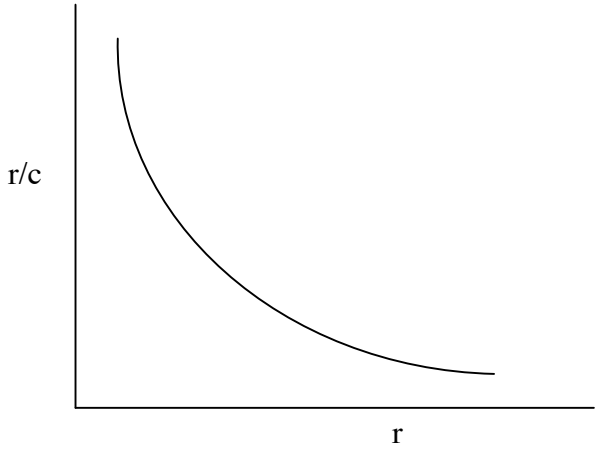
**Problem Set #2** **Molecular Immunology** **Fall 2006**

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

2) What factors effect the immunogenicity of a protein?

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?



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?

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?

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 rabbit polyclonal antiserum reacts strongly with Factor M but the monoclonal antibody does not give a signal. Why might this be?

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

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?

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

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?