

JANEWAY'S IMMUNOBIOLOGY, 9TH EDITION CHAPTER 2: INNATE IMMUNITY: THE FIRST LINES OF DEFENSE

Anatomic barriers and initial chemical defenses

2-1 Infectious diseases are caused by diverse living agents that replicate in their hosts

2.1 **Multiple choice:** Antibodies, complement proteins, and phagocytic cells provide effective protection against all of the following types of infections in **Figure Q2.1**, except:

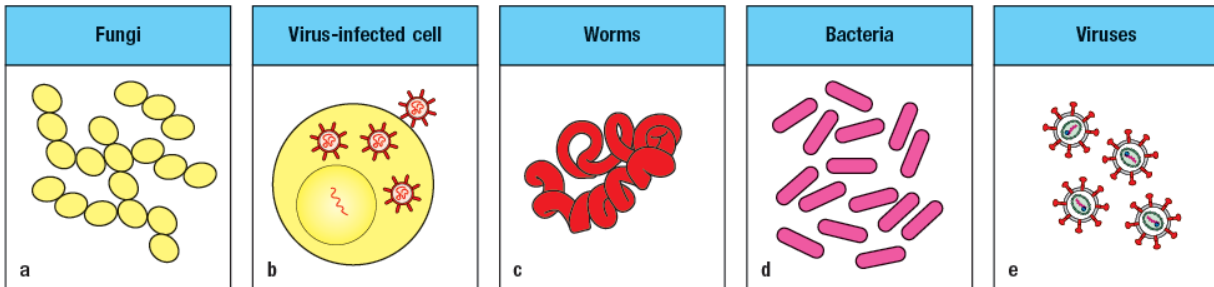


Figure Q2.1

2.2 **Multiple choice:** Pathogenic infections induce damage to the host by a variety of mechanisms. While many mechanisms are direct effects of the pathogen, some damaging mechanisms result from the immune response to the infection, as illustrated in **Figure Q2.2**. Examples of damage caused by the host immune response are:

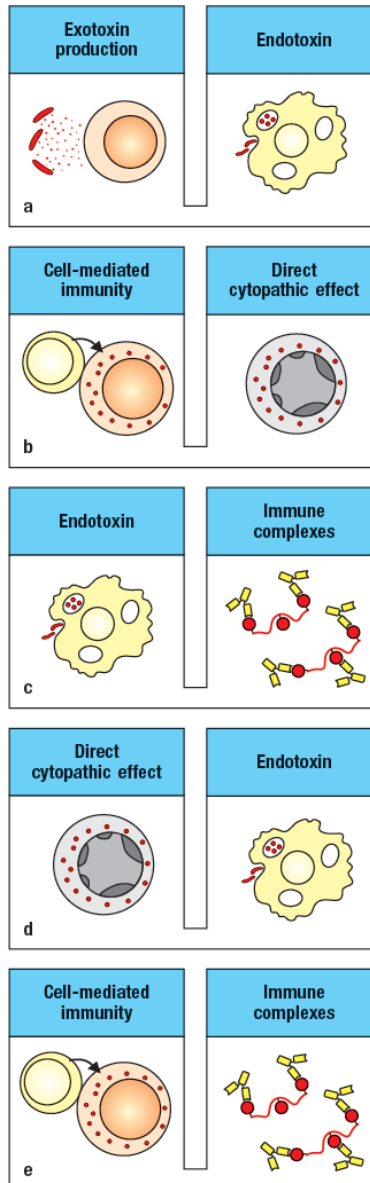


Figure Q2.2

2.3 True/False: Mucosal surfaces and external epithelia are major routes of pathogenic infection. Mucosal surfaces are found in tissues such as the gastrointestinal tract, the reproductive tract and the mouth and respiratory tract. While the mouth and respiratory tract are routes of virus but not bacterial infections, the gastrointestinal tract is the route for bacterial but not virus infections.

2-2 Epithelial surfaces of the body provide the first barrier against infection

2.4 Multiple choice: Epithelial surfaces provide the first line of defense against infection by the use of several types of mechanisms. One of the chemical mechanisms used by epithelia is:

- A. Joining of epithelial cells by tight junctions
- B. Secretion of antimicrobial peptides by epithelial cells

- C. Production of mucus, tears, or saliva in the nose, eyes, and oral cavity
- D. Movement of mucus by cilia
- E. Peristalsis in the gastrointestinal tract

2.5 Multiple choice: Women with urinary tract infections caused by *E. coli* are generally treated with a course of antibiotics. A common complication of the antibiotic treatment is the occurrence of a vaginal yeast infection caused by *Candida albicans*, an organism that is normally present in very low numbers in the human vaginal tract. This complication occurs because:

- A. The *E. coli* infection damages the reproductive epithelium, causing a breach in the tight junctions and allowing invasion by the *Candida albicans*.
- B. The *E. coli* infection induces adhesion molecule expression on the reproductive epithelium, allowing attachment of the yeast.
- C. The antibiotic treatment kills all strains of fungi present in the reproductive tract, except the *Candida albicans*.
- D. The *E. coli* infection causes gastrointestinal distress leading to diarrhea.
- E. The antibiotics kill many of the commensal organisms in the reproductive tract, allowing overgrowth of the fungus.

2-3 Infectious agents must overcome innate host defenses to establish a focus of infection

2.6 Short answer: Our environment contains masses of microorganisms, many of which reside as commensal organisms on our body's mucosal and epithelial surfaces without causing disease. What two features distinguish a pathogenic microbe from these commensal microbes?

2-4 Epithelial cells and phagocytes produce several kinds of antimicrobial proteins

2.7 Multiple choice: *Streptococcus pneumoniae* is a Gram-positive bacterium that colonizes the mucosal surface of the upper respiratory tract in humans. The presence of this bacterium in the nose and throat is widespread in the population, and in most people, colonization with *Strep. pneumoniae* is asymptomatic. **Figure Q2.7** shows a comparison of *in vitro* growth curves of the wild-type strain of *Strep. pneumoniae*, as well as a *Strep. pneumoniae* mutant strain with a defect in one bacterial gene. The graph on the right shows the growth curve following addition of lysozyme during the logarithmic phase of bacterial growth.

