

Bio257 Immunology Practice Questions #6 key

1. Patients with the **autoimmune disease** systemic lupus erythematosus frequently have **high levels of anti-DNA antibodies** in their blood. One surprising aspect of this symptom is that although **DNA** is a reasonable **B-cell antigen**, **DNA** on its own is **not very immunogenic**.

a. **Why would DNA not be very immunogenic?** (think about what **interactions** are **required** for **B-cell activation**)

Since **B-cell activation** requires **signals (membrane protein interactions and cytokines)** from **helper T cells**, the **B cell must present T-cell peptide antigens on MHC class II molecules to interact with and activate helper T cells**. Since **DNA is not presented on MHC molecules**, it **cannot elicit T cell help and therefore would not be immunogenic, even if it could bind to B-cell receptors**.

Given this dilemma, Moens and coworkers wrote: "the immunogenicity of DNA *in vivo* may depend upon **other structures** or **processes** that may **render DNA immunogenic**." (Moens et al., 1995, p. 12393) To test this hypothesis, they constructed **plasmids** that contained the **gene encoding the large T-antigen (T-Ag) DNA-binding protein** from simian virus 40 (SV40).

Plasmid I contained the **T-Ag gene downstream** of a cytomegalovirus **promoter** (which functions in most cell types).

Plasmid II contained the **T-Ag without a promoter**.

Plasmid III contained a **promoter** upstream of a **T-Ag gene** with a **single point mutation** that changed one amino acid. This mutation **completely eliminates strong**, specific DNA binding by T-Ag, but still **allows about 60%** of **weak**, non-specific **DNA binding**.

Plasmid IV contained a **promoter** upstream of the **gene for luciferase (LUC)**, which is **NOT a DNA-binding protein**.

Each of these **plasmids** was **injected** into a set of mice and **10 weeks after injection sera** were collected from all mice. The **relative amounts** of **anti-T-Ag**, **anti-DNA**, and **anti-histone** antibodies in the sera were determined by ELISA, as shown in the table below:

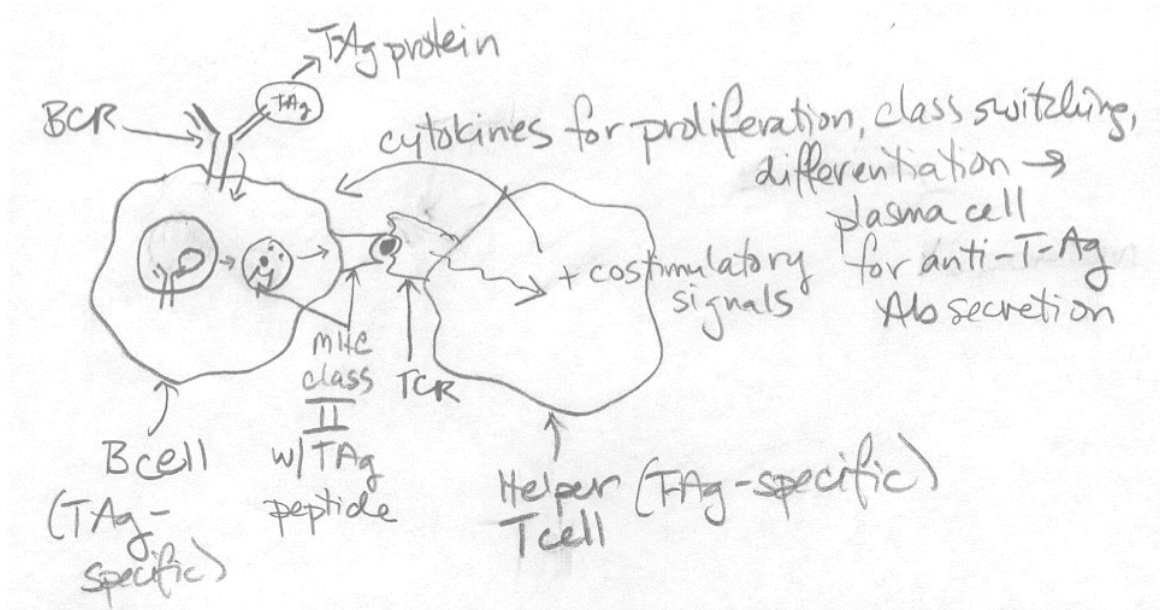
<u>DNA vaccine</u>	<u>Antibody titer</u>		
	<u>anti-T-Ag</u>	<u>anti-DNA</u>	<u>anti-histone</u>
I: T-Ag gene+promoter	573±34	360±46	259±52
II: T-Ag gene-promoter	0	0	0
III: mutant T-Ag gene+promoter	442±44	~50-100	0
IV: LUC gene+promoter	0	0	0

b. **Explain why** there are **no anti-T-Ag antibodies** if the plasmid used for vaccination does **not** contain a **promoter**.

