Janeway's Immunobiology, 9th Edition
Chapter 4: Antigen Recognition by B-Cell and T-Cell Receptors

The structure of a typical antibody molecule

4-1 IgG antibodies consist of four polypeptide chains

4.1 **True/False:** The antibody protein has two functional domains, one for antigen binding and a second to confer specific effector functions. These two functional domains are encoded by the antibody light chain and antibody heavy chain polypeptides, respectively.

4.2 **Multiple choice:** Amino acid sequence analysis of all of the peptides found in a single IgG antibody would reveal unique peptide sequences totaling ~600–700 amino acids. Using this estimate, the predicted molecular weight of an antibody protein would be ~70–75 kDa. Yet, an intact antibody protein has a molecular weight of ~150 kDa. The explanation for this discrepancy is:

A. IgG antibodies have many more heavy amino acids in them than most other proteins.
B. Each IgG antibody is a complex of two identical light chains and two identical heavy chains.
C. IgG antibodies tend to aggregate together during purification, thereby distorting molecular weight estimates.
D. Each IgG antibody is a complex of four identical polypeptides.
E. IgG antibodies are produced as dimers of two identical IgG monomers.

4-2 Immunoglobulin heavy and light chains are composed of constant and variable regions

4.3 **Multiple choice:** Antibody heavy and light chain polypeptides consist of repeated domains, each of which is ~110 amino acids and folds up into a compact three-dimensional structure known as an ‘immunoglobulin domain.’ These immunoglobulin domains are:

A. Mixed and matched between different antibody heavy and light chains to produce variability
B. Always identical to each other within a single antibody heavy chain or light chain polypeptide
C. Always differ in amino acid sequence between different light chain polypeptides in both of the two light chain immunoglobulin domains
D. Similar but not identical in amino acid sequence when comparing the domains in a single heavy chain polypeptide
E. Identical in amino acid sequence for every domain when comparing different antibody heavy chain polypeptides to each other

4-3 The domains of an immunoglobulin molecule have similar structures

4.4 **Short answer:** Each immunoglobulin (Ig) domain is composed of a structure known as a 'β-sandwich,' which consists of two β sheets covalently linked by a disulfide bond. Only a subset of the ~110 amino acids in each domain are required to establish this overall structure, and it is these amino acids that are highly conserved when comparing Ig domains to each other. What might be the advantage of this structure for use as antibody variable domains?

4-4 The antibody molecule can readily be cleaved into functionally distinct fragments

4.5 **Multiple choice:** Early studies analyzing the antibody protein fragments generated after proteolytic cleavage revealed important information about the overall structure of the antibody molecule. Which cleavage pattern (indicated by the red triangles in **Figure Q4.5**) yields a fragment that has the same antigen-binding avidity as the intact antibody, but is unable to activate complement after binding to a pathogen?

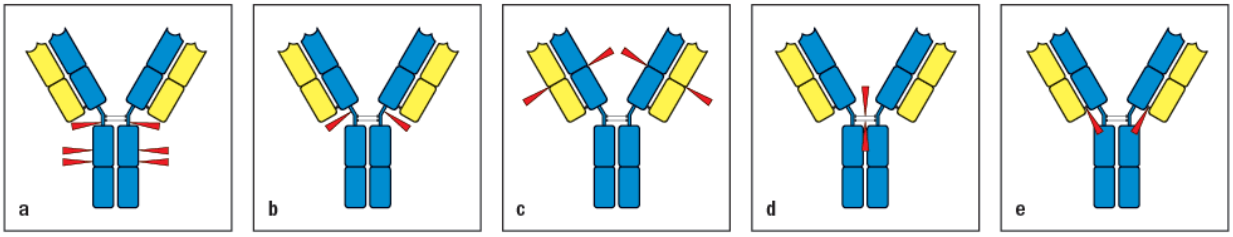

Figure Q4.5

4.6 **Multiple choice:** When a mixture of different IgG antibody proteins are treated with the enzyme papain, each antibody is cleaved into three roughly equal size fragments. From each original antibody, two of the three fragments are identical to each other, and represent the 'arms' of the antibody 'Y'. These fragments are known as Fab fragments. The third fragment is known as the Fc region, because this fragment will crystallize when purified. The reason a mixture of Fc fragments will crystallize is because:

A. It is the only part of the antibody protein that can easily be purified at the high concentrations needed for crystallization.
B. It has no disulfide bonds holding the domains together, as disulfide bonds will inhibit crystallization.
C. It is the only fragment of the antibody that still has disulfide bonds, so it remains intact during the crystallization process.
D. The Fc fragments of IgG are much more water soluble than the Fab fragments.
E. All Fc fragments generated from a mixture of IgG molecules have the identical amino acid sequence.

4-5 The hinge region of the immunoglobulin molecule allows flexibility in binding to multiple antigens

4.7 **True/False:** Antibody binding to a pathogen surface is greatly enhanced when both antigen-binding sites of the antibody are engaged at once, a feature known as bivalent binding. It is possible for antibodies to bind bivalently to a wide variety of components on many different pathogen surfaces due to the flexibility in the protein at the hinge region and at the V–C junction.

The interaction of the antibody molecule with specific antigen

4-6 Localized regions of hypervariable sequence form the antigen-binding site

4.8 **Multiple choice:** In **Figure Q4.8**, which close-up view of these two V domains has the amino acid sequences most important for antigen-binding highlighted correctly in red?

