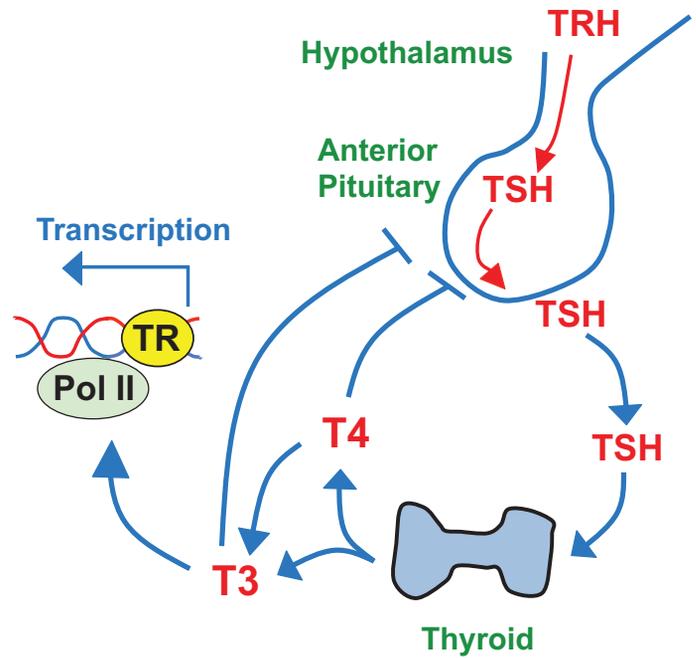


## Autoimmunity

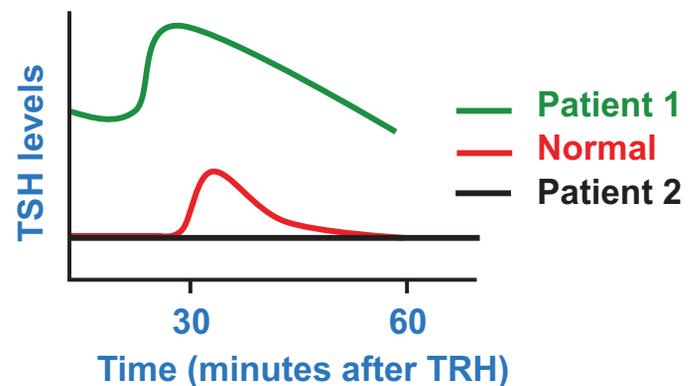
Multiple mechanisms have been posited for the development of autoimmunity: 1) The sequestration of antigens during development in places such as the eye lens and central nervous system shields these tissues and their epitopes from pre-existing auto-reactive antibodies. Upon injury or infection and consequent release of an antigen from sequestration, a self immune response is triggered; 2) “Molecular mimicry” suggests that when foreign peptides from exogenous antigens with sufficient similarities to a host peptide are recognized as self, a cross-reactive B and T cell response is observed.

Grave’s and Hashimoto’s disease are two thyroid autoimmune disorders. Normally, the pituitary-hypothalamus axis regulates secretion of the thyroid-stimulating hormone (TSH) through thyroid releasing hormone (TRH) (Fig. 1). Circulating auto-antibodies in Grave’s disease stimulate thyroid function through activating the TSH receptor. These antibodies cause increased production of the thyroid pro-hormone thyroxine (T4) and the more active hormone T3 triiodothyronine. Hashimoto’s patients possess large numbers of lymphocytes that infiltrate the thyroid and cause chronic inflammation. In some cases of Hashimoto’s, patients generate auto-antibodies against the TSH receptor that block its function.

In a diagnostic thyroid test, two patients were subjected to intravenously administered TRH. After ~30 minutes, TSH levels normally rise and then fall to baseline after one hour. However, in two patient samples, the levels of TSH as measured through an ELISA assay were markedly different, indicating thyroid dysfunction (Fig. 2).



