JANEWAY'S IMMUNOBIOLOGY, 9TH EDITION
CHAPTER 10: THE HUMORAL IMMUNE RESPONSE

B-cell activation by antigen and helper T cells

10-1 Activation of B cells by antigen involves signals from the BCR and either T_{FH} cells or microbial antigens

10.1 **Multiple choice:** A mouse is immunized with the tetanus toxoid protein (inactivated toxin) in adjuvant. One week later, the entire population of splenic B cells are isolated from the mouse and mixed with tetanus toxoid-specific CD4 T_{FH} cells plus the purified tetanus toxoid protein. Four days later, the total number of B cells in the culture and the number of tetanus toxoid-specific B cells are determined and compared to the starting population on the day of isolation. The results are shown in **Figure Q10.1**.

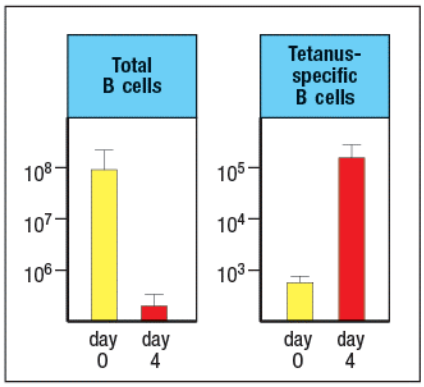

Figure Q10.1

The tetanus-specific B cells preferentially survive and proliferate because:

A. They are the only B cells that express CD40.
B. They are the only B cells that express the receptor for IL-21.
C. They are the only B cells that proliferate in response to their antigen.
D. They are the only B cells presenting the tetanus peptide to the T_{FH} cells.
E. They are the only B cells that received a TLR stimulus during priming.

10-2 Linked recognition of antigen by T cells and B cells promotes robust antibody responses

10.2 **Multiple choice:** The vaccine to *Haemophilus influenzae* type b is called a conjugate vaccine. It is composed of the tetanus toxoid protein conjugated to the capsular polysaccharide of the *H. influenzae* type b bacteria. When used to vaccinate infants, the antibody response generated by this vaccine would include:

A. Antibodies to the bacterial polysaccharide and the tetanus toxoid
B. Antibodies to the tetanus toxoid only
C. Antibodies to the bacterial polysaccharide only
D. Antibodies that only bind to the protein-polysaccharide conjugate in the vaccine
E. Antibodies that recognize the polysaccharide capsule when shed by the bacteria

10-3 B cells that encounter their antigens migrate toward the boundaries between B-cell and T-cell areas in secondary lymphoid tissues

10.3 **Short answer:** When vesicular stomatitis virus (VSV) is used to infect mice via footpad injection, viral particles are trapped in the draining lymph node (the popliteal lymph node) within 5 minutes of injection. These viral particles are then retained in the lymph node for many hours, where they can be visualized on cells that are interacting with B cells. The cells retaining the viral particles in the lymph node are not tissue-resident dendritic cells that have migrated to the lymph node with the virus, as this process takes much longer than 5 minutes. In which region of the lymph node would you expect to find the trapped viral particles and on which cells?

10.4 **Multiple choice:** CXCR5 is the receptor for the chemokine CXCL13, secreted by follicular stromal cells and follicular dendritic cells in the B cell zones (i.e., lymphoid follicles) of secondary lymphoid organs. A conditional knockout mouse in which CXCR5 was specifically deleted only in T cells would have:

A. No defects in any type of antibody response
B. Defects in the initial activation of all B cells
C. A lack of discrete B cell and T cell zones in the lymphoid organ
D. A defect in T cell-dependent antibody responses
E. An increased number and size of germinal centers

10-4 T cells express surface molecules and cytokines that activate B cells, which in turn promote T_{FH}-cell development

10.5 **Multiple choice:** Patients with the disease X-linked lymphoproliferative syndrome (XLP) lack expression of the small adapter protein SAP, which associates with receptors of the SLAM family. One characteristic of this disease is an inability of cytotoxic T cells to control infections with a virus, Epstein–Barr virus (EBV), that replicates in B cells. This defect in control of EBV results from:

A. A defect in antibody responses to EBV due to impaired T cell help for B cells
B. A defect in adhesion of cytotoxic T cells to EBV-infected B cells
C. Impaired migration of activated B cells to the germinal center
D. Impaired survival of activated B cells, normally induced by CD40 stimulation
E. A defect in T_{FH} differentiation, normally induced by ICOS on B cells binding to ICOS-ligand on T_{FH} cells

10-5 Activated B cells differentiate into antibody-secreting plasmablasts and plasma cells

10.6 **True/False:** Once B cells begin secreting antibodies, they cease dividing and have a life-span of only a few days.

10-6 The second phase of a primary B-cell immune response occurs when activated B cells migrate into follicles and proliferate to form germinal centers

10.7 **Multiple choice:** The germinal center is a region within the secondary B cell follicle where sustained B cell proliferation and differentiation take place. The processes of B

cell proliferation and differentiation, including affinity maturation and class switching, require periodic interactions of the germinal center B cells with CD4 T_{FH} cells. These periodic interactions between the B cells and T_{FH} cells can occur:

A. When B cells cycle between the dark zone and the light zone of the germinal center
B. When B cells leave the germinal center and migrate through the T-cell zone on their way to the blood
C. When B cells migrate and form a primary focus of antibody-secreting plasmablasts in the medullary cords of the lymph node
D. When B cells migrate to the border between the T-cell zone and the B-cell zone of the lymph node
E. When B cells up-regulate CXCR4 and migrate into the dark zone of the germinal center

10-7 Germinal center B cells undergo V-region somatic hypermutation, and cells with mutations that improve affinity for antigen are selected

10.8 Multiple choice: In germinal centers, proliferating B cells undergo a process called somatic hypermutation, in which mutations are introduced into the V regions of the antibody heavy and light chain genes. When this process is complete after several weeks, the overall affinities of the antibodies produced are greatly increased compared to those present early in the primary response. The somatic hypermutation process leads to increased antibody affinity because:

A. Mutations that decrease the antibody affinity lead to an arrest of B cell proliferation.
B. B cells making higher affinity antibodies receive more help from T_{FH} cells.
C. Somatic hypermutations only take place in the sequences encoding the CDR1, CDR2, and CDR3 regions.
D. Mutations that increase antibody affinity lead to an increased rate of B cell proliferation.
E. The majority of nucleotide changes introduced by AID don't change the amino acid coding sequence.