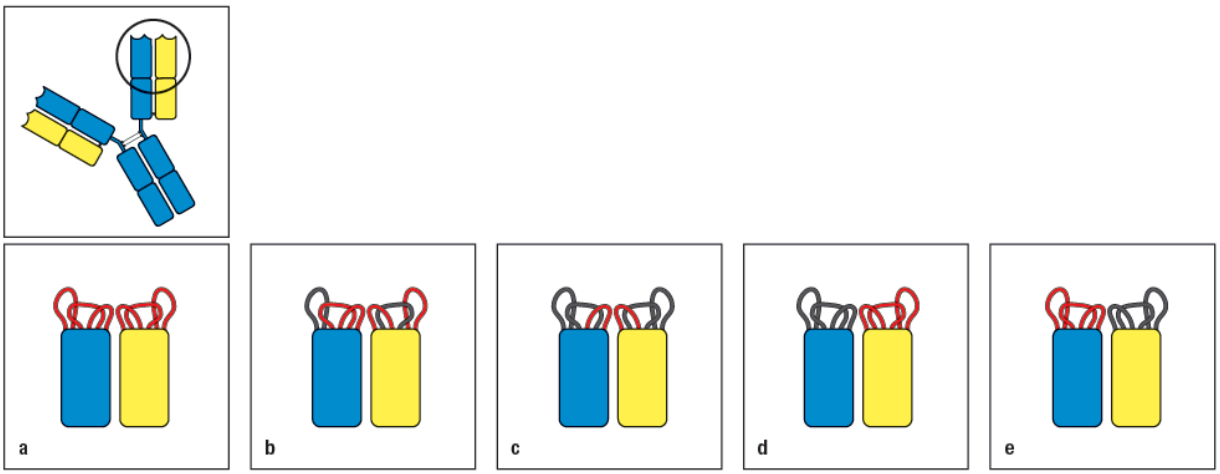

Figure Q4.8

4.9 **Short answer:** Which of the two antibodies shown in **Figure Q4.9** are most likely to have the same antigen-binding specificity? Explain your reasoning.

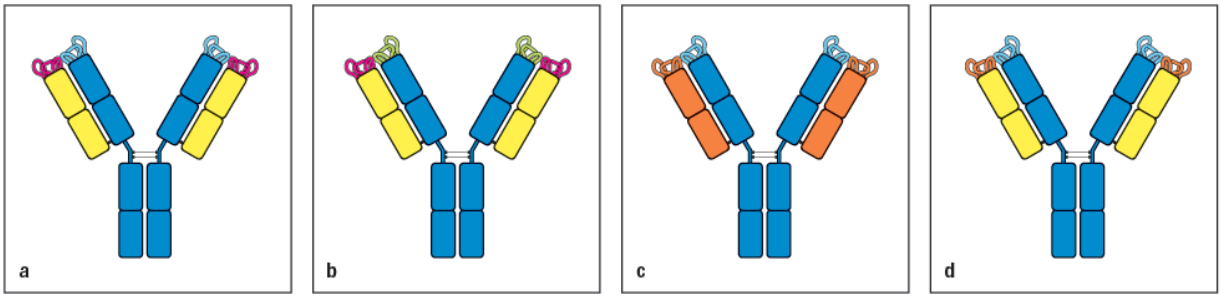

Figure Q4.9

4-7 Antibodies bind antigens via contacts in CDRs that are complementary to the size and shape of the antigen

4.10 **Multiple choice:** The antibody surface involved in antigen binding varies depending on the size and nature of the antigen. This surface can be concave or flat, and sometimes, can have extended protrusions. This is accomplished by:

A. Flexibility in the hinge regions of the antibody allowing rotation of the antigen-binding sites
B. Some antibodies using V region framework sequences instead of the CDRs to bind antigen
C. The ability of different CDR sequences to form many structurally distinct shapes and surfaces
D. The ability of the same heavy chain to pair with different light chains

E. The differential usage of κ versus λ light chains, as κ chains form concave binding sites whereas λ chains make flatter surfaces

4-8 Antibodies bind to conformational shapes on the surfaces of antigens using a variety of noncovalent forces

4.11 **True/False:** Like innate sensors of infections (TLRs, NLRs, RLRs), antibodies frequently recognize nucleic acids of pathogenic organisms.

4-9 Antibody interaction with intact antigens is influenced by steric constraints

4.12 **Multiple choice:** The drawing in **Figure Q4.12** shows antibodies bound to repetitive epitopes on the surface of a bacterial pathogen. Even though all of these epitopes are identical, not all of them have antibodies bound to them.

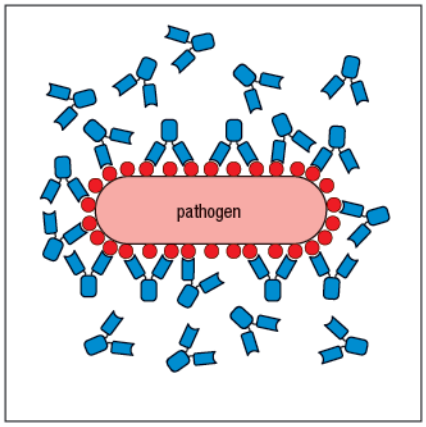

Figure Q4.12

The most likely explanation for this failure of antibodies to bind to every possible epitope on the surface of the pathogen is:

A. There is an insufficient amount of antibody to saturate all the epitopes.
B. The pathogen has an immune evasion strategy to avoid antibody binding to all epitopes.
C. Some of the epitopes cannot bind antibody due to steric hindrance.
D. The antibodies are only able to bind when both antigen-binding sites are engaged on the pathogen surface.
E. The epitopes on the pathogen are not all in the same conformation, so not all will bind the same antibody.