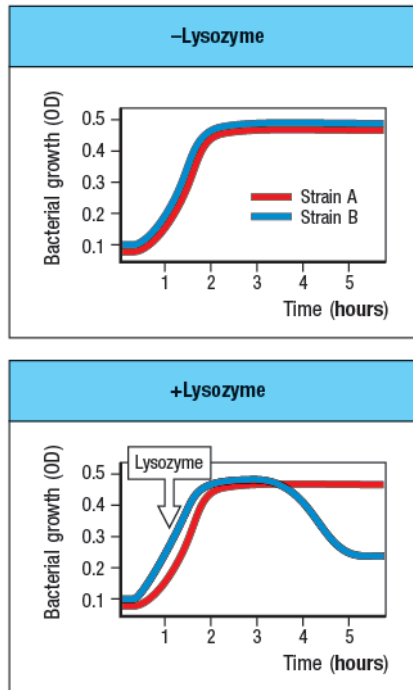


Figure Q2.7

Which statement could account for the data in these graphs?

- Strain B is wild-type *Strep. pneumoniae*, and strain A is a mutant that cannot modify its peptidoglycan to be lysozyme-resistant.
- Strain B is wild-type *Strep. pneumoniae*, and strain A is a mutant that expresses increased levels of LPS.
- Strain A is wild-type *Strep. pneumoniae*, and strain B is a mutant that cannot modify its peptidoglycan to be lysozyme-resistant.
- Strain A is wild-type *Strep. pneumoniae*, and strain B is a mutant that secretes an enzyme that inactivates lysozyme.
- Strain A is wild-type *Strep. pneumoniae*, and strain B is a mutant that cannot grow well *in vitro*.

- 2.8 Multiple choice:** The production of antimicrobial peptides is one of the most evolutionarily ancient mechanisms of defense for multicellular organisms, and most eukaryotic species make many different forms of these proteins. For instance, human paneth cells in the gastrointestinal epithelium make 21 different defensins. The reason for this diversity of antimicrobial peptides is:
- Epithelial cells make different forms than those made by neutrophils.
 - Neutrophils make many different defensins and store them as inactive proteins in their secretory granules.
 - Most of them are produced only in response to infection.
 - The production of different peptides is induced following a bacterial infection versus a fungal infection.
 - Each one has distinct activities against Gram-negative bacteria, Gram-positive bacteria, or fungi.

- 2.9 True/False:** Neutrophils regulate the production of active cathelicidins (a class of antimicrobial peptides) by segregating the inactive propeptide from the processing

enzyme that cleaves and activates it in two different types of cytoplasmic granules. These two types of granules are induced to fuse with phagosomes after ingestion of microbes, bringing the processing enzyme and the propeptide together.

The complement system and innate immunity

2-5 The complement system recognizes features of microbial surfaces and marks them for destruction by coating them with C3b

2.10 Multiple choice: Although the complement cascade can be initiated by antibodies bound to the surface of a pathogen, complement activation is generally considered to be an innate immune response. This is because:

- A. Two of the three pathways for complement activation are initiated by constitutively produced recognition molecules that directly interact with microbial surfaces.
- B. When the complement cascade leads to the formation of a membrane-attack complex, the pathogen is killed.
- C. Several of the soluble products generated by complement activation lead promote the inflammatory response.
- D. Complement proteins bound to the pathogen promote uptake and destruction by phagocytic cells.
- E. The C3 convertase is only produced when complement activation is initiated by antibody binding to a pathogen.

2.11 Multiple choice: The formation of the C3 convertase is a key step in complement activation that occurs in all three complement pathways. This enzyme cleaves C3 in blood plasma, leading to a conformational change in the C3b fragment that exposes its reactive thioester group. The activated C3b is potentially harmful to the host, if it becomes covalently attached to a host cell, rather than to the surface of a pathogen. This deleterious outcome is largely avoided by:

- A. The inability of active C3b to diffuse away in the blood plasma.
- B. The inability of active C3b to covalently attach to the membranes of eukaryotic cells.
- C. The rapid hydrolysis of active C3b in solution, rendering it inactive.
- D. The tight binding of active C3b to the C3 convertase.
- E. The ability of active C3b to recruit phagocytic cells.

2-6 The lectin pathway uses soluble receptors that recognize microbial surfaces to activate the complement cascade

2.12 Short answer: Infants and young children with deficiencies in specific complement components present with recurrent respiratory infections caused by extracellular bacteria. The peak age of susceptibility is between 6 and 12 months after birth. At this time, as shown in **Figure Q2.12**, maternal antibodies acquired by the child during fetal gestation are nearly gone, but the child is not yet generating robust antibody responses to new infections, as indicated by the low circulating levels of IgG and IgA. As children with this immunodeficiency get older, they outgrow this disease and show no further evidence of these recurrent infections. Based on this information, name one likely gene deficiency (in the complement system) that could cause this primary immunodeficiency, and the specific complement pathway likely to be affected. Explain your answer.