10-8 Positive selection of germinal center B cells involves contact with T_{FH} cells and CD40 signaling

10.9 Multiple choice: Studies show that about 50–100 different B cells initially seed each germinal center (d7 post-infection). These different B cells are represented by different colored circles in a white oval (germinal center) in **Figure Q10.9**. Which of the choices shown best represents the B cell population that would be found in the same germinal center approximately two weeks later?

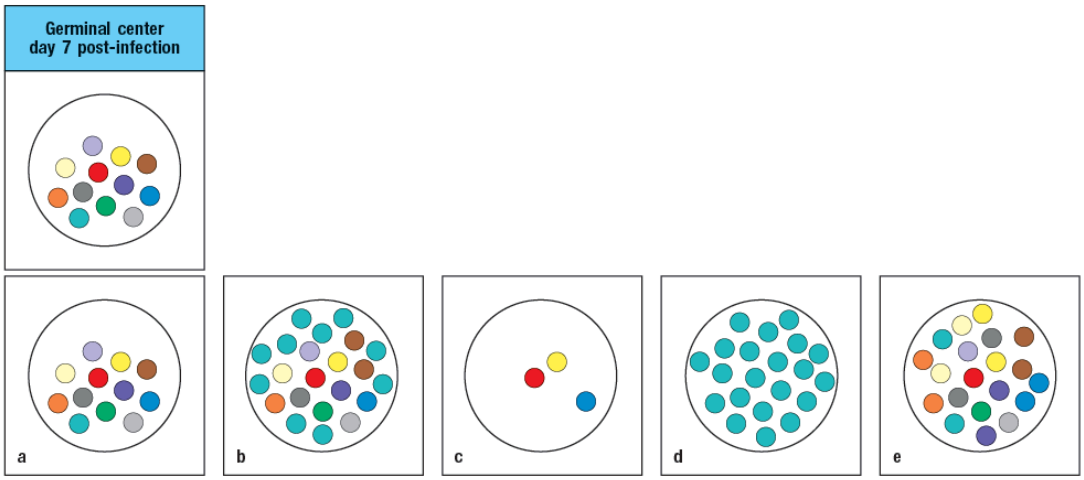

Figure Q10.9

10-9 Activation-induced cytidine deaminase (AID) introduces mutations into genes transcribed in B cells

10.10 Short answer: Mice and humans with inactivating mutations in the gene encoding activation-induced cytidine deaminase (AID) have an immunodeficiency disease known as 'hyper IgM type 2'. Since AID is the enzyme that catalyzes the conversion of cytosines in the DNA to uracils, thereby initiating the process of somatic hypermutation, why do individuals with this deficiency only produce IgM antibodies?

10-10 Mismatch and base-excision repair pathways contribute to somatic hypermutation following initiation by AID

10.11 Multiple choice: The process of somatic hypermutation of antibody V regions sequences is initiated by the enzyme AID. This enzyme targets cytidine residues in the DNA sequence that are normally part of a G:C pair in the double-stranded DNA. Yet the hypermutation process generates mutations at both G:C and A:T base pairs of the original sequence because:

A. Following AID action, a double-stranded DNA break plus chewing back of the ends occurs before re-ligation of the sequence.
B. The error-prone polymerase repairs the sequence by inserting random nucleotides.
C. There are two different pathways of repair target, one targeting G:C and one targeting A:T base pairs.
D. B cells are the only cells to express the enzyme uracil-DNA glycoslyase (UNG).
E. During active transcription both A:T and G:C base pairs are temporarily single-stranded.

10-11 AID initiates class switching to allow the same assembled VH exon to be associated with different CH genes in the course of an immune response

10.12 Multiple choice: Unlike somatic hypermutation, class switching occurs in discrete sequence regions upstream of the immunoglobulin heavy chain coding sequences

(called switch regions). One key element in directing the enzyme AID to a specific switch region is the opening of the DNA duplex combined with polymerase stalling during active transcription in that region. A second key feature of directing AID to a specific switch region is:

A. The processed RNA from the switch region guides AID to this site in the DNA
B. The binding of DNA-PK's to the switch region sequence in the DNA
C. The presence of double-stranded breaks in the DNA in this region
D. The binding of AID to the RNA polymerase that is transcribing the switch region
E. The predominance of G:C base pairs in the switch sequence

10-12 Cytokines made by T_{FH} cells direct the choice of isotype for class switching in T-dependent antibody responses

10.13 Multiple choice: In cell culture experiments, purified B cells expressing IgM can be induced to switch to producing IgE by stimulating them with an antibody to CD40 (a stimulatory antibody) plus the cytokine IL-4. In an individual undergoing an immune response, these signals would normally be provided by:

A. Germinal center stromal cells
B. Other B cells in the germinal center
C. Follicular dendritic cells in the germinal center
D. Any CD4 T cell in the same lymph node
E. T_{FH} cells in the germinal center

10-13 B cells that survive the germinal center reaction eventually differentiate into either plasma cells or memory cells

10.14 Short answer: Irradiation of mice with a dose of 600 rad of total body irradiation eliminates 95% of total lymphocytes from spleen and lymph nodes and also eliminates all antigen-specific memory B cells. Nonetheless, when Influenza A-infected mice are subjected to this irradiation at 60-days post-infection, and then reconstituted with bone marrow cells from a naive mouse (this replenishes all of the lymphocyte populations), the levels of circulating anti-Influenza A IgG antibodies show nearly no decline when mice are monitored for the following year. What is the explanation for this finding?

10.15 Multiple choice: In humans, IgA is produced in copious amounts, estimated to be a rate of 3 g/day. Nearly all of the IgA secreting plasma cells are found in the gastrointestinal (GI) tract where the secreted IgA is transported across the GI epithelium into the lumen of the gut. There, this antibody protects the GI epithelium against intestinal pathogens. In contrast, none of the GI resident long-lived antibody secreting cells produce antibodies of the IgG class. The differential localization of long-lived antibody secreting cells producing IgA compared to those producing IgG is likely due to:

A. Their interactions with T_{FH} cells specific for pathogens that infect the gut
B. The lack of germinal centers in the mucosal lymphoid organs
C. The absence of S1PR1 expression on IgA-secreting plasma cells
D. Their priming and differentiation in mucosal lymphoid organs
E. Their inability to access the bone marrow compartment

10-14 Some antigens do not require T-cell help to induce B-cell responses

10.16 Multiple choice: Two different vaccines have been developed that protect vaccinated individuals against pneumococcal disease, a bacterial infection that causes pneumonia, meningitis and sepsis (blood stream infection). This disease is caused by the bacteria, *Streptococcus pneumoniae*.