4-10 Some species generate antibodies with alternative structures

4.13 **Multiple choice:** Some species, like camels, alpacas, and llamas, have evolved variant forms of immunoglobulin proteins that retain the ability to bind to antigens. While overall the antibodies made by these animals are simpler than human or mouse antibodies, an important feature conserved among all of these antibodies is:

A. The presence of both heavy and light chain polypeptides

B. Antigen-binding sites comprised of V_H and V_L sequences
C. The presence of exactly three constant region domains
D. The presence of two antigen-binding sites per antibody
E. The presence of multiple disulfide bonds linking antibody light chains to heavy chains

Antigen recognition by T cells

4-11 The TCRα:β heterodimer is very similar to a Fab fragment of immunoglobulin

4.14 **Short answer:** α:β TCRs are membrane-bound proteins comprised of two polypeptides linked by a disulfide bond. Both polypeptide components of the α:β TCR are members of the immunoglobulin superfamily, and each of their domains share structural similarity with regions of antibody proteins. However, due to the different functions of TCRs versus antibodies, the overall domain organization of the TCR is not the same as for an antibody. In the cartoon in **Figure Q4.14**, describe three features that are incorrect illustrations of the α:β TCR.

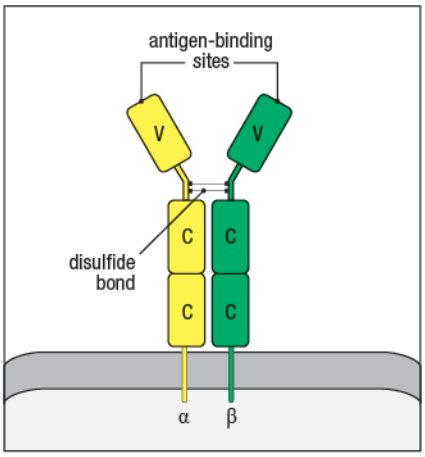

Figure Q4.14

4-12 A T-cell receptor recognizes antigen in the form of a complex of a foreign peptide bound to an MHC molecule

4.15 **Multiple choice:** The innate immune response together with antibodies are generally not effective at clearing infections established by pathogens that replicate inside host cells. The evolution of T cells has provided a means for the immune response to 'see' intracellular infections based on the ability of T cells to:
A. Secrete cytokines that diffuse into the infected tissue
B. Activate type I interferon production by macrophages and dendritic cells
C. Activate macrophages to induce inflammation
D. Recognize pathogen-derived peptides on host MHC surface molecules
E. Express cytoplasmic sensors for detecting pathogen-derived nucleic acids

4-13 There are two classes of MHC molecules with distinct subunit compositions but similar three-dimensional structures

4.16 **Multiple choice:** Both MHC class I and MHC class II molecules are highly polymorphic genes in the human population, with tens to hundreds of different alleles co-existing in the population. This means that a comparison of the MHC protein sequences between two individuals would reveal amino acid differences between one individual and the next. However, these amino acid differences are not randomly distributed along the entire protein, but are clustered in certain locations. The diagram in **Figure Q4.16** that most correctly indicates the regions of greatest variability between different MHC proteins (shown by the red highlights) is:

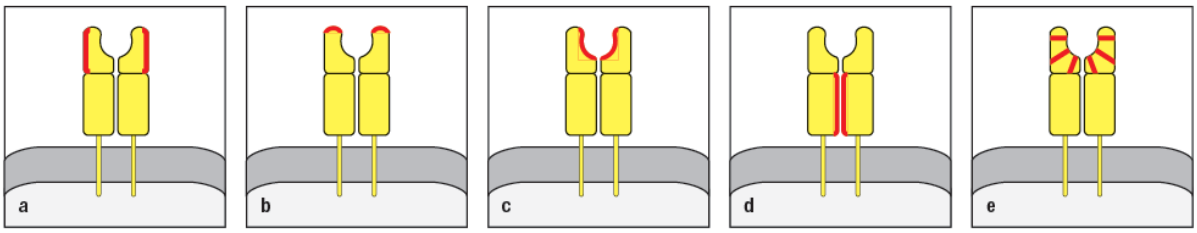

Figure Q4.16

4.17 **Short answer:** MHC genes are the most polymorphic genes in the human genome. This means that, within the population, few individuals share exactly the same sequences for all of their MHC proteins. What deleterious outcome might occur if all humans shared exactly the same sequence for their MHC proteins?

