Problem Set #2 **Molecular Immunology** **Spring 2006**

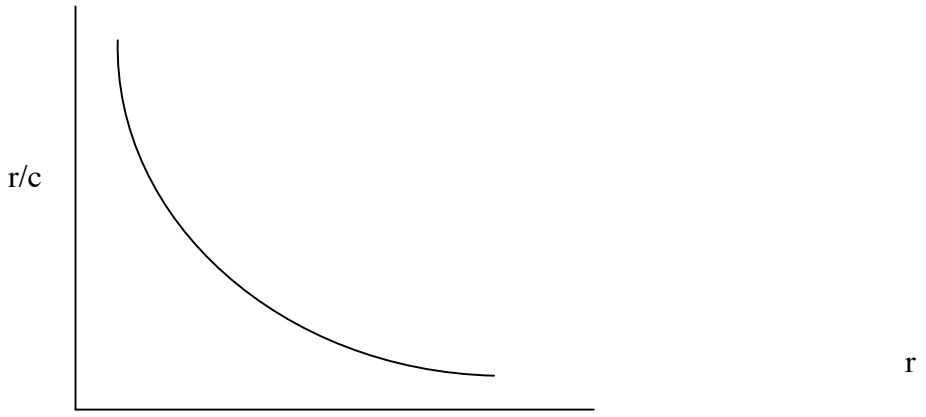
1) What is the difference between an antigen and an immunogen?

An antigen is any substance which can be recognized by an antibody (or a T cell). An immunogen is an antigen which can activate an immune response. Some antigens (i.e. haptens) cannot activate an immune response on their own.

2) What factors effect the immunogenicity of a protein?

Size, foreigness, complexity, route of administration, induction of inflammation

3) The ideal Scatchard plot of binding data involving an antigen-antibody interaction is a straight line. In practice, however, when one performs a Scatchard analysis of binding using a pure antigen and a crude antiserum, the plot is a curve (below). Why is this? Are Scatchard plots useful for following changes in affinity during an immune response. How?



the line is curved since it represents the sum of many straight lines of different slope. This is because a crude antiserum contains many different antibodies against the target antigen, each with a distinct affinity.

4) You are studying the behavior of a newly discovered protein (called Factor M). As part of your research project, you purify the protein from a cultured mouse cell line and inject some into a rabbit, a mouse, and a rat in order to generate an antiserum. Several weeks later, you take blood samples from each animal and purify serum to test for antigen reactivity.

a) Describe how you would use the purified protein to set up an ELISA to screen for anti-Factor M antibody in each serum sample? What controls would you need to perform to assure the validity of your assay?

Many ways to do this. One example: fix antigen to plastic well in multiwell plate. Add test serum. Wash. Add enzyme-labeled anti-Ig antibody. Wash. Develop color assay. Controls include using pre-immune serum, irrelevant protein, soluble antigen as inhibitor.

b) You find that the rabbit serum reacts strongly in your assay, but the mouse and rat sera do not. Is this surprising? Why or why not?

This is not surprising since the antigen came from mouse cells, and rats are closely related to mice.

c) You find out that another scientist is also studying Factor M and has made a monoclonal antibody specific for the protein. You send her a sample of your rabbit anti-serum and she sends you some of her monoclonal antibody. You perform a Western blot analysis using either your serum or your colleague's monoclonal and find that the monoclonal antibody reacts strongly with Factor M but your rabbit antiserum does not give a signal. Why might this be?

Antiserum was raised against native protein, whereas western blot is against denatured protein. Monoclonal may have been raised against denatured antigen.

d) Which reagent would you expect to work better for immunoprecipitation of Factor M from cell extracts? Why?

Your polyclonal serum would likely work better since it contains many different antibodies, some of which would be directed against surface of native protein. Monoclonal may or may not recognize surface determinant on native protein.

5e) How would you use either of these reagents to determine the sub-cellular localization of Factor M? Which reagent (the antiserum or the monoclonal) would you expect to work more reliably in such an assay? What controls would be required to assure the validity of your results?

I would use immunofluoresence. The antiserum would probably work best. Need pre-immune serum control.

f) Describe one way in which you could use your antiserum in an attempt to clone the gene encoding Factor M.

Make an cDNA expression library using RNA purified from mouse cell line. Screen the library with the antiserum.

6) The spleen consists of a mixture of B cells, T cells and non-lymphoid cells. Given antibodies specific for CD19 (a B cell-specific transmembrane protein) and CD3 (a T cell specific transmembrane protein complex), how would you go about purifying B and T cells from spleen?

Several approaches possible. These include using flow cytometry on fluorescent antibody-stained spleen. FACS can sort anti-CD19 stained cells vs. anti-CD3 stained cells. Same thing can be done with biotinylated antibodies and streptavidin magnetic beads.