One vaccine, PPSV23, is a mixture of polysaccharides isolated from 23 different serotypes of *S. pneumoniae*. The second vaccine, PCV13, is a conjugate vaccine made from polysaccharides of 13 different serotypes of the bacteria conjugated to diphtheria toxoid (inactivated toxin protein). The PPSV23 vaccine is only given to adults, whereas infants and small children are given PCV13. This is because:

A. Adults are likely exposed to more different strains (serotypes) of *S. pneumoniae* than infants.
B. Adult B cells don't require T_{FH} cells to make antibody responses.
C. Infant B cells are immature and don't respond to TI-2 antigens.
D. Adult B cells respond more robustly than infant B cells to B-cell mitogens.
E. Infant B cells are more dependent on the cytokine BAFF.

The distributions and functions of immunoglobulin classes

10-15 Antibodies of different classes operate in distinct places and have distinct effector functions

10.17 Short answer: *Borrelia hermsii* is a spirochete bacterium, transmitted by tick bites, that causes an illness characterized by a relapsing fever. The bacteria enter the host bloodstream and replicate there. Studies in mice show that episodes of bacteremia (bacteria in the blood) are efficiently controlled by anti-bacterial antibodies, but interestingly, follicular B cells are not required for this response, nor is the response impaired by splenectomizing the mice (i.e., removing the spleen). Which B cells are most likely responsible for this antibody response?

10.18 Multiple choice: Individuals with a genetic polymorphism in the Fcγ receptor, FcγRIIa (CD32), have an increased susceptibility to bacterial meningitis (inflammation of the membranes (meninges) surrounding the brain and spinal cord) caused by the encapsulated bacterium, *Neisseria meningitidis*. This polymorphism reduces the efficiency with which the phagocytes expressing FcγRIIa bind to the constant region of this receptor's target antibody. The reason this FcγRIIa-dependent response it the major form of protection against *Neisseria meningitidis* is because:

A. T cells are unable to enter the brain and spinal cord.
B. Mast cells are unable to localize to the meninges.
C. IgG antibodies are the major isotype able to diffuse into tissues.
D. IgM antibodies do not have high enough affinity to provide protection.
E. Neutrophils are unable to phagocytose encapsulated bacteria.

10-16 Polymeric immunoglobulin receptor binds to the Fc regions of IgA and IgM and transports them across epithelial barriers

10.19 Multiple choice: Wild-type mice infected with one strain of Influenza A virus (PR8) by intranasal inoculation are protected from intranasal infection by a related Influenza A virus (Beijing), a phenomenon known as cross-protection. These infections are generally localized to the upper respiratory tract. Mice with a homozygous single gene defect in 'gene X' have greatly impaired cross-protection to Influenza A-Beijing following immunization with Influenza A-PR8 by the intranasal route. Gene X likely encodes:

A. Fc receptor, FcγRIIa
B. Complement receptor, CR1
C. TLR adapter protein, MyD88
D. Poly Ig receptor
E. AID

10-17 The neonatal Fc receptor carries IgG across the placenta and prevents IgG excretion from the body

10.20 Multiple choice: Infants born with the immunodeficiency disease X-linked agammaglobulinemia (XLA) have a block in B cell development, and fail to produce mature B cells. As a result, these infants lack the ability to produce antibodies. After birth, babies with XLA first begin to show symptoms of recurrent and persistent extracellular bacterial infections due to common environmental pathogens when they are 4–6 months of age. The reason these infants are healthy for the first 4–6 months after birth is because:Newborn infants have high circulating levels of maternal IgG at birth.

A. Newborn infants are not exposed to bacterial pathogens for the first 4–6 months of life.
B. Newborn infants are born with high circulating levels of complement proteins to protect them.
C. Newborn infants are born with high circulating levels of antimicrobial peptides to protect them.
D. Newborn infants are vaccinated against these common bacterial pathogens.

10-18 High-affinity IgG and IgA antibodies can neutralize toxins and block the infectivity of viruses and bacteria

10.21 Multiple choice: Neutralizing antibodies are effective at preventing infection or toxicity mediated by pathogens or their toxic products. In fact, nearly all vaccines currently in use function by eliciting neutralizing antibodies. One example is the tetanus vaccine, in which neutralizing antibodies are generated against an inactivated form of the tetanus toxin (i.e., the tetanus toxoid). The most important feature of a neutralizing antibody is:

A. Having a high degree of multi-valency, such as being a pentamer or hexamer of immunoglobulin monomers
B. Having high affinity for the antigen
C. Being present at a high concentration in the circulation
D. Being efficient at activating the complement cascade
E. Having a long half-life in the body

10-19 Antibody:antigen complexes activate the classical pathway of complement by binding to C1q

10.22 Multiple choice: IgM antibodies are much more efficient than IgG at activating the complement cascade. However, under certain circumstances, IgG antibodies will activate the complement pathway. One example of a situation in which IgG binding to its antigen will not trigger the complement cascade is when:

A. The IgG antibodies bind to a multivalent soluble antigen in solution, such as a polysaccharide structure shed from a bacterial pathogen.
B. The IgG antibodies bind to a viral capsid protein that is present in more than 100 copies on the viral particle surface.
C. The IgG antibodies bind to a bacterial surface by recognizing a repetitive polysaccharide component of the bacterial capsule.
D. The IgG antibodies are binding self-antigens such as chromatin released from dead cells.
E. The IgG antibodies are neutralizing a bacterial toxin protein by blocking the receptor-attachment site on the toxin.

10-20 Complement receptors and Fc receptors both contribute to removal of immune complexes from the circulation

10.23 Short answer: Surprisingly, individuals with defects in the early components of the classical complement cascade (i.e., C1, C2, or C4) suffer from an autoimmune type of kidney damage, rather than from an immunodeficiency leading to increased susceptibility to infections. Why do these complement defects lead to autoimmune kidney damage?