4-14 Peptides are stably bound to MHC molecules, and also serve to stabilize the MHC molecule on the cell surface

4.18 **Multiple choice:** Individuals or mice with defects in the biochemical pathways needed for loading peptides onto MHC molecules show greatly increased susceptibility to virus infections. Experiments examining the MHC molecules present on the surface of host cells in these individuals would show:

A. Normal numbers of MHC molecules expressed on host cells, but no peptides bound to them.
B. Very low levels of total MHC proteins expressed on the cell surface.
C. Normal numbers of MHC proteins on the surface but all of them bound to self-peptides not pathogen peptides.
D. Very high levels of total MHC proteins expressed on the cell surface.
E. Only virus-infected cells expressing high levels of MHC proteins on the cell surface.

4.19 **Multiple choice:** Once expressed on the surface of host cells, an MHC protein remains stably associated with its bound peptide for several days. This highly stable peptide binding behavior is important because:

A. It prevents peptide exchanges on the cell surface, ensuring that peptide:MHC complexes are reliable indicators of the proteins present inside that host cell.
B. If the MHC protein lost its peptide it would become unstable, and would be rapidly internalized and degraded.
C. Pathogens would otherwise evade the immune response by making decoy peptides that mimic host cell peptides.

D. Pathogens would be able to evade the T cell response by making proteases that cleave MHC proteins inducing peptide release.
E. Immune responses to infection often induce noxious chemicals that damage surface MHC proteins, and might result in peptide loss.

4-15 MHC class I molecules bind short peptides of 8–10 amino acids by both ends

4.20 **True/False:** MHC class I molecules generally bind peptides that are 8–10 amino acids. Each allelic variant has preferences for the amino acid residues at key anchor positions, but will not bind every possible peptide containing the correct anchor residues.

4-16 The length of the peptides bound by MHC class II molecules is not constrained

4.21 **Multiple choice:** One strategy for vaccine development currently under investigation is the use of pathogen-derived T cell epitopes as a component of the vaccine. For viral pathogens, implementing this strategy involves scanning the predicted amino acid sequences of the viral proteins for likely peptide epitopes that would bind to MHC class I and MHC class II molecules. In addition to the complication of MHC sequence polymorphism in the human population, another complication of this strategy for peptide epitopes that would bind to MHC class II proteins is:

A. The importance of viral proteins containing peptides that are cleaved into 8–10 amino acid long fragments.
B. The ability of viruses to mutate their proteins to avoid MHC anchor residue sequences.
C. The fact that long peptides (>13 amino acids) are rapidly degraded in cells.
D. The fact that MHC class II proteins are intrinsically stable, even in the absence of binding to a peptide.
E. The absence of defined sequence motifs that predict peptide binding to MHC class II molecules.

4-17 The crystal structures of several peptide:MHC:T-cell receptor complexes show a similar orientation of the T-cell receptor over the peptide:MHC complex

4.22 **Multiple choice:** One striking feature of TCR interactions with peptide:MHC complexes is that amino acid residues in the MHC protein are as important to the TCR binding strength as are amino acid residues in the pathogen-derived peptide. This feature is in contrast to antigen recognition by antibodies, which is a direct interaction that is independent of other host proteins. Based on the different functions of T cells versus antibodies in the adaptive immune response, the fact that TCRs recognize components of both the MHC and the bound peptide exists to:

A. Prevent TCRs from binding only to surface exposed epitopes of native pathogens
B. Prevent immune evasion by a pathogen that has mutated the sequences required for antibody recognition
C. Put constraints on T cell recognition, due to the potentially damaging effector molecules made by activated T cells
D. Ensure that TCRs are focused on recognizing antigens associated with host cells, and not those that are free in solution
E. Ensure that the pathogen has already been destroyed by the host cell before the T cell will recognize it

4-18 The CD4 and CD8 cell-surface proteins of T cells directly contact MHC molecules and are required to make an effective response to antigen

4.23 **Short answer:** α:β TCRs generally have a binding preference for either peptide:MHC class I or peptide:MHC class II complexes. However, on occasion, one α:β TCR might actually be able to recognize either class of peptide:MHC complexes. When such an α:β TCR is expressed on a CD4 T cell, it will only activate its T cell after binding to peptide:MHC class II complexes. Why is this the case?

4-19 The two classes of MHC molecules are expressed differentially on cells

4.24 **Multiple choice:** The cellular distribution of MHC class I versus MHC class II molecules is quite different, with MHC class II molecules generally expressed on a very limited set of cell types. This is because:

A. It would be detrimental to have CD8 T cells killing macrophages or B cells.
B. CD4 T cells generally secrete cytokines that act on macrophages and B cells.
C. Viruses generally do not infect and replicate in macrophages and B cells.
D. Dendritic cells are more important in stimulating CD4 than CD8 T cell responses.
E. CD4 T cells can only kill macrophages, dendritic cells, and B cells.

4.25 **Multiple choice:** Hepatitis C is a virus that infects hepatocytes, which are non-immune cells of the liver. Currently, patients with chronic Hepatitis C infections are treated with repeated administration of type I interferon, predominantly interferon α. One aspect of this treatment that might aid the patient's immune system in clearing this virus infection is:

A. Up-regulation of MHC class I expression levels on hepatocytes
B. Activation of macrophages to produce noxious compounds that might kill the virus
C. Induction of an inflammatory response to promote neutrophil trafficking to the liver
D. Production of TNF-α in response to type I interferon leading to vasodilation in the liver
E. Induction of IL-1 and IL-6 leading to the acute phase response

4-20 A distinct subset of T cells bears an alternative receptor made up of γ and δ chains

4.26 **True/False:** T cells expressing γ:δ TCRs are distinct from those expressing α:β TCRs in that they do not generally recognize host cell responses to infections or tissue damage; rather they recognize components of the pathogen directly.

