be:

A. A neutralizing antibody to IL-12
B. A neutralizing antibody to IL-4
C. A neutralizing antibody to TSLP
D. A neutralizing antibody to STAT4
E. A neutralizing antibody to IL-13

11-13 Effector T cells demonstrate plasticity and cooperativity that enable adaptation during anti-pathogen responses

11.15 Multiple choice: *Salmonella typhimurium* is a Gram-negative bacterial pathogen that infects its host via the gastrointestinal (GI) tract. Early in infection, the bacteria enter and replicate in gut epithelial cells, where the infection provokes a type 3 response, including the development of T_H17 cells, in the GI tract. However, this type 3 response in the GI tract does not eradicate the pathogen, as *S. typhimurium* has evolved strategies to evade the T_H17 response and to spread systemically by infecting and replicating in macrophages. Therefore, a second phase of the immune response is required to completely eliminate the pathogen from the body, as has been demonstrated in mouse models of *S. typhimurium* infection. These experiments in mouse models likely showed that:

A. IFN-γ is required to clear *S. typhimurium* from the body.
B. IL-17 is required to clear *S. typhimurium* from the body.
C. IL-22 is required to clear *S. typhimurium* from the body.
D. IL-13 is required to clear *S. typhimurium* from the body.

E. IL-4 is required to clear *S. typhimurium* from the body.

11-14 Integration of cell- and antibody-mediated immunity is critical for protection against many types of pathogens

11.16 True/False: In some infectious diseases, antibodies specific for the pathogen are not essential for clearing a primary infection with that pathogen, but are essential in preventing re-infection by the same pathogen. This protective role of pathogen-specific antibodies is not useful for any clinical applications.

11-15 Primary CD8 T-cell responses to pathogens can occur in the absence of CD4 T-cell help

11.17 Multiple choice: In the cases of some infections, such as mice infected with adenovirus, the generation of effector cytotoxic CD8 T cell responses needed to clear the infection is dependent on the antigen-presenting dendritic cells receiving stimulation through the CD40 receptor on their surface, a process known as dendritic cell 'licensing'. In this infection system, the dendritic cell would likely receive CD40 receptor stimulation from:

A. The activation of a TLR expressed in the dendritic cell
B. The up-regulation of CD40 ligand by virus-infected host cells
C. The activation of cytosolic nucleic acid sensors in the dendritic cell
D. The interaction with a CD4 effector cell expressing CD40 ligand
E. The phagocytosis of apoptotic cell debris resulting from the virus infection

11-16 Resolution of an infection is accompanied by the death of most of the effector cells and the generation of memory cells

11.18 Multiple choice: The kinetics of a typical CD8 T cell response to an acute virus infection in mice is shown in **Figure Q11.18**.

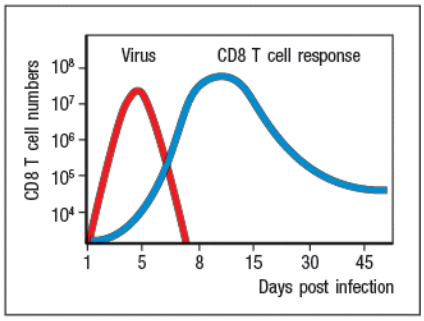

Figure Q11.18

In this example, the virus is cleared by ~day 7 post-infection, and starting at ~day 10 post-infection, the majority of the virus-specific CD8 T cells die. The death of these virus-specific CD8 T cells is caused by:

A. Lysis from the virus infection
B. Engulfment and destruction by phagocytes in the body
C. Destruction by cytotoxic T cells
D. Natural killer cell lysis
E. Fas-induced death or cytokine withdrawal

Immunological memory

11-17 Immunological memory is long lived after infection or vaccination

11.19 Multiple choice: Immunological memory in humans has been examined by assessing responses in individuals who were given the vaccinia virus to induce immunity against smallpox. Antiviral CD4 and CD8 T cell responses could be detected many years after the vaccinia immunization, but declined with an estimated half-time of about 10 years. In contrast, antiviral antibody responses were maintained at a relatively constant level, with a barely detectable decline over decades. The persistence of antiviral antibodies for years after immunization is likely due to:

A. The presence of CD4 T cell help for memory B cells
B. The presence of long-lived antibody secreting plasma cells
C. The periodic reactivation of memory B cells by low levels of antigen exposure
D. The persistence of the virus in the immunized host
E. The presence of pro-survival cytokines, such as IL-7 and IL-15

11-18 Memory B-cell responses are more rapid and have higher affinity for antigen compared with responses of naive B cells