In the absence of a **promoter**, **no T-Ag mRNA or protein would be produced even in cells that contain the plasmid**. In the absence of **antigen**, there is **nothing for T-Ag-specific B or T cells to recognize to elicit an antibody response**.

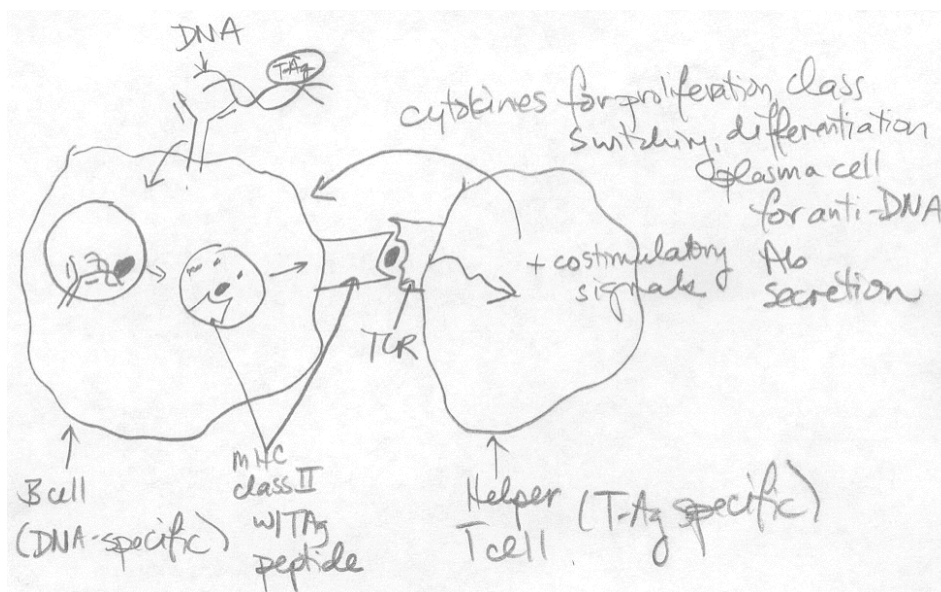
c. Draw the molecular and cellular interactions that lead to the production of anti-T-Ag antibodies in I.

Costimulatory signals (which I didn't have room to draw) include B7 on B cells interacting with CD28 on T cells and CD40 on B cells interacting with CD40 ligand on T cells.



d. Describe a model to explain why the expression of T-Ag leads to the production of anti-DNA antibodies. Use a picture of key molecular and cellular interactions in your model.

The binding of DNA to a DNA-specific B-cell receptor allows the internalization not only of DNA, but also of the T-Ag protein bound to the DNA. This associated T-Ag can then be processed in the B cell and T-Ag peptides presented on MHC class II molecules for T-Ag-specific helper T cells to recognize (which may have already proliferated due to the activation shown in (c)). This recognition, combined with the costimulatory signals described in (c), allows activation of the DNA-specific B cell.



e. Describe a model to explain why the expression of T-Ag leads to the production of **anti-histone antibodies**. Use a picture of **key molecular and cellular interactions** in your model.

The ability of T-Ag expression to elicit anti-histone antibody relies on intermolecular interactions similar to those shown in (d). The difference is that a histone-specific B-cell receptor binds to a histone that is only indirectly associated with T-Ag, since both are bound to DNA. The T-Ag can therefore still be internalized and peptides presented to activate T-Ag-specific T cells, which can then activate the histone-specific B cell as described in (d). Here's a picture of what's happening at the surface of the histone-specific B cell (the rest is directly parallel to the picture in (d)).



f. Given your model in (d), explain why expression of the **mutant T-Ag** leads to **lower levels** of **anti-DNA antibodies**.

Since mutant T-Ag does not bind DNA as well, less T-Ag is internalized with the DNA and fewer T-Ag peptides can be presented to helper T cells. Thus activation of B cells to produce anti-DNA antibodies is reduced due to lower levels of signals from T-Ag-specific helper T cells.

g. Given your models in (d and e), explain why they **could detect anti-DNA antibodies** but could **not detect anti-histone antibodies** after expression of the **mutant T-Ag**.

As described in (e), histones are only indirectly associated with T-Ag, so even less T-Ag will be internalized, eliciting even less T-cell help. (it is also possible that negative selection may be more effective in eliminating anti-histone B cells than anti-DNA B cells, but that's just speculation).

h. Why did they use **plasmid IV**?

Since plasmid IV expresses luciferase, which doesn't bind to DNA, it acts as a negative control to demonstrate that it isn't expression of any protein from the plasmid that can elicit an anti-DNA antibody response.