4.27 **Synthesis question:** Several vaccines against viral infections are made by isolating purified surface proteins of the viral particle, mixing them with an adjuvant to stimulate an innate immune response, and injecting the mixture into people. Two examples of this are the vaccine against Hepatitis B virus, and the vaccine against Human Papilloma Virus (the 'cervical cancer' vaccine). One interesting property of vaccines of this type (known as 'subunit vaccines') is that there is a requirement for a CD4 T cell response to the vaccine antigen in order to generate antibodies to the innocuous protein in the vaccine.

In the case of the Hepatitis B vaccine, the viral protein included in the vaccine is the Hepatitis B surface antigen (HepB-SAg), a protein that is approximately 200 amino acids in length. The graph in **Figure Q4.27** shows the data from immunizing individuals with this vaccine, and monitoring their production of protective antibody responses to the viral protein.

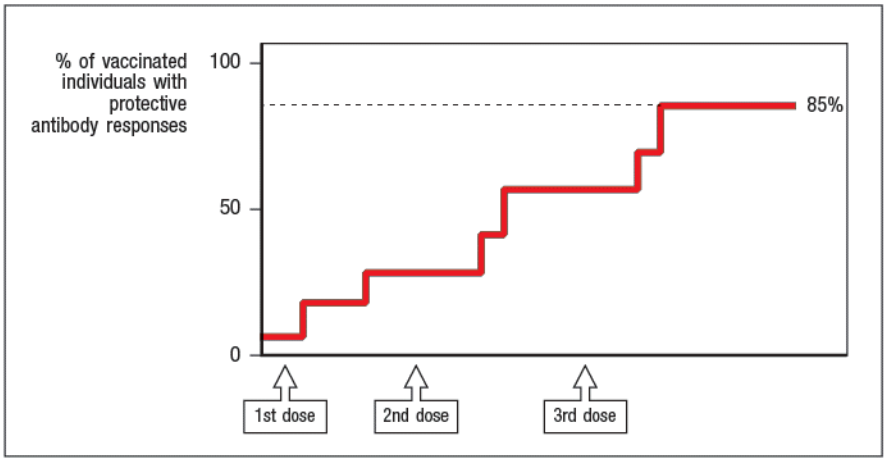

Figure Q4.27

a) What results would be predicted if experiments were performed to examine the CD4 T cell responses to the HepB-SAg in these same individuals? In particular, indicate whether all individuals would show similar responses. Explain your reasoning.

b) Most vaccines, particularly those made by immunizing individuals with purified pathogen components, are designed to elicit robust antibody responses to the immunizing antigens. For vaccines against viral infections (like the HepB vaccine), the immune responses generated are only protective when administered prophylactically, i.e., before the individual is ever exposed to the pathogen. Why is it essential to vaccinate against viral infections before the individual is ever exposed to the virus?

4.28 **Synthesis question:** For the last five years, the seasonal flu vaccine has contained a mixture of two Influenza A strains and one Influenza B strain. The Influenza A strains were categorized as H1N1 and H3N2 subtypes (**Figure Q4.28A**). This nomenclature refers to the sequences of the two surface glycoproteins on the Influenza A virus particle, the hemaglutinin (H) and the neuraminidase (N). Antibodies specific for these glycoproteins are known to be effective at preventing flu infection.

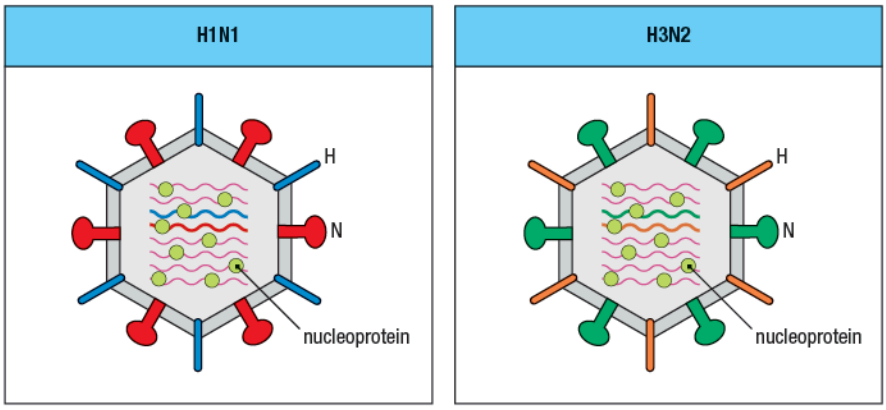

Figure Q4.28A

a) The highly pathogenic Asian avian flu causes a fatal infection in about 60% of the individuals infected. The seasonal flu vaccine does not provide protection against this strain of Influenza. Is the highly pathogenic Asian avian flu likely to be an H1N1 or H3N2 strain of influenza? Why or why not?

In the seasonal vaccine, the two strains of Influenza A, H1N1 and H3N2, are both required to provide protection to individuals exposed to one or the other of the viral strains. Currently, research efforts are focused on trying to generate protective CD8 T cell responses to Influenza A, with the goal of generating a 'universal' or broadly neutralizing vaccine that would provide protection against multiple strains of the virus, even those not included in the vaccine.

b) Based on the information provided in the cartoon of the two viral strains shown in **Figure Q4.28A**, what is the reasoning for expecting CD8 T cell responses to be protective against multiple different strains of Influenza A?