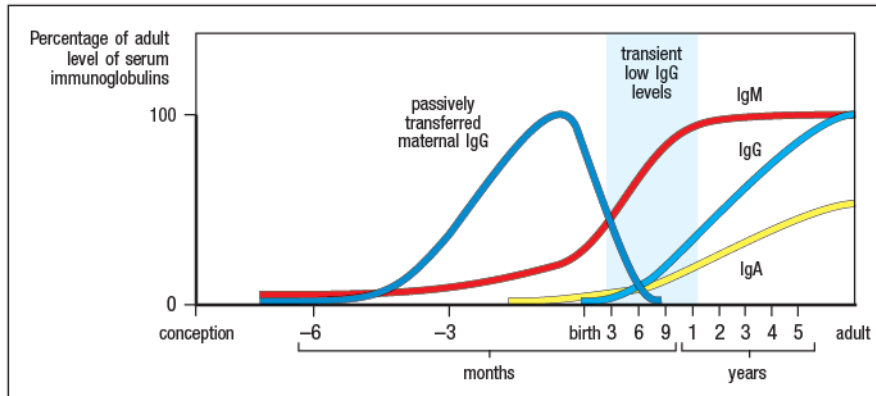


Figure Q2.12

2.13 Multiple choice: Mannose binding lectins (MBL) and ficolins are the two classes of proteins that can initiate the lectin pathway of complement activation. These proteins are selective for activating complement on the surfaces of microbial pathogens rather than host cells because:

- Their higher-order oligomeric structure can be assembled only after the monomers first bind to pathogen membranes.
- They only recruit MASP (MBL-associated serine proteases) proteins when bound to pathogen surfaces and not when bound to host cells.
- They only undergo the conformational change needed to activate MASP proteins when bound to a pathogen and not when bound to a host cell.
- They only bind to carbohydrate side chains and oligosaccharide modifications found on pathogen surfaces but not on host cell membranes.
- The activated MASP proteins are rapidly inactivated by hydrolysis when present on the surface of a host cell.

2-7 The classical pathway is initiated by activation of the C1 complex and is homologous to the lectin pathway

2.14 Multiple choice: The classical complement pathway is initiated by C1q binding to the surface of a pathogen. In some cases, C1q can directly bind the pathogen, for instance by recognizing proteins of bacterial cell walls, but in most cases C1q binds to IgM antibodies that are bound to the pathogen surface. How does this IgM-binding feature of C1q contribute to rapid, innate immune responses rather than to slow, adaptive responses?

- C1q induces B lymphocytes to begin secreting antibody within hours of pathogen exposure.
- Natural antibody that binds to many microbial pathogens is produced prior to pathogen exposure.
- C1q binds to C-reactive protein which then binds to IgM on the pathogen surface.
- C1q directly induces inflammation, recruiting phagocytes and antibodies from the blood into the infected tissue.
- C1q binds to dendritic cells in the infected tissue, inducing them to secrete inflammatory cytokines.

2.15 True/False: The classical and lectin pathways of complement activation converge at the step of C3 activation. However, the initiating steps of each pathway use protein components and enzymatic mechanisms that share no similarity with each other.

2-8 Complement activation is largely confined to the surface on which it is initiated

2.16 Multiple choice: Opsonization of pathogens by both antibodies and complement proteins (C3b) leads to uptake and destruction of the pathogen by phagocytic cells that express both Fc receptors and complement receptors. Which of the following in **Figure Q2.16** is the most efficient form of dual opsonization of the pathogen by antibody and C3b to maximize phagocytosis?

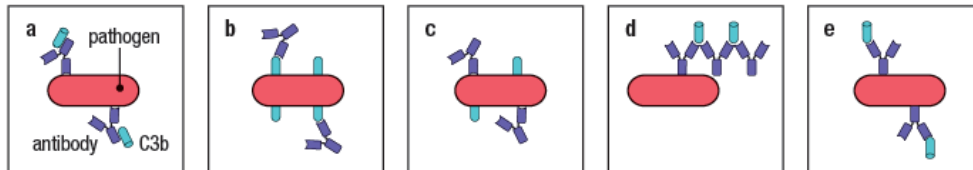


Figure Q2.16

2-9 The alternative pathway is an amplification loop for C3b formation that is accelerated by properdin in the presence of pathogens

2.17 Multiple choice: The alternative pathway of complement activation has an important role in innate immunity, due to its ability to greatly amplify the amount of C3b deposited onto the pathogen surface. This amplification occurs because:

- A. The C3 convertase of the alternative pathway is much more active than those of the classical and lectin pathways.
- B. The C3 convertase of the alternative pathway works as a soluble enzyme in the plasma.
- C. The C3 convertase of the alternative pathway cannot be inactivated by complement regulatory factors in the host.
- D. The C3 convertase of the alternative pathway is more efficiently recruited to pathogen surfaces than the C3 convertases of the classical and lectin pathways.
- E. The C3 convertase of the alternative pathway contains C3b, and can generate more of itself.

2.18 True/False: The C3 convertase of the alternative complement pathway amplifies the overall magnitude of complement activation regardless of which of the three pathways initiated the complement activation initially.

2.19 Multiple choice: Patients with recurrent infections of *Neisseria meningitidis*, an extracellular bacterial pathogen that causes meningitis, were examined to determine the underlying cause of their immunodeficiency. A subset of these patients were found to have defects in complement activation on the bacterial surface, a process that for this bacterium is dominated by alternative complement activation leading to C3b deposition on the pathogen surface. When neutrophils from these patients were examined *in vitro*, the results in **Figure Q2.19** were obtained.