2. **Rheumatoid arthritis**, another autoimmune disease, is frequently diagnosed by **testing for the presence of rheumatoid factor** using the **Waller-Rose hemagglutination assay**. This assay requires an **antiserum** produced by **inoculating rabbits with sheep red blood cells (RBC)**. The **steps of the assay** are as follows:

1. **Sheep red blood cells** are "sensitized" by **incubation with rabbit anti-sheep RBC antibodies**.
2. The **sensitized RBC** are then **mixed with human serum** from people suspected of having rheumatoid arthritis.
3. If **rheumatoid factor is present**, the **sheep RBC agglutinate** (clump) and the test is **positive**.

a. **Explain why rheumatoid factor would cause agglutination of RBC in this assay. Describe the molecules involved.**

In rheumatoid arthritis, antibodies (also referred to as "rheumatoid factor") can be produced that recognize the Fc region of human antibodies. Since the RBC have bound to the variable regions of the rabbit antibodies, the Fc region of the rabbit antibodies are exposed on the surface of the RBC. The binding of one variable region of a "rheumatoid factor" antibody to a rabbit Fc on one RBC and the binding of the other variable region to a rabbit Fc on another RBC allows cross-linking of these cells and therefore a huge network can form (agglutination).

Moens *et al.* used this assay to test for the presence of rheumatoid factor in their mouse sera (see question 1). In the mice injected with plasmid I, the Waler-Rose assay results were negative (other sera were not tested).

b. **What does this result indicate? Does this result make sense? Why or why not?**

This result indicates that the expression of T-Ag does not lead to the activation of Fc-specific B cells, which makes sense. Since the mechanism of T-Ag inducing self-antibodies involves its binding to DNA, we have no reason to expect that it would be internalized and presented by B cells that bind to Fc portions of antibodies (i.e. we have no reason to suspect that T-Ag would bind non-specifically to antibodies).

3. The immunology class has decided to start producing vaccines to protect people against *Staphylococcus aureus* infection.

a. **Name two groups of people who would benefit from a *Staphylococcus* vaccine. Explain:**
i. **why each group would benefit from the vaccine more than the general population.**
ii. **when you would recommend administering the vaccine to each group.**

Football/wrestling teams, nursing home residents, prisoners, homeless shelter residents, military
Outbreaks of community-acquired methicillin-resistant *Staphylococcus aureus* have been detected in each of these groups. Therefore vaccines to prevent infection in these groups could help prevent disease in group members as well as preventing spread of this pathogen both within the group (note that all groups can involve people living/working/playing in close contact with one another) and to individuals outside the group. Vaccines should be administered before the pathogen is detected in the population.

The class divides itself into three groups and each group takes a different approach in designing its vaccine.

Group I grows up gallons of *Staphylococcus* culture, strips the polysaccharide capsule off the bacteria and uses the polysaccharide-containing supernatant as an immunogen.

Group II also grows up gallons of *Staphylococcus* culture and strips the polysaccharide capsule off the bacteria. Next, however, they mix the polysaccharide with an exotoxin from another bacterium and treat with a chemical crosslinker. They purify the linked polysaccharide-exotoxin and use it as an immunogen

Group III grows up gallons of *Staphylococcus* culture, collects the cells by centrifugation, lyses the cells and purifies the four major soluble proteins from the lysate. They use this protein mixture as an immunogen.

b. Which group's vaccine do you think will be the most effective for prevention of *Staphylococcus* infection? Explain why you chose this vaccine.

Group II's vaccine should be the most effective. Although polysaccharides are found on the surface of *S. aureus*, they are not good T-cell antigens. By linking polysaccharides to an exotoxin, B cells that can bind to the polysaccharide can present peptides from the exotoxin (a protein) to helper T cells and to allow T-cell-dependent activation of polysaccharide-specific B cells and class switching, rather than relying on weaker T-independent B-cell activation. The greater B-cell activation means that upon later exposure there will be more polysaccharide-binding antibody in the body to opsonize the bacteria for phagocytosis/other Ab-dependent cell-mediated cytotoxicity or to target the bacteria for destruction by complement.

c. Of the other two vaccine options, which group do you think will have better success at eliciting an anti-*Staphylococcus* immune response with their antigen? Why?

Group I's vaccine should work better than Group III's vaccine. T-cell independent activation of polysaccharide-specific B cells should allow the production of some anti-polysaccharide antibodies that can bind to bacteria and allow ADCC or complement fixation to rid the body of the bacteria as described above. Although B cells that recognize the four proteins in Group III's vaccine should be able to be activated through a T-cell-dependent mechanism, the antibodies produced would be relatively useless because no proteins are exposed on the surface of the bacterium, given its polysaccharide capsule.

d. Another classmate wants to design a DNA vaccine to prevent *Staphylococcus* infections. Explain why you think this option will or will not work to create an effective vaccine.

A DNA vaccine is not a great option to prevent *S. aureus* infection. A DNA vaccine works by host cells taking up the DNA that encodes a protein antigen of interest and then expressing that antigen, frequently for presentation to Tc cells. Since *S. aureus* is an extracellular bacterium, a cytotoxic T-cell response will not be helpful. In addition, even if the protein antigen were to escape cells to elicit a B/helper T cell response, it is the polysaccharide coat that needs to be targeted for effective ADCC and expression of bacterial genes would likely not be able to cause the assembly of a polysaccharide capsid on mammalian cells.