From year to year, the Influenza A strains circulating in the population undergo a process known as 'antigenic drift' in which mutations accumulate in the viral genes, leading to modest changes in the amino acid sequences of the viral proteins. Due to this antigenic drift, different isolates of the H1N1 or H3N2 strains are included in the annual flu vaccine. Shown in **Figure Q4.28B** are some of the regions of viral proteins that often undergo antigenic drift from year to year.

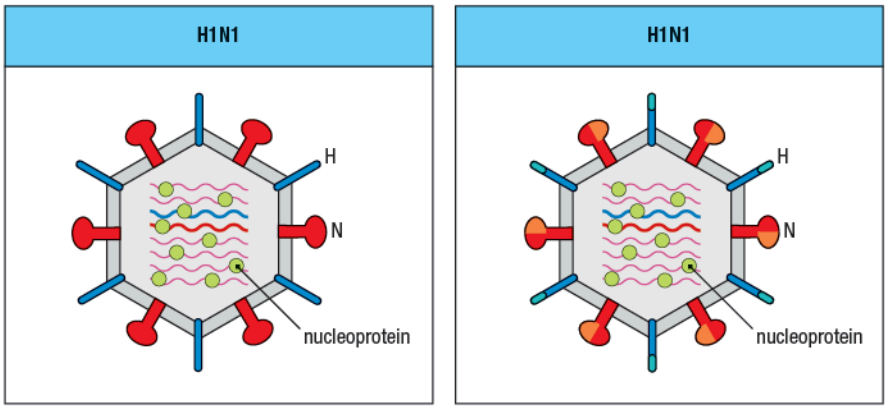

Figure Q4.28B

c) What is the minimum number of amino acids that needs to change for a neutralizing antibody to the neuraminidase of the H1N1 strain on the left to no longer bind to the neuraminidase of the H1N1 strain on the right?

ANSWERS

4.1: False.
The antigen-binding portions of the antibody protein are encoded by the variable domains of both the heavy and light chain polypeptides. Each of these polypeptides contains a variable domain, and the variable domain from one heavy chain and one light chain polypeptide pair to form a single antigen-binding site. The effector functions of the antibody are determined by the heavy chain constant region sequences. There is no contribution of light chain polypeptides to this portion of the antibody.

4.2: B.
Each IgG antibody consists of four polypeptides, two identical light chains and two identical heavy chains. Each light chain is ~25 kDa and each heavy chain ~75 kDa, adding up to ~150 kDa.

4.3: D.
Each immunoglobulin domain is similar, but not identical to other immunoglobulin domains based on amino acid sequence. This is the case when comparing domains within the heavy chain or within the light chain polypeptide, or when comparing heavy chains to light chains. When comparing one IgG antibody to another, the differences in amino acid sequence all reside in the variable domains of the light chains or heavy chains. The constant domains of the heavy chain and light chain are always identical, as long as one compares to the same subtype (i.e., for light chains, kappa to kappa or lambda to lambda; for heavy chain, for instance, IgG2a to IgG2a).

4.4: Since only a subset of the amino acids in a single Ig domain are required to assemble the β-sandwich structure, this allows the remaining amino acids to vary between antibody variable domains while still retaining the same overall structure. In general the conserved amino acids lie in the sequences encoding the β strands that form each β sheet, and the cysteine residues used for the disulfide bond. The loops between the β strands can then vary in amino acid sequence between different antibody polypeptides.

4.5: A.
The cleavage pattern shown in (A) represents the action of the protease pepsin. Pepsin cuts on the carboxy-terminal side of the disulfide bonds linking the two heavy chains together, and makes several cuts in each heavy chain polypeptide. The resulting fragment comprising the amino-terminal portion of the molecule is essentially a dimer of two Fab fragments, thus has the same binding avidity for antigen as the intact antibody (since it is still bivalent). However, this fragment lacks the constant region sequences that mediate effector functions such as recruiting complement. This amino-terminal fragment is known as an $F(ab')_2$.

4.6: E.
A mixture of IgG molecules will differ in the variable domains of both the heavy and light chains. In addition, some IgG molecules will contain κ light chains, and others will be comprised of λ light chains. For both of these reasons, the Fab fragments made from a mixture of IgG molecules will vary in amino acid sequence, and therefore will not crystallize. In contrast, the Fc fragments, which are composed exclusively of heavy chain sequences, will be identical in the mixture, and therefore will crystallize.

4.7: True.
The hinge region between the Fab and the Fc portions of the antibody is flexible, allowing a range of motion between the two antigen-binding sites. In addition, some flexibility is also found at the junction between the V and C domains, allowing bending and rotation of the V domain relative to the C domain. These two features allow antibodies to bivalently bind to components of a pathogen surface that are varying distances apart.

4.8: A.
In general, all three hypervariable loops of both the heavy chain and light chain V regions are important for antigen-binding.