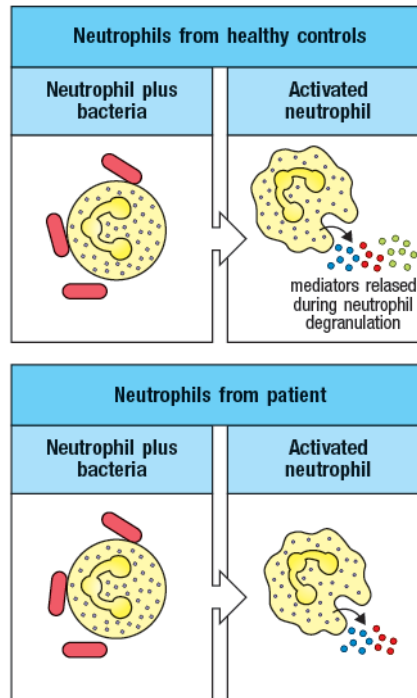


Figure Q2.19

Based on these data, the identity of the green neutrophil mediator in **Figure Q2.19** is likely to be:

- A. Complement factor B
- B. The C3 convertase
- C. Factor P (properdin)
- D. C3b
- E. Mannose-binding lectin (MBL)

2-10 Membrane and plasma proteins that regulate the formation and stability of C3 convertases determine the extent of complement activation

2.20 Multiple choice: One form of anemia results when individuals have a deficiency in the enzyme phosphatidylinositol glycan A (PIGA). This enzyme is required for the membrane attachment of proteins anchored by glycolipids to the plasma membrane, using what is called a 'GPI-linkage.' Included in the group of GPI-linked cell surface proteins is DAF/CD55. These individuals become anemic because:

- A. DAF/CD55 prevents the lysis of red blood cells by infecting pathogens.
- B. DAF/CD55 normally prevents the spleen from clearing healthy red blood cells from the circulation.
- C. In the absence of PIGA, the red blood cell membrane is bare of proteins allowing increased access of complement activating proteins to attach to the cell membrane.
- D. DAF/CD55 is a complement inhibitory protein that inactivates any C3 convertase that may form on host cell surfaces.
- E. In the absence of PIGA, red blood cells are unable to synthesize high levels of hemoglobin.

2-11 Complement developed early in the evolution of multicellular organisms

- 2.21 Short answer:** In vertebrates, complement activation generally involves a pathogen recognition step followed by a proteolytic cascade that produces the effector proteins that function in opsonization, membrane attack, and inflammation.
- a) Which of these is likely to be the most evolutionarily primitive aspect of the complement system?
 - b) Which pathway of complement initiation is likely to be the one that most recently evolved?

2-12 Surface-bound C3 convertase deposits large numbers of C3b fragments on pathogen surfaces and generates C5 convertase activity

- 2.22 True/False:** The C3 convertase amplifies the process of complement activation by generating large amounts of C3b and cleaving large numbers of C5 molecules.

2-13 Ingestion of complement-tagged pathogens by phagocytes is mediated by receptors for the bound complement proteins

- 2.23 Multiple choice:** Even when the complement cascade fails to proceed beyond generating the C3 convertase, complement activation is effective at inducing pathogen uptake and destruction. This process of immune protection is mediated by:
- A. Activation of complement inhibitory receptors on phagocytes that promote pathogen uptake
 - B. Activation of soluble proteases in the serum that disrupt pathogen membranes
 - C. Engagement of complement receptors on phagocytes by C3b and its cleavage products which promotes phagocytosis
 - D. Engagement of complement receptors on B cells that promotes antibody production
 - E. Stimulation of antimicrobial peptide secretion by phagocytes
- 2.24 Multiple choice:** B cells express a complement receptor that binds to C3b cleavage products, such as iC3b and C3dg. When a B cell with an antigen receptor that specifically recognizes that pathogen also has its complement receptor stimulated because the pathogen is opsonized with these C3 fragments, B cell activation is greatly enhanced. Due to this mechanism, B cells can be activated by much lower concentrations of antigen (in this case, the pathogen) than if the antigen is devoid of complement components. This mechanism functions to:
- A. Ensure that pathogens are readily detected by the adaptive immune system before they replicate to high levels in the host
 - B. Prevent B cells from being activated in response to antigens that are not pathogens
 - C. Allow B cells to phagocytose the pathogen and help destroy it
 - D. Induce increased rounds of B cell replication to make more pathogen-specific B cells
 - E. Allow the B cell to block pathogen replication by interfering with multiple pathogen surface functions

2-14 The small fragments of some complement proteins initiate a local inflammatory response

- 2.25 Short answer:** Recent studies using mouse models of pulmonary inflammation (a model

for human asthma) have found that mice deficient in the C3a receptor have greatly reduced disease symptoms when challenged with inhaled preparations containing extracts of the fungal pathogen *Aspergillus fumigatus*. Specifically, the C3a receptor-deficient mice showed reduced influx of granulocytes and lymphocytes into the lung and reduced fluid in the lung after challenge. What is the explanation for these findings?

2-15 The terminal complement proteins polymerize to form pores in membranes that can kill certain pathogens

- 2.26 Multiple choice:** The terminal components of the complement pathway assemble to form a membrane attack complex that can induce pathogen lysis and death. Yet, evidence indicates that this feature of complement is less important than the earlier steps that promote pathogen opsonization and induce inflammation. This conclusion is based on:
- A. *In vitro* experiments showing that very few species of bacteria are susceptible to lysis by the membrane attack complex
 - B. Experiments indicating that only bacteria, but not viruses or fungi, are susceptible to lysis by the membrane attack complex
 - C. The very low levels of terminal complement components in the serum
 - D. The fact that other mammalian species lack the terminal components of the complement pathway needed to form the membrane attack complex
 - E. The limited susceptibility to infections of patients with deficiencies in terminal complement components

2-16 Complement control proteins regulate all three pathways of complement activation and protect the host from their destructive effects

- 2.27 Multiple choice:** Multiple pathways for regulating complement activation limit the potential damage caused by complement deposition on host cells or caused by the spontaneous activation of complement proteins in the plasma. Genetic deficiencies in these mechanisms often lead to chronic inflammatory diseases, but in some cases can paradoxically lead to increased susceptibility to bacterial infections. This latter outcome may occur because:
- A. Complement regulatory proteins have dual functions in inhibiting and promoting complement activation.
 - B. Uncontrolled complement activation leads to the depletion of serum complement proteins.
 - C. The inhibition of the membrane attack complex by complement regulatory proteins normally leads to enhanced activation of the early steps of the complement pathway.
 - D. Complement regulatory proteins normally cause the rapid depletion of plasma complement factors.
 - E. Uncontrolled complement activation recruits the majority of phagocytic cells, leaving few remaining to fight infections in the tissues.
- 2.28 Short answer:** Although homozygous deficiencies in complement regulatory proteins cause serious diseases, more subtle alterations in the balance of complement activation versus inhibition have been found to contribute to disease susceptibility. Describe the genetic evidence linking subtle alterations in complement regulatory proteins to disease susceptibility.