11.20 Multiple choice: A set of mice are each immunized with one of the following as shown in **Figure Q11.20**.

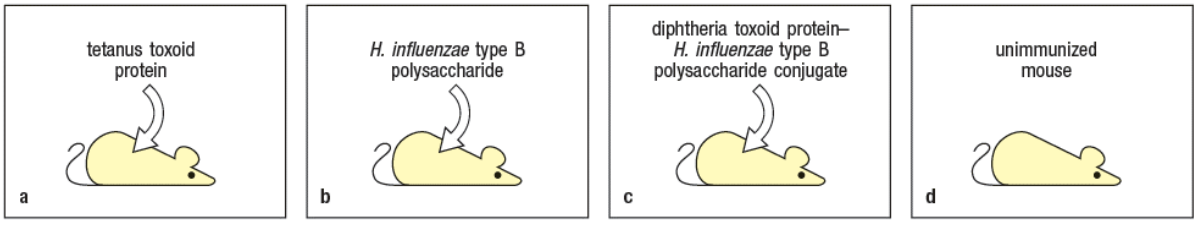

Figure Q11.20

Mouse A is immunized with tetanus toxoid protein. Mouse B is immunized with the *Haemophilus influenzae* type b polysaccharide antigen. Mouse C is immunized with a conjugate of the diphtheria toxoid protein linked to *H. influenzae* type b polysaccharide. Mouse D is left unimmunized (naive). Four weeks later the spleen cells from each mouse are isolated, and B lymphocytes and T lymphocytes from each spleen cell population are purified. When mixed together in culture together with a conjugate antigen of the tetanus toxoid protein linked to the to *H. influenzae* type b polysaccharide, which combination of spleen cells would generate a memory B cell response?

A. B lymphocytes from mouse B plus T lymphocytes from mouse B
B. B lymphocytes from mouse A plus T lymphocytes from mouse A
C. B lymphocytes from mouse C plus T lymphocytes from mouse A
D. B lymphocytes from mouse C plus T lymphocytes from mouse D
E. B lymphocytes from mouse B plus T lymphocytes from mouse D

11-19 Memory B cells can re-enter germinal centers and undergo additional somatic hypermutation and affinity maturation during secondary immune responses

11.21 Multiple choice: It is well documented that antibody affinities for an immunizing antigen continue to increase upon successive rounds of immunization (i.e., secondary, tertiary, etc.). This is due to the fact that:

A. At each round of immunization, new naive B cells are recruited into the response.
B. At each round of immunization, the expression of AID increases, leading to higher rates of somatic hypermutation.
C. Memory B cells express higher levels of AID than naive B cells, leading to higher rates of somatic hypermutation.
D. Memory B cells can re-enter germinal centers and undergo additional somatic hypermutation.
E. At each round of immunization, germinal centers become larger and have increased numbers of B cells in them.

11-20 MHC tetramers identify memory T cells that persist at an increased frequency relative to their frequency as naive T cells

11.22 Short answer: One of the first studies using peptide:MHC class I tetramers to measure the frequencies of virus-specific CD8 T cells in an acute virus infection in mice demonstrated the remarkable finding that more than 50% of all the CD8 T cells in the mouse were virus-specific at the peak of the response (day 8 post-infection). This study used the virus lymphocytic choriomeningitis virus (LCMV). One peptide:MHC tetramer used in this study, H-2-D^b loaded with the LCMV nucleoprotein peptide $NP_{396\text{-}404}$, bound to ~20% of the CD8 T cells in the spleen at day 8 post-infection. Why did this tetramer only stain ~20% of the CD8 T cells if more than half of the CD8 cells in the spleen were virus-specific?

11-21 Memory T cells arise from effector T cells that maintain sensitivity to IL-7 or IL-15

11.23 Multiple choice: Following an acute virus infection in which the host clears the virus by approximately one week post-infection, a population of virus-specific memory CD8 T cells is maintained and can be detected for months to years post-infection. In mice with a knockout of a single cytokine, virus-specific memory CD8 T cells cannot be maintained, and disappear over time as shown in **Figure Q11.23**.

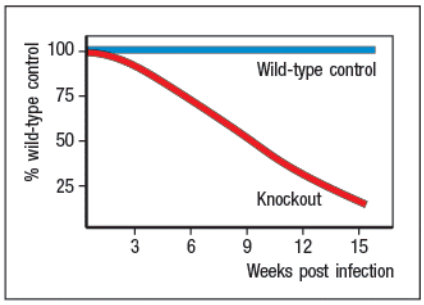

Figure Q11.23

The most likely identity of the cytokine that is missing in these knockout mice is:

A. IL-15
B. IL-2
C. IL-21
D. IL-23

E. IL-4

11-22 Memory T cells are heterogeneous and include central memory, effector memory, and tissue-resident subsets

11.24 Multiple choice: Studies in mice have shown that resident memory cells (T_{RM}) most often take up permanent residence in the tissue where the initial infection that produced those memory cells occurred. In this location, they are poised to respond rapidly should that infection re-occur in that same location. In contrast, central memory cells (T_{CM}) are primarily found in secondary lymphoid organs, where they can be activated to proliferate and differentiate into effector cells when stimulated by antigen-bearing dendritic cells following re-infection. The third subset of memory cells, effector memory cells (T_{EM}), are recirculating cells that can readily enter tissues at sites of inflammation or infection and are poised to rapidly respond to re-infection. The subset of T_{EM} cells provides an important component of protective immunity to re-infection by the same pathogen because:

A. They are the only memory cell subset that can produce effector cytokines within a few hours of antigen re-encounter.
B. They are able to respond to S1PR1 and enter the blood circulation rapidly upon re-infection.
C. They express the integrin $\alpha_E\beta_7$ that binds to integrin ligands expressed on epithelial cells.
D. They can protect against re-infection that occurs in a different site in the body than the primary infection.
E. They can simultaneously express cytokines associated with all three effector T cell lineages.