**Figure 1.** The hypothalamus-pituitary axis regulates the production of thyroid stimulating hormone (TSH). TR = thyroid hormone nuclear receptor; Pol II = RNA polymerase II.



**Figure 2.** TRH stimulation test. TSH levels were measured in two patients and compared to a normal control sample after treatment with TRH.

1) Which of the following is the most consistent diagnosis regarding the patients who underwent TRH treatment?

- A. Patient 1 has hypothyroidism, consistent with Grave's.
- B. Patient 1 has hyperthyroidism, consistent with Hashimoto's.
- C. Patient 2 has hyperthyroidism, consistent with Grave's.
- D. Patient 2 has hypothyroidism, consistent with Hashimoto's.

2) Thyroid disorders can be caused by defects in the thyroid (primary) or in the pituitary (secondary). Which of the following diagnoses is most consistent regarding the patients who underwent TRH treatment?

- A. Patient 1 has primary hypothyroidism.
- B. Patient 2 has secondary hypothyroidism.
- C. Patient 2 has primary hyperthyroidism.
- D. Patient 1 has secondary hyperthyroidism.

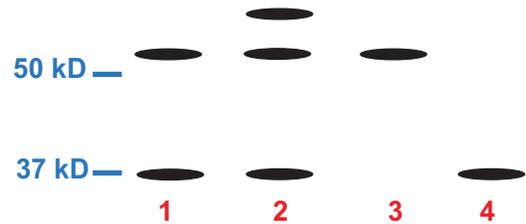
3) Multiple sclerosis can be explained by both genetic and viral-mediated mechanisms. Which of the following models of autoimmune disease development are consistent with this?

- A. The sequestration model is consistent with genetic, but not viral-mediated mechanisms.
- B. The mimicry model is consistent with viral- but not genetic-mediated mechanisms.
- C. Both mechanisms are consistent with sequestration and the mimicry models.
- D. Neither mechanism is consistent with the sequestration and mimicry models.

4) In some autoimmune diseases, multiple antibody classes recognizing the same epitopes are affected. To generate various antibody classes, the isotype IgM switches to another isotype structure that has equivalent:

- A. constant regions.
- B. variable regions.
- C. variable and constant regions.
- D. primary structure.

5) Reducing agents such as dithiothreitol (DTT) can alter antibody structure. Extensive treatment of purified IgG with DTT would generate which of the following SDS electrophoresis patterns?



- A. 1
- B. 2
- C. 3
- D. 4

6) Which of the following observations would be most consistent with sequestration-driven autoimmune disease?

- A. Detection of antibodies directed against red cell surface proteins in a patient with hemolytic anemia.
- B. Failure to negatively select autoantibodies that cause autoimmune hyperthyroidism.
- C. Development of autoimmune myocarditis in a patient with a history of cardiac ischemic attacks.
- D. Detection of anti-nuclear antibodies in the estrogen-driven development of lupus.

# Autoimmunity

## Annotations.

1) C 2) A 3) C 4) B 5) A 6) C

	Foundation 1: Biochemistry-A			Foundation 3: Other Systems-B		
	A			B		
Concepts						
Reasoning				3,6		
Research						
Data	5			1,2		

**Big Picture.** Clearly, immunology is an important subject in medicine. However, immunology is a vast and complicated topic. What should you know and at what depth should you know it for the MCAT? Just know the fundamentals, including the basic knowledge of B and T cells in adaptive (humoral) immunity, antibody structure and diversity, and autoimmune disorders. This passage focuses on autoimmunity in the thyroid, yet has questions that also probe your comprehension of antibody structure. The passage did not test an understanding of adaptive immunity and its distinction from innate immunity.