2-17 Pathogens produce several types of proteins that can inhibit complement activation

2.29 Multiple choice: The importance of complement activation as an innate immune defense against infections is illustrated by:

- A. The evolution of complement avoidance strategies by many pathogens
- B. The large number of proteins involved in the complement pathway
- C. The large number of complement regulatory pathways expressed by the host
- D. The existence of three different mechanisms for initiating complement activation
- E. The ability of the membrane attack complex to lyse some pathogens

2.30 True/False: Several pathogens produce proteins, either membrane-bound or secreted, that inactivate C3b that might be deposited on the pathogen surface. C3b is specifically targeted due to its central position in all three complement pathways.

2.31 Synthesis question: Four different clinical isolates of the Gram-positive bacterium, *Staphylococcus aureus*, are tested for their abilities to resist innate immune defense mechanisms. For these experiments, each bacterial strain is first grown in culture to achieve log-phase replication, and then cultures are supplemented with dilutions of human serum containing normal serum proteins as well as antibodies capable of binding to *S. aureus*. One hour later, the cultures are analyzed and the numbers of live bacteria are quantitated. The data from this experiment are shown in **Figure Q2.31A**.

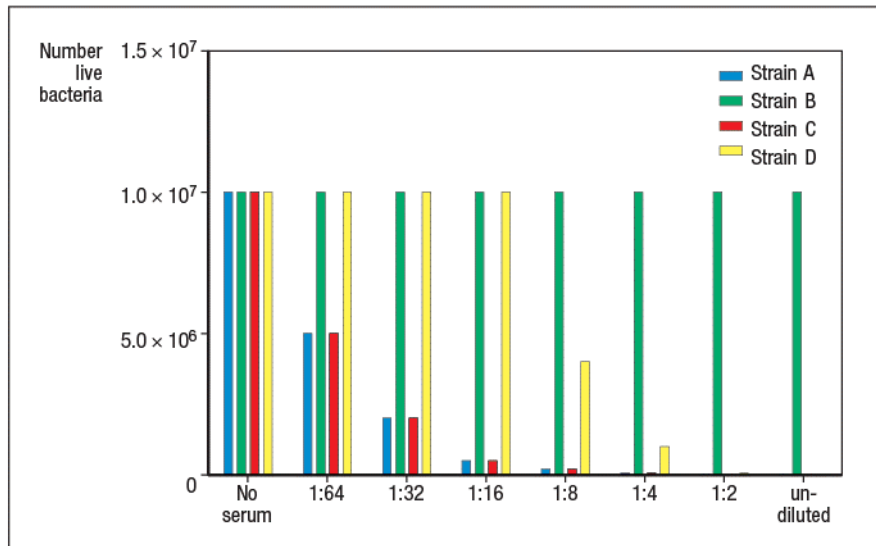


Figure Q2.31A

a) From these data, what general conclusions can be reached about the four strains of *S. aureus*?

To identify the bactericidal mechanisms killing each strain of *S. aureus*, the serum is depleted of complement C3 by running it over an anti-complement C3 antibody affinity column. The experiment above is then repeated and the data are shown in **Figure Q2.31B**.

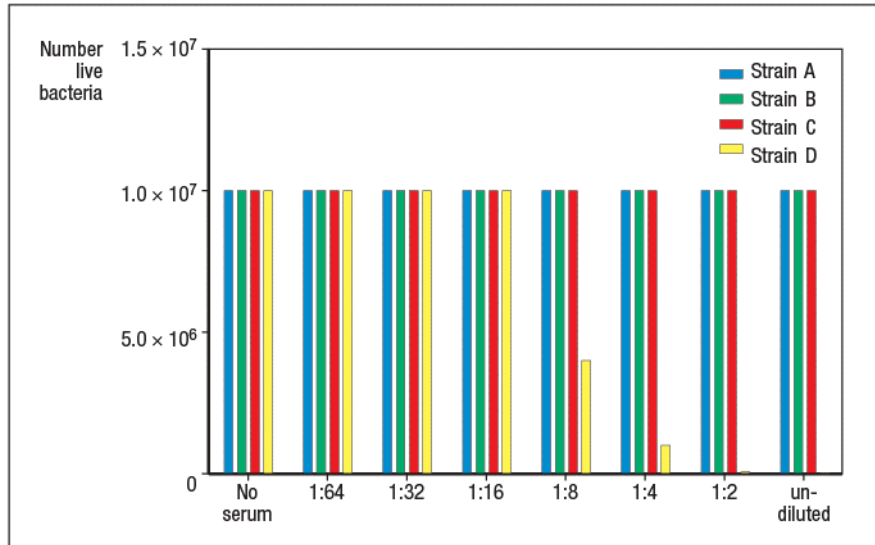


Figure Q2.31B

b) What is the most likely mechanism accounting for the killing of strain D in this experiment?

To determine whether strains A and C are susceptible to the same microbicidal pathway, the serum is depleted of antibody by running over an anti-human immunoglobulin affinity column. Following this treatment, it is found that strain A, but not strain C is still killed by incubation with the serum.

c) From these data, what is the most likely mechanism killing *S. aureus* strain A? What about strain C?