4.9: C and D.
Antibodies C and D have identical heavy chains, but their light chains differ in constant region and V region framework amino acid sequences. Nonetheless, these two light chains share identical amino acid sequences in their CDR1, CD2, and CD3 loops. Therefore, it is likely that these two antibodies would bind to the same antigen. The other antibodies differ in CDR sequences and therefore would be unlikely to bind the same antigen, regardless of sharing light chain C region and V region framework sequences.

4.10: C.
The enormous variability in amino acid sequence in the CDR regions is sufficient to generate many structurally distinct shapes and surfaces. For the CDR3 region, some of this variability is also in the length of the CDR3 sequences.

4.11: False.
Antibodies provide protection by binding to intact pathogens or toxins. Therefore, antibodies generally recognize surface structures on the pathogen. For extracellular bacteria, antibodies often recognize the carbohydrate structures of the bacterial polysaccharide coat. For viruses, antibodies often recognize viral coat proteins important in virus attachment and entry into host cells. For toxins, the antibody generally recognizes amino acid sequences on the surface of the folded toxin protein.

4.12: C.
When identical epitopes are packed closely together on the surface of a pathogen, it is likely that some epitopes will not have antibody bound to them. This is due to steric hindrance, where the presence of one antibody bound will block the access of another antibody to a nearby epitope. In addition, some antibodies may bind to the pathogen with only one of their two antigen-binding sites, if the orientation of the epitopes on the pathogen surface are not compatible with bivalent antibody binding.

4.13: D.
Despite having different domain organizations and lacking light chain proteins, camelid antibodies and shark antibodies share one important feature with human antibodies. That feature is the presence of two antigen-binding sites per antibody protein. It is likely that this feature is conserved to provide enhanced binding avidity of antibodies to their antigens, even in cases where the monomeric affinity of the interaction is low.

4.14:

1. α:β TCRs have only one V and one C region domain per polypeptide.
2. α:β TCRs have a disulfide bond at the C-terminal side of the single Cα and Cβ domains, just amino-terminal to the transmembrane region.
3. α:β TCRs have only one binding site for antigen (i.e., peptide:MHC).
4. The antigen-binding site of the α:β TCR is comprised of both the Vα and Vβ domains together.

4.15: D.
The T cell antigen receptor does not recognize intact antigens or pathogens. Instead, this receptor only recognizes small fragments of degraded pathogen proteins, and only when those peptides are bound to host cell MHC molecules. Since MHC molecules are cell-surface glycoproteins, the T cells can detect a host cell infected with an intracellular pathogen by recognizing pieces of the pathogen bound to these cell surface MHC molecules. This prevents pathogens from hiding from the immune system by replicating inside host cells, a location that is inaccessible to antibodies and to many soluble innate mediators.

4.16: C.
The membrane distal domains of the MHC fold to form a peptide-binding cleft. The major differences in amino acid sequence between different alleles are located in the amino acids that line the peptide binding cleft. The differences in these sequences between alleles determines the peptide-binding specificity of each allelic form of the MHC protein.

4.17: If all humans shared invariant MHC proteins, there would be a limited diversity of peptides that would bind to these proteins. A clever pathogen could evade recognition by T cells by making proteins whose peptides could not bind to the invariant MHC molecules. It is likely that the polymorphism of these proteins in the population as a whole ensures that a subset of individuals are always able to respond to an infecting pathogen.

4.18: B.
An important feature of the binding of peptides to MHC molecules is that the peptide is bound as an integral part of the MHC molecule's structure. Therefore, MHC molecules are highly unstable in the absence of bound peptide. These unstable proteins are poorly expressed on the cell surface. Consequently, efficient surface expression of MHC proteins depends on a constant source of peptides to be incorporated into the peptide binding cleft of each MHC protein as it is synthesized. When a source of intracellular peptides is lacking, total MHC protein expression on the cell surface is substantially reduced.

4.19: A.
The ability of T cells to recognize and respond to host cells harboring intracellular pathogens is dependent on the host cell retaining pathogen peptides on the surface MHC molecules. If MHC molecules did not bind peptides stably, peptide exchange could occur on molecules present at the cell surface. Should this happen, the surface MHC molecules would no longer be providing the T cells with an indicator of the internal proteins present in the host cell, but instead, would be displaying peptides from extracellular proteins in the environment.

4.20: True.
Each allelic variant of MHC class I proteins has preferences for certain amino acid residues at

two anchor positions. Whereas changing an anchor residue to one that is not preferred by that allele will in most cases prevent the peptide from binding, not every synthetic peptide of suitable length that contains these anchor residues will bind the appropriate MHC class I molecule. Instead, the overall binding also depends on the nature of the amino acids at other positions in the peptide. In some cases, particular amino acids are preferred in certain positions, whereas in others the presence of particular amino acids prevents binding. These additional amino acid positions are called ‘secondary anchors.’ These features of peptide binding enable an individual MHC class I molecule to bind a wide variety of different peptides, yet allow different MHC class I allelic variants to bind different sets of peptides.

4.21: E.
Unlike for peptides binding to MHC class I molecules, there are not well-defined sequence motifs that predict peptides binding to MHC class II molecules. This is because the binding pockets of MHC class II molecules accommodate a greater variety of side chains than those of MHC class I molecules, making it more difficult to define anchor residues and to predict which peptides will be able to bind a particular MHC class II variant.