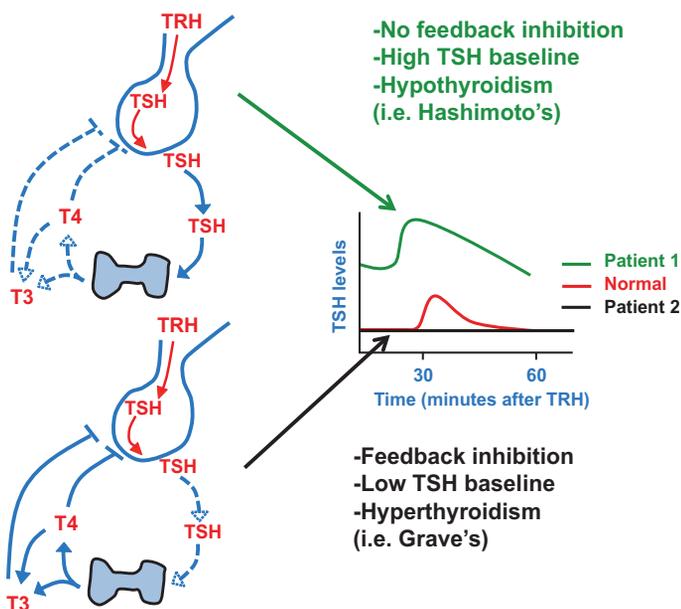


Figure 3. TSH levels in both hyper- and hypothyroidism.

1) **C. Patient 2 has hyperthyroidism, consistent with Grave's.** Deduce that Grave's disease results in hyperthyroidism as the passage states: "In Grave's, circulating auto-antibodies stimulate thyroid function through activating the TSH receptor." **This eliminates choices A and D** (hypothyroidism).

Now, how to distinguish between Grave's and Hashimoto's? In Grave's, stimulation of TSH would generate T3 and T4 as shown in Figure 3. Recall from the passage that the thyroid hormones limit their own synthesis through feedback (negative) regulation. This is also shown in the Figure 1. Increased T3 and T4 levels would suppress the release of pituitary TSH. In terms of the TRH test discussed in the passage, patients with Grave's would be expected to respond poorly to administration of TRH as TSH release is abrogated through high levels of T3 and T4 that result from Grave's. This is consistent with Patient 2. **Choice C is the correct answer.**

2) **A. Patient 1 has primary hypothyroidism.** As per above, Patient 2 has hyperthyroidism, **eliminating choice B.** Deduce that Patient 1 has hypothyroidism as would be seen in Hashimoto's. Recall from the passage that, "Hashimoto's patients possess large numbers of lymphocytes that infiltrate the thyroid and cause chronic inflammation." The resulting thyroid impairment causes hypothyroidism through lack of T3 and T4. **This eliminates choice D.**

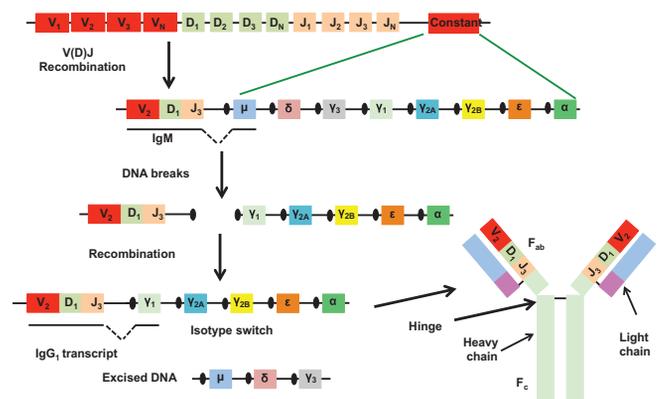
Ok, so what about the distinction between primary and secondary thyroid disease? The question stem defines the terms for you: "Thyroid disorders can be caused by defects in the thyroid (primary) or in the pituitary (secondary)." In Hashimoto's disease, the thyroid hormones (T3 and T4) are not made in the thyroid. Therefore, Hashimoto's (patient 1) is a primary thyroid disease. This **makes choice A correct.** To make sure, let's examine choice C: "Patient 2 has secondary hypothyroidism." The data re-presented in Figure 3 shows that Patient 2 fails to respond to TRH treatment, meaning that the thyroid is active and shutting down T3 and T4 hormone synthesis via feedback regulation. This, as discussed above, is hyperthyroidism. However, the pituitary is failing to secrete TSH under these circumstances, indicating that Patient 2 has a secondary thyroid condition.