2.32 Synthesis question: *Pseudomonas aeruginosa* is a Gram-negative bacterium that causes severe, and often life-threatening infections in immunocompromised individuals. In susceptible individuals, *P. aeruginosa* can establish infections in a wide range of tissues, including the lung, the GI tract, the eye, the ear, the urinary tract, the skin, and the blood. This bacterium is common in the environment, and is found on the skin of approximately 5% of healthy individuals. It is often found on hospital equipment, such as ventilators and catheters, and as a consequence, *P. aeruginosa* accounts for ~10% of hospital-acquired infections. To study the role of complement in the early innate immune response to *P. aeruginosa*, the following studies in mice were performed. Mice deficient in complement C3 or C5 (C3^{-/-} or C5^{-/-}, respectively) were infected by intratracheal inoculation with 10^5 colony forming units (CFU) of *P. aeruginosa*, and survival was monitored over the first 72 hrs post-infection. The data from these studies are shown in **Figure Q2.32**. Genetic data from human population studies also indicate that Individuals with genetic deficiencies in one of the collectins or ficolins show increased susceptibility to *P. aeruginosa* infections.

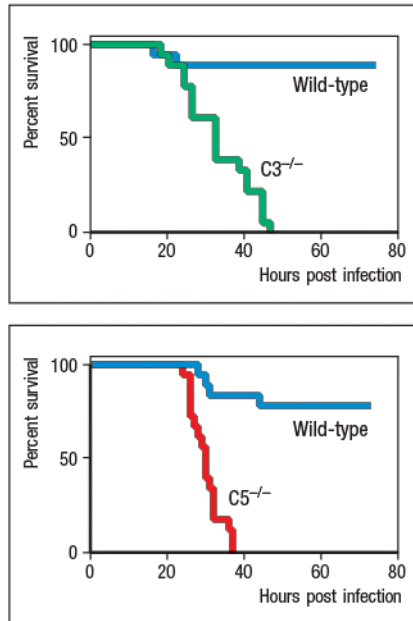


Figure Q2.32

a) Based on these data, evaluate the importance of complement in protection against *P. aeruginosa* infection, and describe the most likely complement pathway(s) involved in pathogen recognition and in pathogen destruction.

Another group of individuals that are highly susceptible to *P. aeruginosa* infections are patients with the disease cystic fibrosis. These individuals suffer from the production of a thick mucus secretion in their lungs, which clogs the bronchial tubes. A similar increase in viscosity of bodily secretions is seen in these patients' sweat, digestive fluid, and gastrointestinal mucus. In these patients, the most common form of lung infection is that of *P. aeruginosa*.

b) What is the most likely explanation for the increased susceptibility of cystic fibrosis patients to *P. aeruginosa* and other infections?

ANSWERS

2.1: B.

All extracellular forms of pathogens are targets for antibodies, complement, phagocytic cells and antibody-dependent immune clearance mechanisms. Once a pathogen, such as a virus or intracellular protozoan, invades a cell and begins replicating in the cell, these mechanisms are no longer able to clear the infection. These intracellular stages of pathogenic infection require cellular responses, such as those mediated by T cells or NK cells.

2.2: C.

Pathogens cause direct tissue damage by the production of exotoxins or endotoxins, as well as by direct cytopathic effects. Tissue damage caused by the host immune response include damage caused by cell-mediated immunity and by the accumulation of immune complexes.

2.3: False.

Both bacterial and virus infections can use both the mouth and respiratory tract and the gastrointestinal tract. There is no route of infection that is specific for a single category of pathogen.

2.4: B.

Anti-microbial peptides are produced by epithelia at all mucosal and epidermal surfaces. These chemicals are important in immune protection against microbial pathogens. All other choices are mechanical mechanisms by which surface epithelia protect against infections, not chemical mechanisms.

2.5: E.

Commensal organisms associated with all epithelial surfaces provide protection against colonization by pathogenic microbes. One mechanism is by competition for nutrients as well as for attachment sites on epithelial surfaces. Another mechanism is by producing metabolites that are toxic to other organisms. When these commensal microorganisms are eliminated by antibiotic treatment, pathogenic microbes are able to step into the void and establish an infection.

2.6: The first important feature of a pathogenic microbe is that it must establish a replicating colony of organisms in our body. This can occur by the pathogen crossing an epithelial barrier and replicating in the tissue, or by attaching to the epithelial surface and establishing a colony there. The second feature is that the pathogen needs to have special mechanisms to evade the innate immune response.

2.7: C.

Strep. pneumoniae is a Gram-positive bacterium that readily colonizes the human nose and mouth, due to the resistance of its peptidoglycan to degradation by lysozyme, an enzyme that is abundant in tears, saliva, and mucus. Wild-type *Strep. pneumoniae* is naturally lysozyme-resistant because a substantial proportion of GlcNAc residues in its peptidoglycan are deacetylated, and no longer substrates for lysozyme-mediated hydrolysis. This accounts for the prevalence of *Strep. pneumoniae* in the upper respiratory tract of healthy humans, where lysozyme is present at high concentrations. When one of these enzymes is missing, as in strain B, the bacteria become lysozyme-sensitive, and are killed by the lysozyme. fungi.

2.8: E.

The diversity of antimicrobial peptides is a reflection of the diversity of microbial pathogens that they attack. Some antimicrobial peptides are active against Gram-negative bacteria, while others are only active against Gram-positive bacteria. Other antimicrobial peptides are only active against fungal pathogens, and some are able to disrupt the membrane envelopes of some viruses.

2.9: True.

All antimicrobial peptides, including cathelicidins, are produced as inactive propeptides. The active forms of the peptides are generated following proteolytic cleavage of the propeptides. Neutrophils constitutively produce cathelicidins, which are synthesized as inactive propeptides. The inactive cathelicidin propeptides are stored in secondary granules, whereas the cleavage enzyme, neutrophil elastase, is stored in primary granules. These two types of granules are induced to fuse with phagocytic vesicles, called phagosomes, after the neutrophil has engulfed a pathogen. This fusion brings the cleavage enzyme together with the cathelicidin propeptide, leading to cathelicidin activation.

2.10: A.