The destruction of antibody-coated pathogens via Fc receptors

10-21 The Fc receptors of accessory cells are signaling receptors specific for immunoglobulins of different classes

10.24 Multiple choice: The upper respiratory tract of many individuals is colonized by *Streptococcus pneumoniae* bacteria. Infections caused by these bacteria, including pneumonia, meningitis, otitis media (ear infections), and sinusitis, are thought to occur in individuals lacking protective antibodies. For many years, IgG was thought to be the major antibody class responsible for protective immunity to *S. pneumoniae*, due to the ability of anti-bacterial capsule IgG antibodies to opsonize the bacteria and promote phagocytosis. However, in addition to IgG, pneumococcal polysaccharides elicit robust IgA antibody responses. It was traditionally thought that these IgA antibodies functioned in neutralization, by blocking bacterial attachment to mucosal epithelial cells. It is now known that IgA antibodies, like IgG, can function as opsonins, to induce phagocytosis and killing of IgA-coated pathogens. This function of IgA antibodies depends on:

A. The production of high affinity anti-bacterial IgA antibodies
B. The dimeric form of IgA antibodies to bind to multivalent sites on the bacteria
C. The ability of dimeric IgA binding to recruit and activate the complement cascade
D. The presence of IgA-specific Fc receptors on neutrophils and macrophages
E. The ability of IgA antibodies to efficiently cross the epithelial surface and enter the airways

10-22 Fc receptors on phagocytes are activated by antibodies bound to the surface of

pathogens and enable the phagocytes to ingest and destroy pathogens

10.25 Multiple choice: Some individuals with repetitive exposure to high doses of *Schistosoma mansoni* develop resistance to re-infection by this helminthic parasite. In contrast, other individuals remain highly susceptible. Population studies showed that resistant individuals had increased numbers of circulating eosinophils in their blood compared to susceptible individuals, and further, that these eosinophils had increased levels of:

A. Complement receptor, CR1
B. Mannose binding lectin
C. FcεRII, the low affinity IgE receptor
D. FcγRII-B, the inhibitor IgG receptor
E. Anti-microbial ficolins

10-23 Fc receptors activate NK cells to destroy antibody-coated targets

10.26 Multiple choice: Antibody-dependent cell-mediated cytotoxicity (ADCC) is an important effector mechanism in immunity to virus infections. This immune pathway has also been exploited for clinical applications. For instance, patients with various disorders, including rheumatoid arthritis and some B cell lymphomas, are treated with an antibody directed at CD20, a surface receptor expressed on all B cells. This antibody leads to the depletion of B cells from the patients by the actions of:

A. Natural killer (NK) cells
B. Cytotoxic CD8 T cells
C. Cytotoxic CD4 T cells
D. Activated macrophages
E. The complement cascade membrane attack complex

10-24 Mast cells and basophils bind IgE antibody via the high-affinity Fcε receptor

10.27 Short answer: Individuals with allergies often mount IgE antibody responses to non-infectious environmental antigens. Examples are peanut allergies, allergies to house dust mites, and allergic responses to bee stings. In all of these cases, the allergic response is mediated by the cross-linking of the high affinity IgE receptors on mast cells (FcεRI) which are pre-bound to the allergen-specific IgE antibodies. Yet, the symptoms of these different allergic responses can vary greatly, and can include skin rashes or hives, swelling of the throat and difficulty breathing, to gastrointestinal symptoms such as cramps, diarrhea or vomiting. How can different IgE-mediated allergic responses cause such different symptoms?

10-25 IgE-mediated activation of accessory cells has an important role in resistance to parasite infection

10.28 Multiple choice: The W/W^{v} mouse strain is heterozygous for two different alleles of the gene encoding the growth factor receptor, c-kit, an important receptor expressed on hematopoietic progenitor cells in the bone marrow. The major defect in these mice is the absence of single lineage of hematopoietic cells. When these mice are challenged with larval *Haemaphysalis longicornis* ticks, they fail to become resistant to the ticks, in spite

of generating high titers of anti-tick IgE antibodies. The cell type missing in the W/W^{v} mice is most likely:Natural Killer (NK) cells

A. Eosinophils
B. Tissue-resident dendritic cells
C. Tissue-resident macrophages
D. Tissue-resident mast cells

10.29 Synthesis question: MPSV-4 is a tetravalent polysaccharide vaccine designed to elicit antibodies to four different serotypes of *Neisseria meningitidis*, a bacterial infection that causes meningitis and sepsis in susceptible individuals. However, the protection induced by this vaccine is short-lived, and normally wanes to undetectable levels by 2–3 years post-vaccination. In addition, MPSV-4 does not induce mucosal immunity to *Neisseria meningitidis*; instead, it only protects against bacteria that access the bloodstream. Two newly developed vaccine candidates (A and B) are tested in mice for their ability to elicit high concentrations of anti-meningococcal antibodies that would provide mucosal as well as bloodstream protection. Also, the ideal candidate vaccine should also provide long-lasting immunity to the infection. **Figure Q10.29A** is a diagram of the results from the primary immunization with both candidate vaccines:

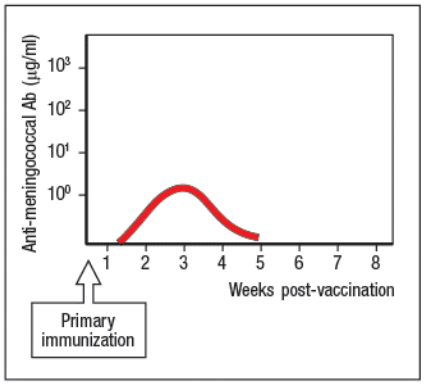

Figure Q10.29A

a) what is the predominant antibody isotype elicited by the primary immunization with these candidate vaccines? In which part of the body is that antibody primarily found?

Figure Q10.29B shows the responses to a primary, followed by a secondary immunization to each of the two candidate vaccines.