3) **C. Both mechanisms are consistent with sequestration and the mimicry models.** Multiple sclerosis is an autoimmune disorder where self-directed antibodies attack the myelin sheath. By its very nature, molecular mimicry models rely upon homologous sequences as mimicry is derived from structure/function. It should readily be seen that a viral protein possessing sequence similarities to self-antigens could be mistaken as self by the immune system. In fact, differences of just a few amino acid sequences can trigger an autoimmune response in hosts. (Because of this, genetic mutations could also generate mimicry.) With respect to sequestration, anything that can break down the barriers that generate the separation of potential self-antigens from the immune system could be causal for disease. Both genetic and viral mechanisms could eliminate barriers (i.e. tissue inflammation, bleeding, etc.), causing autoimmunity from a sequestration mechanism.

4) **B. variable regions.** Isotype switching (also called class switch recombination) changes the constant region of immunoglobulin M (IgM) to one with any of several other isotypes (for example IgG, IgA, IgE). Isotype switching generates a new type of antibody effector. These two antibody classes recognize the same epitope due to possessing equivalent variable regions (Fig. 4), but have different cellular locations. **Choice B is the correct answer.**

You should understand the basic aspects of antibody diversity, including how isotypes are created. Both V(D)J and class (or isotype) switch recombination is required to generate the immunoglobulin heavy chain. In contrast, the light chain (as well as the T cell receptor) is made only via V(D)J recombination. The basics of this are shown in Figure 4. Let's go through this. First, notice that both V(D)J and isotype switching proceed through the generation of DNA breaks. Specifically, during immune cell development, DNA double stranded breaks (DSBs) are generated by RAG enzymes for V(D)J or the enzyme AID for isotype switching. By deleting various portions of DNA, there are numerous combinations of V, D, and J segments that can generate many different structures that recognize antigen. Therefore, V(D)J recombination creates immunological diversity.

You should know that the variable region recognizes epitopes and that the constant region determines the class of antibody. Further, antibodies “start out” as IgM and then can have their constant regions replaced with various effector classes, including IgM, IgA, IgG, IgD, and IgE. The class of antibody largely dictates location within the body. For example, IgA is largely found in saliva, tears, and mucosal membranes. What drives switching? In response to various cytokines, heavy chains can replace the IgM constant portion with another isotype class (Fig. 4). Therefore, IgM antibodies that switch to IgA have identical variable regions, but differential constant regions. **Choices B and C are wrong.** As the constant regions are formed exclusively from the heavy chain, the primary structures of IgM and IgA are different, **eliminating choice D.** The question stem uses the word “epitopes,” but this word is not mentioned in the passage. Epitope is a fundamental term that you should already be familiar with. If not, then what is an epitope? The variable region of an antibody recognizes its antigen by binding to an epitope. Epitopes can be sugars, lipids, nucleic acids, or proteins and peptides. In the context of proteins, which is most relevant for this passage, an epitope can be as little as five contiguous amino acids. As proteins fold in three dimensions (tertiary or 3° structure), two amino acids that are not adjacent to each other in the primary sequence can lie juxtaposed in 3D space. These two residues might contribute to a “conformational” epitope.