There are three pathways for initiating complement activation. One of them, known as the classical pathway, occurs when the pathogen has antibodies bound to its surface, leading to recruitment of C1q. The other two pathways, the lectin pathway and the alternative pathway, are initiated by mechanisms that do not require antibodies directed against the pathogen surface. These latter two pathways are dependent on constitutively produced, and therefore 'innate' recognition molecules that directly bind to pathogen surfaces, initiating complement activation.

2.11: C.

Active C3b is highly labile, and is rapidly inactivated by hydrolysis. This prevents the C3b from remaining active should it diffuse away from the pathogen surface where it was activated by the C3 convertase.

2.12: MBL or MASP.

Infants and small children with defects in MBL or MASP show recurrent upper respiratory infections by extracellular bacteria. This is due to a defect in the lectin pathway of complement activation. When maternal antibodies wane and the child is not yet generating robust antibody responses on its own, complement activation cannot proceed by the classical pathway. During this time, protection against upper respiratory bacterial infections is highly dependent on the lectin pathway, initiated by MBL or collectin binding to the pathogen. The information provided rule out the alternative pathway, which is initiated by spontaneous C3 cleavage. If there was a defect in C3, or a downstream component of the complement cascade shared by all three pathways, the recurrent infections would not disappear as children age.

2.13: D.

MBL and ficolins have binding specificity for carbohydrate side chains and oligosaccharide modifications that are unique to microbial pathogens, and not found on host cells. MBL binds to mannose, fucose, and GlcNAc residues, which are common on microbial glycans; in contrast, MBL does not bind to sialic residues, which terminate vertebrate glycans. Ficolins have specificity for binding to oligosaccharides containing acetylated sugars, a structure also only found on pathogen surfaces, not on host cells.

2.14: B.

Natural antibody, which is primarily of the IgM class, is produced in the body prior to pathogen

exposure. These antibodies are widely reactive with many microbial pathogens, although they generally have low affinity for the pathogen. However, since IgM is a pentamer of IgM monomers, each IgM pentamer has 10 binding sites for antigen, allowing even low affinity antibodies to bind, due to the increased avidity of multiple binding sites. This natural antibody will then recruit C1q, leading to complement activation. Since the natural antibody pre-exists prior to pathogen exposure, this response is rapid and is considered part of the innate immune response.

2.15: False.

The initiating steps of the classical and lectin pathways of complement activation are remarkably conserved in their mechanisms. The pathogen recognition component of the classical pathway, C1q, has structural similarity to MBL and the ficolins. The C1r and C1s components of the classical pathway, that are activated to form the serine protease, are closely related to the MASP proteins of the lectin pathway.

2.16: A.

The most efficient form of opsonization by antibody plus C3b is when the complement protein is covalently linked to the antibody molecule. This leads to efficient engagement of both Fc receptors and complement receptors on phagocytic cells.

2.17: E.

The C3 convertase (C3bBb) of the alternative pathway contains C3b, allowing it to generate more of itself and amplify the overall level of C3b formed. Once additional molecules of C3b are made by C3bBb, these can recruit additional molecules of factor B and the plasma protease factor D. Factor D cleaves factor B, and one of the products, Bb, remains associated with C3b, forming more active C3 convertase.

2.18: True.

The C3 convertase of the alternative pathway contains C3b, allowing it to generate more of itself and amplify the overall level of C3b formed. Since C3b is a common intermediate for all three pathways of complement activation, once the initial C3b is generated by any of the pathways, the recruitment of factor B, and cleavage by factor D can proceed. By this mechanism, the initial C3b generated forms an amplification loop leading to more C3b, regardless of how the initial C3b was made.

2.19: C.

Factor P (properdin) is made by neutrophils and stored in their granules. When neutrophils are activated by the presence of pathogens, factor P is released. Factor P binds to and stabilizes the reactive form of C3 (C3-H₂O) and the C3 convertase C3bBb. In the absence of factor P, the alternative complement pathway is inefficient, due to the rapid spontaneous inactivation of C3-H₂O and C3bBb. This pathway is particularly important in protection against *Neisseria meningitidis*, and patients that are deficient in producing factor P are highly susceptible to infections with this pathogen.

2.20: D.

Host cells express several complement-regulatory proteins on their surface. These proteins function to rapidly inactivate any C3bBb (active C3 convertase) that may form on the host cell membrane. Several of these complement regulatory proteins use GPI-linkages to attach to the host cell membrane. Included in this group is DAF/CD55, which competes with factor B for binding to C3b on the cell surface, and displaces Bb from any active C3 convertase that has already formed. The absence of DAF/CD55 makes host cells susceptible to complement-

mediated lysis. For reasons that are not entirely clear, red blood cells are particularly susceptible to complement-mediated lysis and the absence of the GPI-linked subset of complement regulatory proteins is sufficient to cause red blood cell lysis leading to anemia.

2.21:

- a) The most primitive form of a complement system is one that resembles our alternative complement pathway, with ancestral homologs of C3 and factor B that make a C3 convertase. This provides a mechanism for opsonizing infecting bacteria and increasing their phagocytosis by phagocytic cells. These ancestral homologs of C3 and factor B have been found in echinoderms, and may even have existed in even more primitive organisms such as corals and sea anemones.
- b) The latest evolutionary development in the complement system is the classical pathway, which makes use of antibody binding to initiate complement activation. The adaptive immune system, including the production of antibodies, is only found in vertebrates.

2.22: False.

The C3 convertase does generate large numbers of C3b molecules which become attached to the pathogen surface in the vicinity of the convertase. This enzyme can only cleave C5 when bound to a molecule of C3b, generating the C5 convertase. The generation of the C5 convertase occurs at a much lower level than the C3 convertase, and many fewer molecules of C5 than C3 are cleaved.

2.23: C.

Phagocytes have a variety of receptors that recognize C3b and fragments of C3b, such as iC3b. Engagement of these complement receptors stimulates phagocytosis of the C3b-coated pathogen, leading to pathogen destruction.