4.22: D.
Unlike antibodies, which function to bind pathogens directly and aid in their elimination, the effector functions of T cells are focused on host cells harboring an intracellular pathogen. The functions of the T cells include killing host cells, in the case of virus infections, as well as activating macrophages, in the case of intracellular bacteria and protozoan infections. For these functions, it is essential that the T cell is not distracted by the presence of free pathogens in its environment, but instead, focuses on the host cell that requires assistance in eliminating the pathogen. The fact that TCRs only recognize pathogen-derived peptides when they are bound to surface MHC molecules ensures this single-minded focus of the T cell.

4.23: The co-receptor (CD4 or CD8) expressed on each T cell has an important function in α:β TCR recognition leading to T cell activation. Experiments have demonstrated that the CD4 molecule and the T-cell receptor can bind simultaneously to the same peptide:MHC class II complex. As a result, CD4 enhances sensitivity to antigen, as the T cell is about 100-fold more sensitive to the antigen when CD4 is present. The enhancement process results from the ability of the intracellular portion of CD4 to bind to a cytoplasmic tyrosine kinase called Lck. This brings Lck into proximity with the T-cell receptor complex helps activate the signaling cascade induced by antigen recognition. An analogous situation occurs for CD8 T cells and their recognition of peptide:MHC class I complexes.

4.24: B.
Unlike CD8 T cells that need to recognize any nucleated cell in the body that might be the target of a virus infection, the major function of CD4 T cells that recognize MHC class II molecules is to activate other effector cells of the immune system. Thus, MHC class II molecules are normally found on dendritic cells, B lymphocytes, and macrophages—antigen-presenting cells that participate in immune responses—but not on other tissue cells. The peptides presented by MHC class II molecules expressed by dendritic cells can function to activate naive CD4 T cells. When previously activated CD4 T cells recognize peptides bound to MHC class II molecules on B cells, the T cells secrete cytokines that can influence the isotype of antibody that those B cells will choose to produce. Upon recognizing peptides bound to MHC class II molecules on macrophages, CD4 T cells activate these cells, again in part through cytokines, to destroy the

pathogens in their vesicles.

4.25: A.
While most immune cells constitutively express high levels of MHC class I proteins, other cells in the body often express much lower levels. Treatment with type I interferons (IFNα/β) will up-regulate MHC class I expression on most cells. This would lead to higher levels of viral peptide: MHC class I complexes on the surfaces of infected cells, resulting in more efficient recognition (and killing) of these cells by CD8 T cells.

4.26: True.
Like NK-cell receptor ligands, such as the proteins MIC and RAET1, many of the ligands seen by γ:δ T cells are induced by cellular stress or damage. Additional ligands for γ:δ T cells may include heat-shock proteins and nonpeptide ligands. Recognition of molecules expressed as a consequence of infection, rather than recognition of pathogen-specific antigens themselves, distinguishes intraepithelial γ:δ T cells from other T cells and B cells, and this would place them in somewhere in between adaptive and innate cells of the immune system.

4.27:

a) Interestingly, only about 85% of the individuals immunized with the HepB vaccine respond by making an antibody response that is sufficient to prevent infection. Furthermore, in the non-responding individuals, repeated injection of the vaccine does not improve the antibody response. The most likely explanation for the failure of these individuals to make an antibody response to the HepB-SAg is that they cannot generate a CD4 T cell response to the peptides from this protein. Due to the polymorphism of the MHC class II genes, different individuals will express different allelic versions of these peptide-binding molecules. Some individuals may express MHC class II variants that are unable to bind any of the peptides generated from the HepB-SAg. These individuals would fail to make a CD4 T cell response to the protein, and therefore would not generate antibody responses to the vaccine.

b) Since most vaccines against viral infections are designed to elicit neutralizing antibody responses, the antibodies must be present in the circulation prior to exposure to the virus. When this is the case, free virus particles that enter the body are rapidly bound by antibodies and then eliminated following uptake by phagocytic cells, or on occasion, lysed by complement activation. Once the virus has infected host cells and begun replicating in them, the circulating antibodies are unable to eliminate the infection. At this point, a CD8 T cell response is needed. Since vaccines that are based on purified viral components, rather than infection with live virus, are unable to generate CD8 T cell responses, these vaccines cannot provide protection once a viral infection has been established in the host.

4.28:

a) The highly pathogenic Asian avian flu is an H5N1 strain of influenza. The seasonal flu vaccine strains are designed to elicit neutralizing antibodies to the surface glycoproteins, hemaglutinin and neuraminidase. In the Asian avian flu, the hemaglutinin protein is highly divergent from the H1 and H3 forms in the vaccine strains. This allows the H5N1 strain to evade the antibody response elicited to the vaccine strains.

b) The internal proteins of the virus, such as the Nucleoprotein shown in the cartoon, are often highly conserved between Influenza A variants. Unlike antibodies, T cell responses

can be generated against proteins that are not exposed on the surface of the intact virus particle. Therefore, protective CD8 T cell responses could be generated that recognize peptides of the viral Nucleoprotein. If an individual was vaccinated with the H1N1 strain, and generated protective CD8 T cells against the Nucleoprotein, these T cells would also protect the individual from infection with the H3N2 strain of the virus.

c) One. Antibody epitopes are comprised of a relatively small number of amino acids, on the order of 6–10. Depending on the location and chemical characteristics of the amino acid change, even a single substitution can be sufficient to prevent antibody binding.