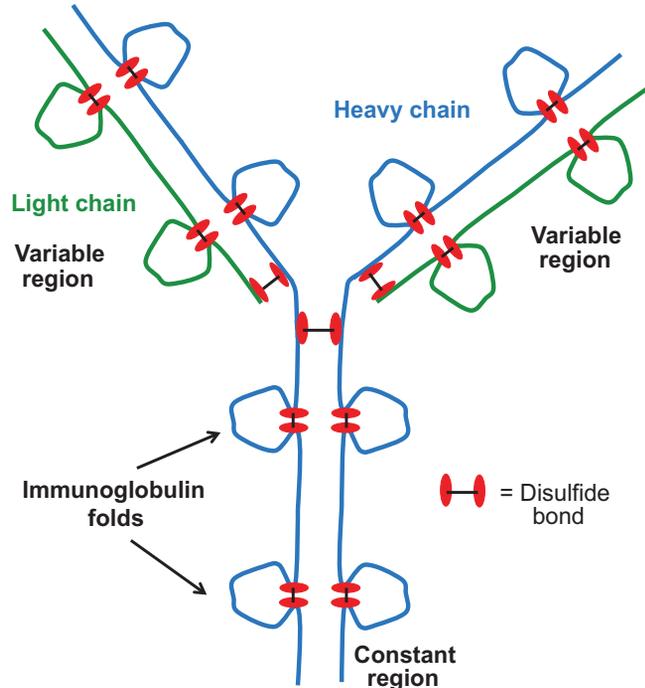


**Figure 4.** V(D)J recombination and isotype switching are two DNA repair pathways used in production of heavy chains. Fab = variable region; Fc = constant region.

## Autoimmunity

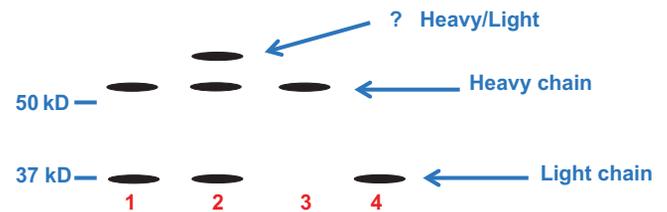
5) **A. 1.** Recall that antibodies are heterodimers composed of two heavy and two light chains linked together by disulfide bonds. (Fig. 5). Intramolecular disulfide bonds are also present and contribute to the formation of immunoglobulin folds. These structural motifs are also observed in MHC and T cell receptor molecules.

DTT treatment of an immunoglobulin would reduce the disulfide bonds in the heterodimer, releasing a heavy chain and a light chain (Fig. 6). Two different protein bands would be expected during electrophoresis. **Choice A is correct.** Extensive treatment with reducing agents such as DTT will cause the oxidized cysteine residues in the disulfide bonds to be reduced back to the thiols present in the cysteine side chain. Note that the heavy chain migrates near 50 kD and the light chain migrates near 35 kD. **Choice B is wrong** because there are three bands. Although one could argue that the high molecular weight band is derived from a heavy-light chain covalently linked structure, this would be unlikely as the stem describes “extensive treatment with DTT”. **C and D are wrong choices** as there is either a heavy or a light chain only.



**Figure 5.** Structure of heterotetrameric immunoglobulin.

6) **C. Development of autoimmune myocarditis in a patient with a history of cardiac ischemic attacks.** As described in the passage, the sequestration model predicts, “Upon injury or infection and consequent release of an antigen from sequestration, a self immune response is triggered.” **The credited answer is choice C;** it is known that damage to cardiac tissue (i.e. injury) can expose antigens such as cardiac myosin that are normally sequestered from auto-reactive antibodies. Although this could also be caused by molecular mimicry, the association between exposed cardiac tissue and injury through heart attack is consistent with a sequestration model. **Choice A is not credited** because it is very hard to imagine how circulating red blood cells could be “sequestered,” particularly as the circulatory system is linked to the lymphatic system. Autoimmune hyperthyroidism is another name for Grave’s disease. The failure to negatively select autoantibodies that are causal for Grave’s disease would result in their circulation into the periphery where they would have access to the thyroid stimulating hormone receptor. **Choice B is not credited. Choice D is not credited** because, in this case, the lupus is driven by estrogen, a hormone that is released into the bloodstream.



**Figure 6.** Gel electrophoresis analysis of immunoglobulins yields two bands on extensive treatment with DTT.