11-23 CD4 T-cell help is required for CD8 T-cell memory and involves CD40 and IL-2 signaling

11.25 True/False: The generation of optimal CD8 T cell memory following a primary infection requires CD4 T cell help for the responding CD8 T cells. This requirement for CD4 T cell help would not be completely replaced by supplying high levels of the cytokine IL-2 during the primary CD8 T cell response.

11-24 In immune individuals, secondary and subsequent responses are mainly attributable to memory lymphocytes

11.26 Short answer: Vaccinia virus, used to immunize individuals against smallpox, has a long history of well-documented safe use in humans. Due to the eradication of smallpox, immunizations with vaccinia virus were halted, and babies born after 1970 no longer received this vaccine. This has led to the proposal to engineer vaccinia virus to express proteins derived from other human pathogens for which there are no current vaccines. For instance, the gene encoding the F protein of the respiratory syncytial virus (RSV) has been inserted into vaccinia virus (VaccV-F), and has been tested for its ability to induce anti-F protein antibody responses following immunization.

Would you expect a different antibody response directed at the RSV F protein in individuals immunized with the recombinant VaccV-F strain if they were born before versus after 1970?

11.27 **Synthesis question:** Hyper-IgE syndrome, also known as Job's syndrome, is an immunodeficiency disease resulting from the lack of function of a single gene (gene 'X'). Patients with this disease are highly susceptible to infections with extracellular bacteria and fungi, most frequently including *Staphylococcus aureus* infections and *Candida albicans* infections in the skin. Analysis of the various immune cell compartments indicates that these patients have normal numbers of each cell lineage (i.e., CD4 and CD8 T cells, B cells, monocytes, dendritic cells, NK cells, granulocytes, etc.), and normal levels of IgG, IgA, and IgM antibodies, but higher than normal levels of IgE.

a) Given this information, name a likely component of the immune response that could be impaired in these patients.

To investigate the immune mechanism impaired in these patients, a mouse model of this gene deficiency was generated. Conditional knockout mouse lines were generated in which gene X was knocked out in either the T cells, the B cells, or the myeloid cells of the mouse. For each conditional knockout line, mice were challenged with *Candida albicans*, and the ability to clear the infection was assessed. In mice, infection of the oral cavity with *Candida albicans* has been shown to be a valid model for mucosal *Candida albicans* infections in humans. After infection, the response was assessed by measuring fungal burden (CFU/g tissue) on the tongue. The resulting data are shown in **Figure Q11.27A.**

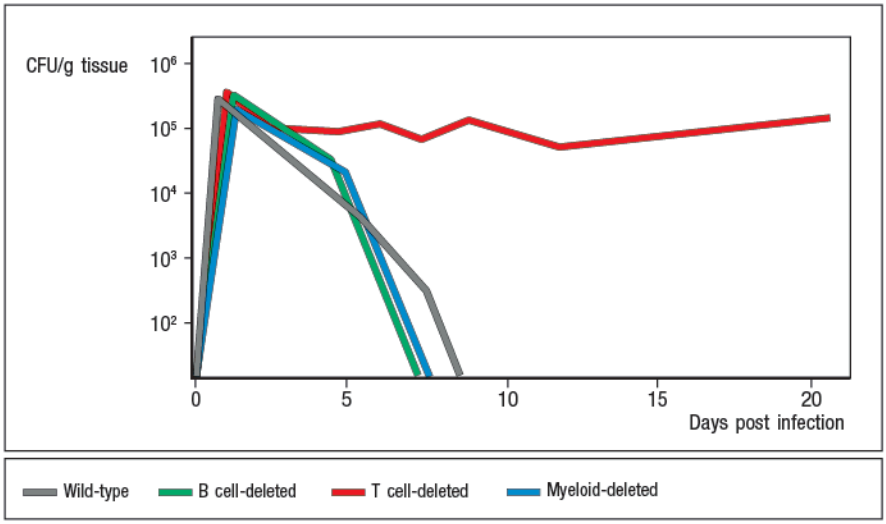

Figure Q11.27A

b) Based on these data, what is the most likely immune function impaired in the Gene X-deficient patients?

Histological examination of tongue sections from *Candida albicans* infected mice were examined, and the numbers of infiltrating leukocytes (white blood cells) were quantified in each microscopic field of each section, and the results are shown in **Figure Q11.27B**.

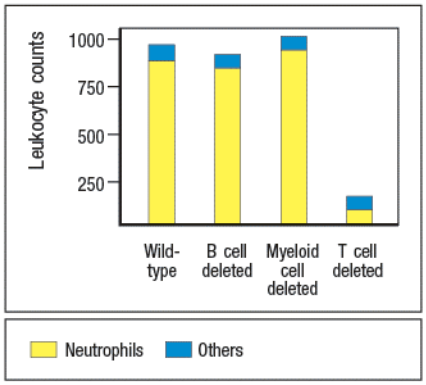

Figure Q11.27B

c) Do these data support or refute your hypothesis stated in response to question (b)? Why or why not?

