

Bio257 Immunology Practice Questions #6

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**)

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:

DNA vaccine	Antibody titer		
	anti-T-Ag	anti-DNA	anti-histone
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**.

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

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.

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.

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

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**.

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

2. **Rheumatoid arthritis**, another autoimmune disease, is frequently diagnosed by **testing for the presence of rheumatoid factor** using the **Waler-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.

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?**

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.

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.

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?**

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**.

From the textbook:

Ch. 16: 2

Ch. 18: 2, 3*, 4, 8*