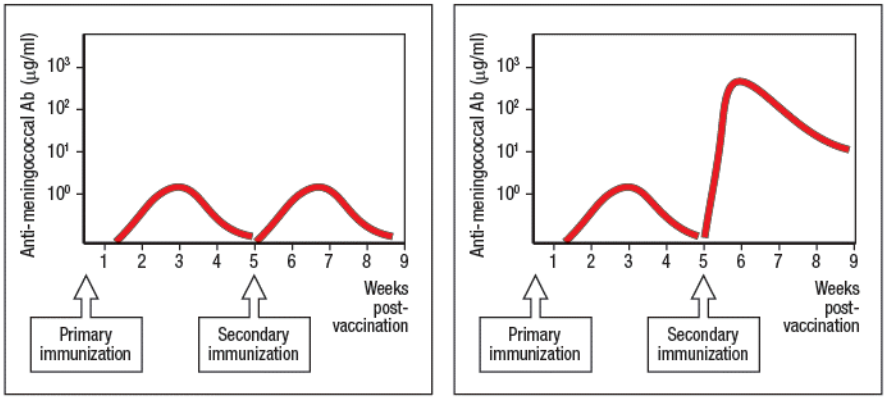

Figure Q10.29B

b) Which candidate vaccine elicits the preferred response? What are the three aspects of the preferred response that make it the candidate vaccine of choice?

The vaccine developers refuse to divulge the components of the candidate vaccines A and B.

c) What is the likely composition of each vaccine and what evidence from the information above are used to lead to your conclusions?

d) To confirm the choice of the preferred candidate vaccine, what type of additional information from the vaccine trials in mice shown above would support this conclusion? Name two additional features of the secondary antibody response to each candidate vaccine that could be assessed, and what results would be expected for each of the candidate vaccines.

e) To assess whether either candidate vaccine might provide mucosal immunity in addition to immunity in the bloodstream, what feature of the response to each vaccine should be examined?

10.30 Synthesis question: Individuals infected with herpes simplex virus (HSV) mount protective antibody responses directed against the surface glycoproteins of the virus. These antibodies are critical to viral clearance, contributing to viral neutralization as well as to complement-mediated and cytotoxic cell-mediated killing of infected target cells. Surprisingly, humans as well as mice deficient in the complement protein, C3, have greatly reduced antibody responses to T cell-dependent antigens, and are impaired in their ability to control HSV infections. When C3-deficient mice are infected with HSV, once at day 0 and then a second time 4 weeks later, their antibody response is altered compared to wild-type mice, as shown in **Figure Q10.30A**.

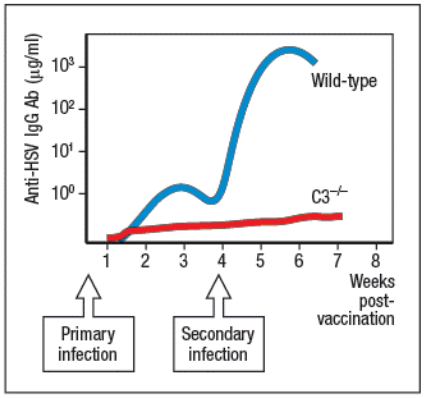

Figure Q10.30A

a) What is a likely explanation for the altered antibody response in the absence of complement C3?

To test your hypothesis, the following experiment is performed. Wild-type and $C3^{-/-}$ mice are each inoculated with a replication-defective form of HSV (HSV-rd). This virus infects

cells, stimulates innate immune responses, but is not able to replicate and spread in the body. For this experiment, mice are infected with varying doses of the HSV-rd virus, and peak IgG responses to the viral surface glycoproteins are measured. The results are shown in **Figure Q10.30B**.

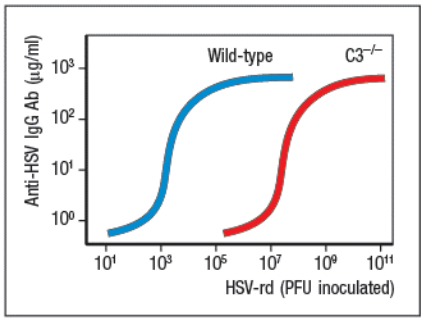

Figure Q10.30B

b) What is the most likely explanation for these data? Do these results impact your answer to part (a)? Explain your reasoning.

To further elucidate the function of complement C3 in the humoral immune response, a knockout mouse line lacking the receptor for C3 fragments is generated. This receptor is encoded by the *Cr2* gene, so knockout mice lacking this gene are known as *Cr2*$^{-/-}$. Previous studies have indicated that the complement receptor encoded by the *Cr2* gene is expressed on B cells and on the follicular dendritic cells that reside in B cell zones of secondary lymphoid organs. To distinguish the functions of the complement receptor on these two cell types, a series of bone marrow chimeras are generated. Wild-type bone marrow is used to reconstitute lethally irradiated *Cr2*$^{-/-}$ mice (wt→Cr2-/-), or vice versa (Cr2-/-→wt). As controls, *Cr2*$^{-/-}$ bone marrow is used to reconstitute Cr2-/- recipients (Cr2-/-→Cr2-/-), and wild-type bone marrow used to reconstitute wild-type recipients (wt→wt). These mice are then inoculated with the HSV-rd virus at 10^6 PFU, once at day 0 and then a second time at day 28, and the anti-HSV IgG responses are measured every 7 days, as shown in **Figure Q10.30C**.

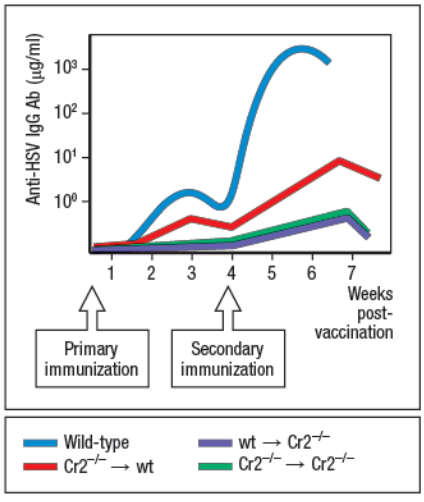

Figure Q10.30C

c) From the data shown above, on which cell type is the expression of the complement receptor most important for humoral immunity?

d) For each of the cell types expressing the complement receptor encoded by *Cr2*, what is the explanation for their importance in humoral immunity to HSV inoculation?

ANSWERS

10.1: D.
To receive T-cell help, the B cell must display antigen on its surface in a form that a T cell can recognize. This occurs when antigen bound by surface immunoglobulin on the B cell is internalized and degraded within the B cell and peptides derived from it are returned to the cell surface in a complex with MHC class II. When the T_{FH} cell recognizes these peptide:MHC complexes, it provides that specific B cell with signals that favor survival and induce proliferation. These signals include the activation of CD40 on B cells by T_{FH} expression of its ligand, CD40L (CD154), and production of various cytokines by T_{FH} cells, including IL-21. CD40 signaling activates the non-canonical NFκB pathway and enhances B-cell survival by inducing the expression of anti-apoptotic molecules such as Bcl-2. IL-21 signaling activates STAT3 and enhances cellular proliferation and differentiation into plasma cells and memory B cells.