To examine the details of T cell responses when Gene X is absent from the T cells, a series of *in vitro* experiments were performed. CD4 T cells were isolated from wild type mice and from T cell-deleted Gene X knockout mice, and were stimulated *in vitro* with a combination of anti-CD3 and anti-CD28 antibodies to activate the T cells. In addition, each culture was supplemented with one of the following cytokine conditions: (1) IFN-γ plus IL-12; (2) IL-4; or (3) IL-6, TGF-β, IL-1 plus IL-23. After four days, the cells were examined for the expression of transcription factors by RT-PCR, as shown in **Figure Q11.27C**. Note that Gene X does not encode T-bet, GATA-3, or RORγt. Instead, these data indicated impaired responses of Gene X-deficient T cells to the cytokines used in these *in vitro* culture experiments.

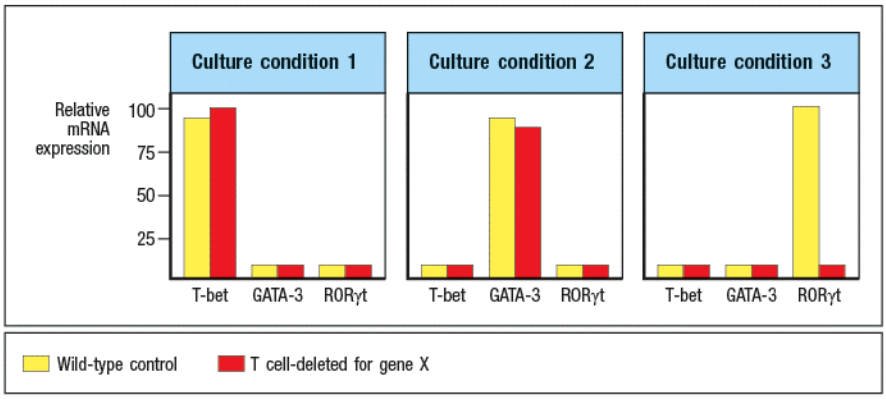

Figure Q11.27C

d) Based on these data, name three candidate genes that could be Gene X.

11.28 Synthesis question: Leishmania parasites are intracellular protozoa that causes skin sores, and in some individuals, infections that spread systemically and cause damage to internal organs. In mice, different strains of inbred mice have varying responses to the

Leishmania parasite *Leishmania major*. Whereas C57BL/6 mice develop self-healing skin lesions following infection, Balb/c mice develop non-healing lesions and ultimately succumb to systemic, fatal disease. An example of such data is shown in **Figure Q11.28A**. For these studies, mice were infected with 2×10^6 *L. major* promastigotes in the footpad, and the sizes of skin lesions and the numbers of parasites per lesion were measured at the indicated times post-infection.

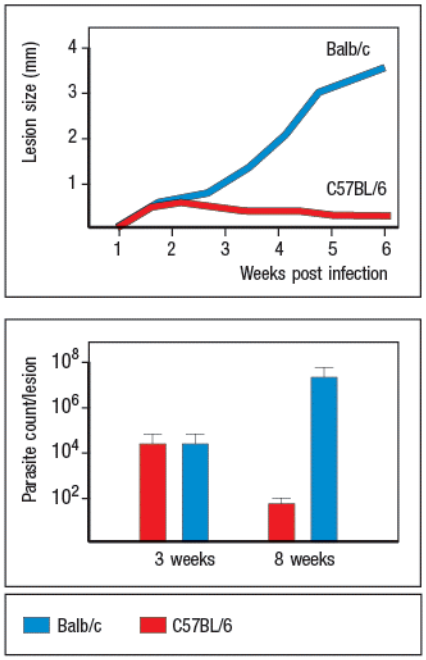

Figure Q11.28A

Early studies in this system indicated a strong bias in the cytokine profiles of effector CD4 T cells elicited in response to *L. major* infection of C57BL/6 versus Balb/c mice, particularly at late stages of the infection (>2 weeks post-infection). An example of the cytokine data from *L. major* infected mice is shown in **Figure Q11.28B**. At the indicated times the percentages of CD4 T cells in the draining lymph node producing IFN-γ versus IL-4 following stimulation with *L. major* antigens were measured by intracellular cytokine staining.