2.24: A.

The complement receptor on B cells, CD21, is often referred to as the B cell co-receptor. When this receptor is engaged together with the B cell antigen receptor, the B cell can be activated by much lower concentrations of antigen compared to antigen lacking ligands for CD21. Experiments have indicated that CD21 stimulation can reduce the concentration of antigen needed to activate the B cell by 100–1000-fold. This allows B cells to detect small numbers of infecting pathogens, to initiate an adaptive response prior to the occurrence of a high pathogen load in the host.

2.25: When complement is activated in the lung in response to the inhaled preparations of the fungus, the C3 convertase generates C3a. C3a induces a local inflammatory response in the lung, by acting on the vascular endothelial cells. This response includes increased vascular permeability, leading to an increase of fluid in the lung, and also acts to up-regulate adhesion molecules on the local vascular endothelium. As a result, there is increased recruitment of granulocytes, monocytes, and lymphocytes into the lung.

2.26: E.

Patients with genetic deficiencies in terminal complement components show only a limited increase in susceptibility to infection. These individuals are more susceptible to infection by *Neisseria* species that cause gonorrhea or meningitis. Otherwise, these individuals show no other increased susceptibility to infection, indicating that formation of the membrane attack complex is a less important aspect of complement activation compared to the earlier steps that lead to opsonization of the pathogen as well as inducing inflammation.

2.27: B.

Individuals with a genetic defect in factor I are subject to recurrent infections with pyogenic (pus-forming) extracellular bacterial infections. This occurs because, in the absence of factor I, uncontrolled complement activation ends up depleting the complement proteins from the plasma. This leads to impaired complement activation on these bacteria, and therefore, to diminished clearance of these infections.

2.28: Two types of genetic alterations in complement regulatory proteins have been linked to disease susceptibility. First, individuals heterozygous for mutations in one of several complement regulatory proteins (MCP, factor I, factor H). These individuals are predisposed to develop a hemolytic disease that leads to damage to platelets and red blood cells, and to kidney inflammation. Second, individuals with particular single-nucleotide polymorphisms in the gene for factor H are predisposed to macular degeneration, an age-related disease that causes blindness. Furthermore, polymorphisms in other complement genes have been found to contribute to the susceptibility to age-related macular degeneration.

2.29: A.

One of the best indicators of the importance of an immune protective mechanism is the development by pathogens of strategies to evade that mechanism. In the case of the complement pathway, many pathogens have evolved strategies to avoid complement activation on their surface. These include the expression of proteins that attract complement regulatory proteins to their surface, in an effort to mimic host cell surfaces that can inactivate complement. An additional strategy is to secrete proteins that directly inhibit components of the complement pathway.

2.30: True.

All three pathways of complement activation converge on the assembly of a C3 convertase that produces C3b bound to the pathogen surface. Therefore, pathogens that can inactivate bound C3b can interfere with complement activation that might be initiated by any of the three pathways. This makes C3b an ideal target for an immune evasions strategy.

2.31:

- a) Strain A and strain C are equally sensitive to a serum component (or set of components) that can kill *S. aureus*. Strain B is resistant to all possible serum components that can kill *S. aureus*. Strain D is also sensitive to a serum component(s) that can kill *S. aureus*, but is killed by a different mechanism than the one(s) killing strains A and C. This latter conclusion is based on the observation that the factor(s) killing strain D are no longer active when the serum is diluted 1:16, whereas the activities that kill strains A and C are still active at this dilution.
- b) Strains A and C are no longer killed when complement C3 is depleted from the serum. This indicates that these two strains are susceptible to complement-mediated lysis. The killing of strain D is not affected by depletion of C3, indicating a distinct mechanism. Strain D is most likely being killed by defensins, or another antimicrobial peptide. It is unlikely that strain D is being killed by lysozyme in these experiments, as lysozyme is predominantly found in tears and saliva, rather than in serum.
- c) Since strain A is still killed following depletion of antibody, but not following depletion of C3, it is likely that strain A is susceptible to the lectin pathway of complement activation, initiated by binding of ficolin (or collectin) to the bacterial surface. Ficolins are the most likely initiators of complement activation in this case, as they are at high concentrations in the serum. Although it is theoretically possible that strain A is killed by the alternative pathway of complement activation, this possibility is unlikely. The spontaneous

hydrolysis of C3 produces a fluid-phase C3 convertase that is very short-lived. This fluid-phase C3 convertase is stabilized by properdin (factor P), which is only produced by neutrophils when they are activated by pathogens. Therefore, there is unlikely to be factor P in the serum used in this study, and therefore, the alternative pathway of complement activation would be very inefficient.

Strain C is no longer killed following depletion of antibody. Therefore, strain C is likely susceptible to the classical pathway of complement activation.

2.32:

- a) Complement is essential for early protection against this dose of *P. aeruginosa* in mice. In the absence of either C3 or C5, all of the infected mice succumb to the infection by 2 days post-infection. Based on the human population studies indicating that individuals deficient in collectins or ficolins are also more susceptible to this bacterial infection, it is likely that the lectin pathway of complement activation is the primary mechanism for initiating the complement cascade. Since C5 is required, this points to an important role for the membrane attack complex in pathogen destruction. However, it is also possible that the requirement for C5 is due to the role of C5a in promoting inflammation, leading to the recruitment of phagocytic cells to the site of infection. In this latter case, pathogen destruction would be due to uptake by phagocytic cells.
- b) Due to their defect in regulating the viscosity of bodily secretions, the mechanical and chemical mechanisms of immune resistance at the body's epithelial barriers do not function normally. Instead of serving as a fluid that flushes away potential pathogens at these barriers, the mucus of cystic fibrosis patients functions as a stagnant reservoir ideal for bacterial colonization. Furthermore, the thick mucus prevents the normal diffusion of antimicrobial enzymes and peptides that are secreted into the mucus.