10.2: A.
To protect infants against *Haemophilus influenzae* type b, an antibody response to the capsular polysaccharide of the bacteria is needed. Infants do not make T cell-independent antibody responses, and therefore, do not respond well to a vaccine that is composed of only the bacterial polysaccharide. For T-dependent antibody responses, the T cells need to be activated by the same antigen as is recognized by the B cells; this is called linked recognition. However, the peptide recognized by the T_{FH} cell can differ from the epitope recognized by the B cell's antigen receptor. For linked recognition to occur, the peptide recognized by the T cell must be physically associated with the antigen recognized by the B cell's receptor, so that the B cell can take up and present the appropriate peptide to the T cell. When these T_{FH} cells are activated by B cells presenting their peptide, they are stimulated to provide specific signals that help B cells to generate antibodies against the antigen. In the case of the conjugate vaccine, the tetanus toxoid-specific T_{FH} cells would provide help to B cells making antibodies that bind to the bacterial polysaccharide, as well as to B cells that make antibodies to the tetanus toxoid protein.

10.3: The virus particles are trapped on macrophages that reside in the subcapsular sinus of the lymph node. These cells can retain antigens on their cell surface without ingesting and degrading the antigen. This allows antigen-specific follicular B cells an opportunity to recognize the antigen via their surface immunoglobulin receptor.

10.4: D.
Naive B cells express CXCR5 and migrate into B cell follicles of secondary lymphoid organs. After activation by antigen, either on a follicular dendritic cell (FDC) or a macrophage, the B cell increases expression of CCR7 and migrates toward the border with the T-cell zone. At the same time, T cells are activated within the T-cell zones by dendritic cells. When naive T cells are activated, some will proliferate, differentiate into effector cells, down-regulate expression of S1PR1, and exit the lymphoid tissue. However, others will induce expression of CXCR5 and migrate to the border with the B-cell follicle. There, T cells can encounter B cells activated during the same response, providing a mechanism for them to recognize linked antigens presented by activated B cells that have recently moved to this location. This process is important for optimal T cell-dependent antibody responses.

10.5: B.

Individuals with a defect in expressing the adapter protein SAP have impaired interactions between B cells and T cells. This defect results in impaired T cell-dependent antibody responses due to failed interactions between T_{FH} cells and B cells in the germinal center. However, for the control of a virus infection, the most critical response is the killing of virus-infected target cells by effector CD8 cytotoxic T cells. Since, in the case of EBV, the infected cells are B cells, the efficient killing of these EBV-infected target cells requires stable adhesion between the effector T cells and the virus-infected B cells, a process that is impaired in the absence of SLAM-receptor signaling through SAP.

10.6: False.
B cells proliferate in the primary focus for several days, and this constitutes the first phase of the primary humoral immune response. Some of these proliferating B cells differentiate into antibody-synthesizing plasmablasts in the primary focus. Plasmablasts are cells that have begun to secrete antibody, yet are still dividing and express many of the characteristics of activated B cells that allow their interaction with T cells.

10.7: A.
The germinal center is a specialized microenvironment in which B-cell proliferation, somatic hypermutation, and selection for strength of antigen binding all occur. Closely packed centroblasts, which express CXCR4 and CXCR5, form the ‘dark zone’ of the germinal center; the less densely packed ‘light zone’ contains centrocytes, which express only CXCR5. Stromal cells in the dark zone produce CXCL12, which attracts the CXCR4-expressing centroblasts. The light zone is the region of the germinal center where the T_{FH} cells are found. Cyclic re-entry describes the process by which B cells can lose and gain expression of CXCR4 and thus move from the light zone to the dark zone and back again.

10.8: B.
After activated B cells interact with T_{FH} cells at the follicle border, they migrate to germinal centers (GCs). In the dark zone of the GC, somatic hypermutation alters the immunoglobulin V regions. In some B cells, the mutated B-cell receptor (BCR) will have low or no affinity for the antigen, while in other B cells the mutated BCR affinity may be higher. After exiting the dark zone, the B cells with higher-affinity BCRs will capture antigen trapped on follicular dendritic cells (FDCs) and then process and present it on MHC class II molecules. B cells with low-affinity BCRs will fail to capture and present antigen. Only those B cells that present linked antigen epitopes to T_{FH} cells will receive help through CD40L and IL-21, which promote survival and proliferation. B cells that lack antigen on MHC class II molecules receive no help and will eventually die.

10.9: D.
The affinity and specificity of B cells is continually refined during the germinal center response, through affinity maturation. The selection process can be quite stringent: although 50–100 B cells may seed the germinal center, most of them leave no progeny, and by the time the germinal center reaches maximum size, it is typically composed of the descendants of only one or a few B cells.

10.10: Individuals with defects in AID have hyper IgM syndrome because AID is required for both somatic hypermutation and for immunoglobulin class switching. When AID deaminates cytidine residues in the immunoglobulin V regions, somatic hypermutation is initiated; when

cytidine residues in switch regions are deaminated, class switch recombination is initiated.

10.11: C.
The uridine produced by AID represents a dual lesion in DNA; not only is uridine foreign to normal DNA, but it is now a mismatch with the guanosine nucleoside on the opposite DNA strand. The presence of uridine in DNA can trigger several types of DNA repair—including the mismatch repair and the base excision repair pathways—which further alter the DNA sequence. These two repair processes lead to different mutational outcomes. In the mismatch repair pathway, the presence of uridine is detected by the mismatch repair proteins MSH2 and MSH6 (MSH2/6). They recruit nucleases that remove the complete uridine nucleotide along with several adjacent nucleotides from the damaged DNA strand. This is followed by a fill-in 'patch repair' by a DNA polymerase; unlike the process in all other cells, in B cells this DNA synthesis is error-prone and tends to introduce mutations at nearby A:T base pairs. If, on the other hand, the original U:G mismatch is recognized by UNG, then an abasic site will be generated in the DNA. If no further modification is made to this site, it can be replicated without instructive base pairing from the template strand by a class of error-prone 'translesion' DNA polymerases that normally repair gross damage to DNA, such as that caused by ultraviolet (UV) radiation. These polymerases can incorporate any nucleotide into the new DNA strand opposite the abasic site, and after a further round of DNA replication this can result in a stable mutation at the site of the original C:G base pair.