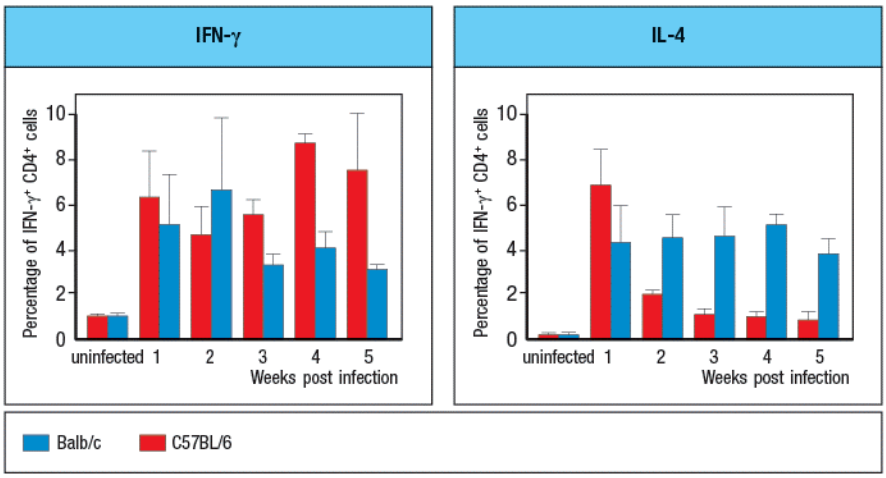

Figure Q11.28B

While subsequent studies have confirmed that the ability of one mouse strain to clear the infection while the other does not is a feature of their relative T cell responses (i.e., the outcome of the infection is dependent on the T cell response), additional data indicate that other differences between the effector T cells in the two mouse strains are also contributing to the ability of one strain to clear the infection, but not the other. For instance, the following results were obtained when the numbers of T cells in the lesions were compared between the two strains following *L. major* infection.

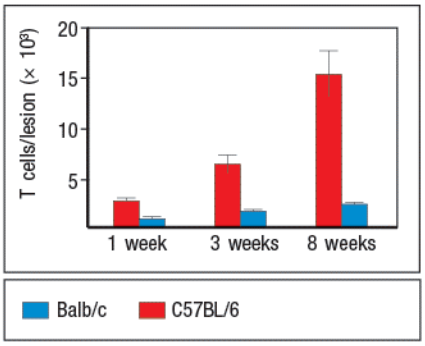

Figure Q11.28C

a) What difference between the C57BL/6 and the Balb/c T cells could account for these data?

b) Name two molecules that could account for the difference noted in the answer to part (a)?

Balb/c mice are not globally immunodeficient, and they are capable of making protective T cell responses to infections that require recruitment of T_H2 cells to the lung, for instance.

c) Do these data alter your responses to part (b)?

Three chemokines—CXCL9, CXCL10, and CXCL11—bind a common receptor, CXCR3 that is up-regulated on T_H1 effector T cells, CD8 effector T cells, and NK cells. These chemokines are normally not detectable in healthy tissues, but are strongly up-regulated during infection, injury or inflammation, in response to IFN-γ production in the tissue.

d) How might this information help explain the divergent ability of C57BL/6 versus Balb/c mice to accumulate increasing numbers of effector T cells into the *L. major* lesions over the long timecourse shown in the data above?

ANSWERS

11.1: E.
The microbe-associated molecular patterns (MAMPs) expressed by different types of pathogens stimulate distinct cytokine responses from innate sensor cells. In the case of intracellular bacterial and protozoan infections, as well as viral infections, tissue-resident dendritic cells, and in some cases macrophages, are stimulated to produce IL-12. This cytokine, in turn, stimulates specific subsets of ILCs to produce different effector cytokines. In this example, IL-12 stimulates ILC1 cells and natural killer (NK) cells to produce IFN-γ that acts to coordinate and amplify the innate response.

11.2: B.
ILC3s play a critical early role in defense against extracellular bacteria and fungi at barrier tissues. An example of such an infection is that of the bacteria *Citrobacter rodentium*. ILC3s are responsive to IL-23 and IL-1β; these cytokines elicit the production of IL-17 and IL-22, which promote early type 3 responses. IL-17 is a pro-inflammatory cytokine that acts on a variety of cells, including stromal cells, epithelial cells, and myeloid cells, to stimulate the production of other pro-inflammatory cytokines (for example, IL-6, IL-1β), hematopoietic growth factors (G-CSF and GM-CSF), and chemokines that recruit neutrophils and monocytes. IL-22 acts on epithelial cells to induce their production of antimicrobial peptides (AMPs) and promote enhanced barrier integrity.

11.3: The differentiation of naive pathogen-specific CD4 T cells into T_H1, T_H2, or T_H17 cells. Each of the three effector CD4 T-cell subsets (T_H1/T_H2/T_H17) evolved to enhance and coordinate the functions of, and integrate adaptive immunity with, different arms of the myelomonocytic pathway for optimal eradication of different classes of pathogens: monocyte/macrophages are enhanced by T_H1 cells; eosinophils, basophils, and mast cells by T_H2 cells; and neutrophils by T_H17 cells. Because of their ability to focus effector cytokines on antigen-bearing target cells and to induce B-cell maturation and the production of class-switched antibodies, effector CD4 T cells also provide an additional layer of licensing of innate effector cells that increases their lethality and ability to achieve microbial clearance.

11.4: D.
T_H1 cells express CCR5, which is also expressed on monocytes that mature into macrophages as they enter the inflammatory site. Thus, both T_H1 cells and the innate effector cells whose effector functions they enhance are recruited to the same tissue site by the same chemokines. T_H1 cells also express CXCR3, which is shared by NK cells and cytotoxic CD8 T cells. In response to CXCR3 ligands—CXCL9 and CXCL10—these cells are recruited to the same inflammatory site to coordinate the cell-mediated killing of targets infected by intracellular pathogens, such as *Listeria monocytogenes*, or certain viruses.

11.5: A.
In the early stage of the adaptive immune response, only a minority of the effector T cells that enter infected tissues will be specific for pathogen. This is because activation of the endothelium of local blood vessels by inflammatory cytokines induces expression of selectins, integrin ligands, and chemokines that can recruit any circulating effector or memory T cell that expresses the appropriate trafficking receptors, irrespective of its antigenic specificity. However, specificity of the reaction is rapidly increased as the number of pathogen- specific T cells

increases and recognition of antigen within the inflamed tissue retains them there.

11.6: B.
Pathogens of all types are ingested by macrophages from the extracellular fluid, and are often destroyed without the need for additional macrophage activation. In several clinically important infections, such as those caused by mycobacteria, ingested pathogens are not killed, and can even set up a chronic infection in macrophages and incapacitate them. Such microorganisms are able to maintain themselves in the hostile environment of phagosomes—shielded from the effects of both antibodies and cytotoxic T cells—by inhibiting the fusion of phagosomes and lysosomes, or by preventing the acidification required to activate lysosomal proteases. Nevertheless, peptides derived from such microorganisms can be displayed by MHC class II molecules on the macrophage surface, where they are recognized by antigen-specific effector T_H1 cells. The T_H1 cell is stimulated to synthesize membrane-associated proteins and soluble cytokines that enhance the macrophage's antimicrobial defenses and enable it to either eliminate the pathogen or control its growth and spread. In patients with AIDS and few CD4 T cells, there are insufficient numbers of T_H1 cells for this mechanism to function to provide protection.

11.7: A.
T_H1 effector cells generated in response to intracellular pathogens migrate into tissues at sites of infection. In the infected tissue, the T_H1 effector cells are activated through their T-cell receptor by recognizing peptide:MHC class II complexes on macrophages or dendritic cells. The effector T_H1 cells produce an array of cytokines and chemokines, as well as other effector molecules. This array of T_H1 products has widespread effects on the body that extend beyond the site of infection. T_H1 cells produce IL-3 and GM-CSF that diffuse to the bone marrow and induce bone marrow progenitor cells to differentiation into monocytes. This causes an increase in monocyte numbers in the circulation.

11.8: True.
Antigen-specific induction of macrophage activation by T_H1 cells is a mechanism that is designed to limit tissue injury, but does not completely eliminate effects on neighboring host cells. However, by targeting only infected macrophages through peptide:MHC recognition, T_H 1 cells minimize 'collateral damage' that might otherwise result to normal components of the inflamed tissue: oxygen radicals, NO, and proteases that are toxic to host cells as well as to the pathogen that is targeted for destruction. Thus, antigen-specific macrophage activation by T_H1 cells is a means of deploying this powerful defensive mechanism to maximum effect while minimizing, but not completely avoiding, local tissue damage.

11.9: C.
Some intracellular pathogens, such as *Mycobacterium tuberculosis* and *Mycobacterium leprae*, are sufficiently resistant to the microbicidal effects of activated macrophages that they are incompletely eliminated by a type 1 response. This gives rise to chronic, low-level infection that requires an ongoing T_H1 response to prevent pathogen proliferation and spread. In this circumstance, chronic coordination between T_H1 cells and macrophages underlies the formation of the immunological reaction called the granuloma, in which microbes are held in check within a central area of macrophages surrounded by activated lymphocytes. A characteristic feature of granulomas is the fusion of several macrophages to form multinucleated giant cells, which can be found at the border of the central focus of activated macrophages and the lymphocytes that

surround them and which appear to have heightened antimicrobial activity. A granuloma serves to ‘wall off’ pathogens that resist destruction.

11.10: A.
In mice whose gene for IFN-γ or CD40 ligand has been deleted by gene targeting, classical macrophage activation is impaired; consequently, the animals succumb to sublethal doses of *Mycobacterium*, *Salmonella*, and *Leishmania* species, as well as *Toxoplasma gondii*. This outcome can also be seen by injecting mice with a neutralizing antibody to IFN-γ. Classical macrophage activation is also crucial in controlling vaccinia virus.

11.11:
1. Gastrointestinal epithelial cells undergo increased proliferation and turnover (shedding) in response to IL-13.
2. Goblet cell production of mucus is increased by IL-13.
3. Smooth muscle cells are induced to contract by IL-13.

11.12: B.
IL-23 is secreted by dendritic cells stimulated by MAMPs found in some extracellular bacteria and fungi. This cytokine plays a critical role in the development of T_H17 responses in the GI tract, a response that is observed in individuals chronically infected with *H. pylori*. T_H17 cells make IL-17 and IL-22. IL-17 acts on stromal cells and epithelial cells to produce chemokines that recruit neutrophils to the site of infection.

11.13: E.
The data indicate a key role for T_H17 cells and for their dependence on IL-23 to induce disease. The antibody neutralization data show that both subunits of IL-23 are essential (IL-12p40 and IL-23p19) and that IL-23, but not IL-12, will exacerbate disease. This indicates a central role for T_H17 cytokines, such as IL-17, and suggests that T_H1 cells are not involved as there is no effect of IL-12.