10.12: A.
Polymerase stalling is connected with the recruitment of AID to specific switch regions being transcribed. Recent evidence indicates that AID is selectively guided to the transcribed switch by an additional mechanism. After an RNA polymerase has completed transcription of one RNA template, the intron RNA harboring the switch region is spliced out. This RNA is processed to generate an RNA structure, called a G-quadruplex , that is based on the G-rich repetitive element of the switch region. This G-quadruplex serves a dual purpose, both binding to AID and also associating with the switch region from which it was transcribed, based on its sequence complementarity. Thus the G-quadruplex guides AID to the appropriate switch region, where particular palindromic sequences, such as AGCT, act as good substrates to allow its cytidine deaminase activity to act on both strands concurrently. In this way, the G-quadruplex functions in a manner similar to the synthetic guide RNAs that deliver the Cas9 endonuclease to specific genomic regions.

10.13: E.
The choice of antibody isotype that is produced during class switching is largely controlled by the cytokines that are produced by T_{FH} cells in the germinal center reaction. Interactions between germinal center B cells and T_{FH} cells are essential for class switching to occur. The required interactions occur through the interplay of CD40 on B cells with CD40 ligand on activated helper T cells. Then, depending on which cytokines that T_{FH} cell produces, switching will be directed to specific antibody classes. IL-4 induces switching to IgE and IgG1.

10.14: Long-lived plasma cells in the bone marrow are resistant to 600 rad of irradiation. These cells survive the treatment, and following reconstitution with bone marrow from a naive individual, the long-lived plasma cells remain functional in the reconstituted bone marrow environment. These long-lived plasma cells continue to produce anti-viral antibodies for the lifetime of the animal.

10.15: D.
Some plasma cells deriving from germinal centers in lymph nodes or spleen migrate to the bone marrow, where a subset live for a long period, whereas others migrate to the medullary cords in lymph nodes or splenic red pulp. B cells that have been activated in germinal centers in mucosal tissues, and which are predominantly switched to IgA production, stay within the mucosal system.

10.16: C.
One class of thymus-independent antigens—TI-2 antigens—consists of molecules that have highly repetitive structures, such as bacterial capsular polysaccharides. These contain no intrinsic B-cell-stimulating activity. Whereas TI-1 antigens can activate both immature and mature B cells, TI-2 antigens can activate only mature B cells; immature B are inactivated by encounter with repetitive epitopes. Infants and young children up to about 5 years of age do not make fully effective antibody responses against polysaccharide antigens, and this might be because most of their B cells are immature. Thus, infants and small children need to be vaccinated with a form of vaccine that contains protein. This provides peptides from the processing of the protein antigen that can elicit CD4 T_{FH} responses to generate a T cell-dependent, rather than a T cell-independent antibody response to the vaccine polysaccharides.

10.17: B-1 B cells.
This antibody response is an IgM response to the *Borrelia hermsii* bacteria. Most IgM is produced by B-1 B cells or marginal zone B cells, rather than follicular B cells. Since the response is maintained in the absence of the spleen, this finding rules out the marginal zone B cells, and the information provided also rule out the follicular B cells. This indicates the IgM response is produced by B-1 B cells.

10.18: C.
IgG is the principal class of antibody in blood and extracellular fluid. IgG efficiently opsonizes pathogens for engulfment by phagocytes and activates the complement system. IgG operates mainly in the tissues, where accessory cells and molecules are available. In contrast, the other antibody classes are generally not found in the tissues.

10.19: D.
In the mucosal immune system, IgA-secreting plasma cells are found predominantly in the lamina propria, which lies immediately below the basement membrane of many surface epithelia. From there the IgA antibodies can be transported across the epithelium to its external surface, for example to the lumen of the gut or of the bronchi. IgA antibody synthesized in the lamina propria is secreted as a dimeric IgA molecule associated with a single J chain. This polymeric form of IgA binds specifically to a receptor called the polymeric immunoglobulin receptor (pIgR), which is present on the basolateral surfaces of the overlying epithelial cells. When the pIgR has bound a molecule of dimeric IgA, the complex is internalized and carried in a transport vesicle through the cytoplasm of the epithelial cell to its luminal surface. This process is called transcytosis. The principal sites of IgA synthesis and secretion are the gut, the respiratory epithelium, the lactating breast, and various other exocrine glands such as the salivary and tear glands. It is believed that the primary functional role of IgA antibodies is to protect epithelial surfaces from infectious agents, such as Influenza A virus.

10.20: A.
Maternal IgG is transported across the placenta directly into the bloodstream of the fetus during intrauterine life; human babies at birth have as high levels of plasma IgG as their mothers, and with the same range of antigen specificities. The selective transport of IgG from mother to fetus is due to an IgG transport protein in the placenta, FcRn (neonatal Fc receptor). By 4–6 months after birth, these maternal antibodies have waned, and healthy infants would be producing their own antibodies.

10.21: B.
Antibodies that bind a toxin's or a virus's receptor-binding site can prevent cell entry and protect cells from attack. Antibodies that act in this way to neutralize toxins or viruses are referred to as neutralizing antibodies. Most toxins are active at nanomolar concentrations: a single molecule of diphtheria toxin can kill a cell. To neutralize toxins, therefore, antibodies must be able to diffuse into the tissues and bind the toxin rapidly and with high affinity. The ability of IgG antibodies to diffuse easily throughout the extracellular fluid and their high affinity for antigen once affinity maturation has taken place, make them the principal antibodies that neutralize toxins or viruses in tissues. High-affinity IgA antibodies similarly neutralize toxins or viruses at the mucosal surfaces of the body.

10.22: E.
In cases where a single IgG antibody is binding to an antigen, as is the case for an antibody that neutralizes a toxin molecule by binding to a single site on the toxin, no complement activation occurs. The binding energy required for C1q activation is achieved only when a single molecule of C1q can bind two or more IgG molecules that are held within 30–40 nm of each other as a result of binding antigen. This requires that multiple molecules of IgG be bound to a single pathogen or to an antigen in solution. For this reason, IgM is much more efficient than IgG in activating complement.

10.23: Complement deposition on immune complexes is important in the clearance from the circulation. In the absence of this mechanism, immune complexes accumulate in the circulation and get deposited in the basement membranes of small blood vessels, most notably those of the renal glomerulus, where the blood is filtered to form urine. Immune complexes then cause kidney damage.

10.24: D.
The FcαRI is expressed on neutrophils and macrophages. When triggered by IgA binding and cross-linking, this receptor promotes phagocytosis of bound antigens, leading to killing of the ingested bacteria. In addition, stimulation of FcαRI induces respiratory burst from the phagocytes, aiding in bacterial killing.

10.25: C.
Some particles are too large for a phagocyte to ingest; parasitic worms are one example. In this case the phagocyte attaches to the surface of the antibody-coated parasite via its Fcγ, Fcα, or Fcε receptors, and the contents of the secretory granules or lysosomes of the phagocyte are released by exocytosis. The contents are discharged directly onto the surface of the parasite and damage it. The principal leukocytes involved in the destruction of large parasites such as helminths are eosinophils, nonphagocytic cells that can bind antibody-coated parasites via

several different Fc receptors, including the low-affinity FcεRII.

10.26: A.
Natural killer (NK) cells can recognize and destroy antibody-coated target cells in a process called antibody-dependent cell-mediated cytotoxicity (ADCC). This is triggered when antibody bound to the surface of a cell interacts with Fc receptors on the NK cell. NK cells express the receptor FcγRIII (CD16), which recognizes the IgG1 and IgG3 subclasses. The killing mechanism is analogous to that of cytotoxic T cells, involving the release of cytoplasmic granules containing perforin and granzymes. ADCC has been shown to have a role in the defense against infection by viruses, and represents another mechanism by which antibodies can direct an antigen-specific attack by an effector cell that itself lacks specificity for antigen.

10.27: Mast cells are present in all mucosal surfaces. Depending on the route of exposure to the allergen, mast cells in different locations in the body will be triggered to degranulate. The effects of histamine release and other mast cell mediators cause different responses depending on the tissue.

10.28: E.
Genetically mast cell-deficient W/Wv mice have a defect in generating resistance against larval *Haemaphysalis longicornis* ticks. Nonetheless, their serum IgE levels increased more than 100-fold after the second tick infestation. The resistance against ticks was detectable only when both mast cells and IgE antibodies were present, indicating a critical role for this pathway in the resistance against *H. longicornis* ticks.

10.29:

a) IgM is the primary antibody isotype produced in the primary infection. It is primarily found in the blood.
b) Vaccine candidate B elicits the preferred response. The key characteristics of the preferred response are visible following the secondary immunization. These include: (1) a shorter lag time post-immunization before antibody responses are detectable, (2) an increased peak magnitude of the antibody response over the primary response, and (3) a long-lasting increase in anti-meningococcal antibody titers.
c) Vaccine candidate A is likely composed of only carbohydrate polysaccharide antigens from the *N. meningitidis* capsule. This is because the antibody response to the vaccine is virtually identical between the primary and secondary responses. The lack of improved response upon secondary immunization is a characteristic of a T cell-independent antibody response. Vaccine candidate B likely has a protein component, and therefore, is likely a conjugate vaccine. The protein in the vaccine will provide epitopes to generate CD4 T cell help for the B cells making the anti-meningococcal antibodies. This will lead to the characteristic improved response upon secondary challenge.
d) The two additional features of the secondary antibody response to each candidate vaccine that could be measured are:
    1. The relative amounts of each antibody isotype contributing to the overall anti-meningococcal antibody titer.
    2. The relative affinities of the antibodies for the *N. meningitidis* capsule.

Vaccine candidate A would show primarily IgM, with only minor components of other isotypes, such as IgG and IgA. Vaccine candidate B would show little IgM, and instead, the

predominant antibody isotypes present in the secondary response would be IgG and IgA.

Antibodies present in response to vaccine candidate A would have lower affinities for the *N. meningitidis* capsule than the antibodies present in response to vaccine candidate B.

e) Levels of anti-meningococcal IgA antibodies.

10.30:

a) There are two possible answers:
   1. Defect in B cell co-stimulation through the complement receptor on B cells that binds fragments of C3. This receptor, CD21 (encoded by the *Cr2* gene), is in a complex with two other proteins (CD19 and CD81) and when engaged, greatly enhances B cell activation to low levels of antigen. This co-stimulatory receptor is engaged when the antigen is decorated with complement fragments of C3.
   2. Defect in retention of immune complexes in the B cell follicle by follicular dendritic cells. These cells also express the complement receptor that binds fragments of C3. This receptor, also encoded by the *Cr2* gene in mice, is a variant of the receptor protein expressed on B cells and is known as CD35.

b) These results indicate the enhanced sensitivity of B cells to antigens that are decorated with complement C3 fragments. These C3 fragments are recognized by the complement receptor CD21 on B cells, and when engaged, lead to ~1000–10,000-fold decrease in the concentration of antigen needed to activate the B cell. This is the most likely answer, although it could still be argued that a defect in the complement receptor on follicular dendritic cells might give a similar outcome.

c) In this experiment, *Cr2* expression on the B cells is determined by the genotype of the bone marrow; alternatively, the *Cr2* expression on the follicular dendritic cells is determined by the genotype of the recipient since follicular dendritic cells are not derived from hematopoietic stem cells. Based on the data, it is clear that the complement receptor encoded by *Cr2* is more important on follicular dendritic cells than on B cells. This can be inferred by the improved response of Cr2-/-→wt bone marrow chimeras, where the follicular dendritic cells (genotype of the recipient) are wild-type and the B cells (genotype of the bone marrow) are Cr2-deficient. In all cases when recipients are Cr2-deficient, and thus the follicular dendritic cells are Cr2-deficient, there is little response to the inoculations.

d) For B cells, the complement receptor functions as a co-stimulatory receptor for B-cell receptor signaling. In this case, the presence of C3 fragments on the antigen greatly enhances the activation of the B cell, resulting in a striking increase in B cell sensitivity to low concentrations of antigen.

For follicular dendritic cells, the complement receptor functions to retain immune complexes in the B cell follicle. This is particularly critical for the germinal center response, which is required for the production of IgG antibodies. In the absence of the retention of immune complexes in the B cell follicle, germinal center responses are greatly impaired and poor class-switched antibody responses are observed.