11.14: C.
Effector T cells acquire the ability to be activated by pairs of cytokines, independently of antigen recognition by their T-cell receptor. The cytokine pairs that mediate this ‘noncognate’ function of differentiated effector cells appear to be the same as those that activate the ILC subset that parallels each T-cell subset. In each case, the pair of stimulating cytokines includes one cytokine that activates a receptor that signals via a STAT factor, and one that activates a receptor that signals via NFκB—typically a member of the IL-1 receptor family. Thus, stimulation of T_H2 cells by TSLP (STAT5) plus IL-33 produces IL-5 and IL-13.

11.15: A.
During the systemic phase of the *S. typhimurium* infection, the T-cell response shifts to become focused on those antigens that enable the intracellular lifestyle of the pathogen in macrophages. Some of these newly expressed antigens appear to activate cytosolic sensors within $CD8\alpha^+$ classical dendritic cells, which produce IL-12 to activate pathogen-specific T_H1 cells and a type 1 response. The pathogen can now be cleared by T_H1-induced macrophage activation directed against these newly expressed antigens, a process that is dependent on production of IFN-γ by the T_H1 cells.

11.16: False.
The protective role of pathogen-specific antibodies is the main goal of nearly all vaccines in current use. Even though antibodies may not be required, or even useful, in eliminating a primary infection of some pathogens, the presence of pre-existing anti-pathogen antibodies can prevent a primary infection from taking place and thereby prevent disease.

11.17: D.
In this virus infection system, the generation of effector cytotoxic CD8 T cell responses to the adenovirus is dependent on CD4 T cell help providing signals to the antigen-presenting dendritic cell. This CD4 T cell help has been shown to require CD40 ligand stimulation on an effector CD4 T cell stimulating the CD40 receptor on the dendritic cell. A stimulatory anti-CD40 antibody can also substitute for the presence of CD40-ligand expressing effector CD4 T cell help.

11.18: E.
When an infection is effectively repelled by the adaptive immune system, most effector T cells undergo 'death by neglect,' removing themselves by apoptosis. The resulting 'clonal contraction' of effector T cells appears to be due both to the loss of pro-survival cytokines that are produced by antigenic stimulation, such as IL-2, and to the loss of expression of receptors for these cytokines. While many effector T cells die from the loss of survival signals and the activation of the Bim-mediated intrinsic pathway of apoptosis, effector T cell death can also occur via the extrinsic pathway of apoptosis that is activated by signaling via members of the TNF receptor superfamily, particularly Fas (CD95).

11.19: B.
The generation of long-lived antibody secreting plasma cells at the time of immunization is thought to be the key to persistence of antiviral antibodies in the vaccinated individuals. Long-lived plasma cells continue to secrete antibody for years, and do not appear to require any re-exposure to antigen.

11.20: C.
The memory B cell response to the *H. influenzae* type b polysaccharide can be elicited *in vitro* by re-stimulating memory B cells primed to *H. influenzae* type b polysaccharide from a mouse that received a primary immunization with an antigen that would generate T cell help for the primary B cell response. This would be mouse C. However, the T lymphocytes isolated from mouse C were not primed to the protein component of the antigen used in the recall response *in vitro*, which is tetanus toxoid. In order to provide the *in vitro* culture with helper T cells primed to the recall antigen, T lymphocytes from mouse A need to be added.

11.21: D.
Besides responding more rapidly, memory B cells can re-enter germinal centers during secondary immune responses and undergo additional somatic hypermutation and affinity maturation. Thus, reactivated memory B cells that have not yet undergone differentiation into plasma cells migrate into the follicle and become germinal center B cells, undergoing additional rounds of proliferation and somatic hypermutation before differentiating into antibody-secreting plasma cells. Since B cells with the higher-affinity antigen receptors will more efficiently acquire and present antigen to antigen-specific T_{FH} cells in the germinal center, the affinity of the antibodies produced during secondary and tertiary responses rises progressively.

11.22: Each peptide:MHC class I tetramer will stain the CD8 T cells whose T-cell receptors are specific for that MHC class I molecule bound to that specific viral peptide. Since the response to the virus overall will include CD8 T cells that are recognizing other viral epitopes, i.e., peptides from other viral proteins or other sequences from the LCMV nucleoprotein, the total antiviral CD8 T cell response will be the sum of all of these different specificities. By using a panel of peptide:MHC class I tetramers to the known LMCV peptide epitopes to stain T cells from the infected mice, one can more closely estimate the total antiviral response, but these experiments cannot necessarily account for all of the virus-specific CD8 T cells in the spleen at the peak of the response.

11.23: A.
The homeostatic mechanisms governing the survival of memory T cells differ from those for naive T cells. Memory T cells divide more frequently than naive T cells, and their expansion is controlled by a shift in the balance between proliferation and cell death. The survival of memory T cells requires signaling by the receptors for the cytokines IL-7 and IL-15. IL-7 is required for the survival of both CD4 and CD8 memory T cells. In addition, IL-15 is critical for the long-term survival and proliferation of CD8 memory T cells under normal conditions. In the absence of IL-15 (or the IL-15R), memory CD8 T cells slowly disappear from the population.

11.24: D.
While re-infection with the same pathogen often occurs in the same location as the initial primary infection, this is not always the case. Therefore, the circulating effector memory T cells can patrol all tissues in the body, and are not restricted to a single tissue of residence.

11.25: True.
The mechanism underlying the requirement for CD4 T cells is not completely understood. It may involve two types of signals received by the CD8 T cell—those received through CD40 and those received through the IL-2 receptor. CD8 T cells that do not express CD40 are unable to generate memory T cells. Although many cells could potentially express the CD40 ligand needed to stimulate CD40, it is most likely that CD4 T cells are the source of this signal. Therefore, in addition to providing the cytokine IL-2, CD4 T cells must interact with the responding CD8 T cells to provide CD40 ligand stimulation of CD40 on the CD8 T cells.

11.26: Yes.
Individuals born before 1970 were immunized with vaccinia virus as children, and therefore will have pre-existing antibodies and memory B cells to the virus. These antibodies will neutralize the virus, and likely prevent the recombinant virus from establishing an infection. The small amount of antigen present in these previously vaccinated individuals will also preferentially activate their memory B cells, producing antibody responses to the vaccinia virus epitopes recognized previously. Most likely these individuals will make a very poor antibody response to the RSV-F protein. In contrast, individuals born after 1970 will be seeing all epitopes of the recombinant virus for the first time, and should make a robust antibody response to both the vaccinia virus epitopes and to the RSV-F protein.

11.27:
a) CD4 effector cell differentiation into T_H17 cells. T_H17 cells are important in immune responses to extracellular bacterial and fungal infections, particularly at barrier surfaces.

Another possibility is the generation of pathogen specific antibodies, possibly IgG or IgA. Although total antibody levels appear generally normal, this fact does not necessarily rule out a specific defect in antibody responses, although making it a less likely alternative.

A third possibility might be an antibody-dependent effector mechanism, such as an Fc receptor or a complement component.

However, the combination of increased susceptibility to extracellular bacteria and fungi strongly suggests a T_H17 defect.

b) T cells require Gene X for the ability to control oral *Candida albicans* infections in mice. The most likely immune function is the generation or effector response mediated by T_H17 cells.

c) These data indicate that the major defect when Gene X is deleted in T cells is the failure of neutrophil recruitment to the site of infection. Since the neutrophil recruitment is T cell-mediated, these data strongly implicate a failure of T_H17 responses in the oral cavity in response to *Candida albicans*.

d) Possible answers:
   1. IL-1 receptor
   2. IL-6 receptor
   3. TGF-β receptor
   4. STAT3
   5. TGF-β receptor signaling protein
   6. IL-23R is not the optimal answer, as IL-23R expression is induced by RORγt. Since the data show that there is defect in RORγt expression, the absence of IL-23R expression would be expected.

Note: The majority of patients with Hyper-IgE syndrome have defects in *STAT3*. To date, the explanation for the elevated IgE in these patients is not known. The susceptibility to skin and other mucosal infections of extracellular bacteria and fungi is believed to be due to defects in T_H17 effector cell differentiation and function, as similar susceptibilities to infection are seen in individuals with genetic defects in IL-17, IL-17R, and an IL-17R downstream signaling protein, ACT1.

11.28:

a) Difference in T cell migration into the infected tissue.

Another possible answer could be that Balb/c T cells entering the tissue fail to proliferate in response to *L. major* antigens that they re-encounter at the site of infection. This is less likely than a defect in migration, because there is no defect in T cell expansion in the Balb/c lymph node, as indicated by similar numbers of IFN-γ-producing T cells in the first three weeks or so post-infection.

b) Answers include: Chemokine receptor on the effector T cells, or an adhesion molecule such as CD44, VLA-4, or a ligand for P- or E-selectin. The difference could not be due to a difference in chemokine expression by cells at the site of infection, as this would not result in a T-cell-intrinsic difference between the two strains of mice.

c) Yes, this information would rule out any general defect in an adhesion molecule such as an integrin ligand or a selectin-ligand. This information makes it most likely that the

Balb/c T cells have a defect, or at the very least a reduction, in expression of a specific chemokine receptor. In fact, studies have shown that effector T cells generated in Balb/c mice in response to *L. major* have greatly reduced expression of CXCR3 compared to those from C57BL/6 mice, and in addition, that *Cxcr3*$^{-/-}$ mice (C57BL/6 background) fail to clear *L. major* due to an impaired effector T cell response in the lesions.

d) An initial partial defect (or reduction) in CXCR3 expression on IFN-γ-producing effector T cells in Balb/c mice would lead to reduced expression of IFN-γ in the infected tissue. This would lead to impaired expression of CXCL9, CXCL10, and CXCL11 in the lesions in Balb/c mice compared to C57BL/6 mice. This in turn would lead to less T cell recruitment to the lesions. So in the end, a modest defect in effector T cell recruitment to the site of infection would over time appear as a major defect due to the absence of the positive feedback loop on the chemokine expression.