

Sobel (15 points) Please keep your answers short.

1. Mice were immunized subcutaneously with (4-hydroxy-3-nitrophenyl)acetyl (NP) conjugated to chicken IgG (NP-CGG). The NP-CGG was mixed with alum (fulfilling a role similar to complete Freund's adjuvant). With regard to the antibody response to NP:

- a. What is the role of CGG in producing an antibody response? (1 pt)
 Chicken IgG (CGG) are foreign proteins and act as the carrier in a hapten-carrier system.
- b. What is the role of NP in producing an antibody response? (1 pt)
 NP is not a protein, but a small molecule that could not by itself produce a T cell dependent, class-switched antibody response. It acts as the hapten for the B cell (Ig receptor).
- c. How does adjuvant work? (1 pt)
 Alum is an adjuvant. It prolongs the presence of the antigen and provides stimulation to antigen presenting cells.
- d. Would you expect an antibody response to CGG? Why or why not? (2 pts)
 Yes, CGG is a foreign protein and can therefore have both T and B cell epitopes.

Using the protocol described above, mice were divided into six groups, two groups receiving a blocking antibody to CD40L, two groups receiving a blocking antibody to CD86 (B7.2), and two groups receiving an irrelevant control antibody. Antibody treatments were started either at the time of immunization (Day 0) or six days later (Day 6). There was no difference in outcome for the control group based on when antibody treatment was started, and the data were combined. The germinal center size is relative to the control group, which is set to 100%. Data are shown in **Table 1**.

Table 1.

Groups	Day Antibody Treatment Started	IgG Anti-NP (µg/ml)	Germinal Center Size
Control	Day 0 or Day 6	880	100%
Anti-CD40L	Day 0	115	<5%
	Day 6	878	<5%
Anti-CD86	Day 0	460	<5%
	Day 6	850	30%

- e. Based on your understanding of immunology, explain why did antibody to CD40L decrease titers of IgG anti-NP (2 pts)
 Antibody to CD40L interrupted interactions between CD40 and CD40L, the major second signal for B cells, and specifically inhibited class-switching.
- f. Based on your understanding of immunology, explain why did antibody to CD86 decrease titers of IgG anti-NP (2 pts)
 Antibody to CD86 interrupted the interactions between CD86 and CD28, the major second signal for T cells and therefore interrupted full activation of T cells for stimulation of B cells.
- g. Why do you think anti-CD40L had a greater effect than anti-CD86? (1 pt)

CD80 (or B7.1) would not have been affected by anti-CD86. There is no equivalent alternative to CD40L.

2. In **Figure 1** below, mice were given 80 million infective units of Adenovirus Type 5 virus via tail vein injection. All mice were on the B6 background, and three types of mice were used: WT (wild type), gld (Fas ligand deficient), and prf (perforin deficient). Eight days later, mice were injected intravenously with **adenovirus-infected, labeled** spleen cells from the same strain of mice. Six hours later, the mice were killed, and the percent lysis was calculated in the spleen and liver relative to an infusion of the same number of uninfected spleen cells.

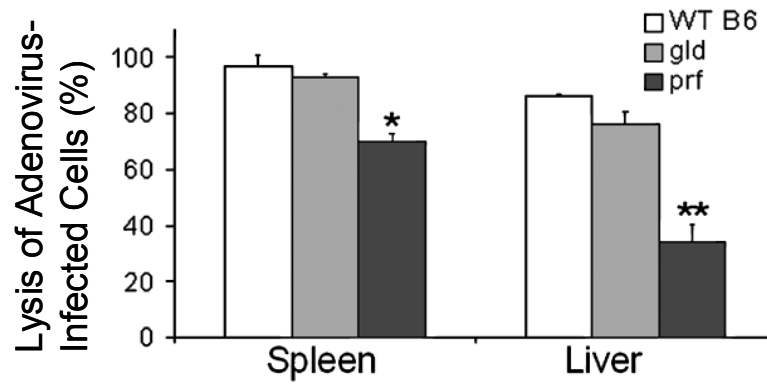


Figure 1

- a. Are the results consistent with your understanding of the role of perforin and Fas ligand in mediating cytotoxicity in viral infections? Why or why not? (2 points)
Yes, they are. Perforin can punch holes in the cell membrane and is required for optimal effectiveness of cytotoxic granules. Fas ligand tends to play a secondary role.
- b. Elimination of perforin affects the function of what other component of cytotoxic granules? (1 point)
Granzymes.
3. In Figure 2, CD4+ T cells from mice were incubated in vitro with purified macrophages from pigs. Additionally, the cells either received no other treatment (untreated), and irrelevant control antibody (Control Ab), or either an antibody neutralizing IFN-gamma (Anti-IFN-gamma) or IL-4 (anti-IL-4). Following incubation, the T cells were tested for the percent of cells expressing either IFN-gamma or TNF-alpha.

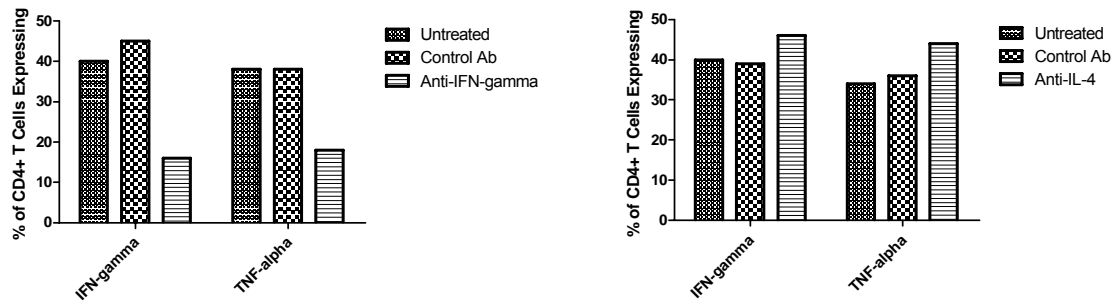


Figure 2

- a. How did the two treatments affect T cell activation? (1 point)

To fully activate macrophages requires a Th1 response by T cells. IFN-gamma is one of the prototypical cytokines and blocked that activation, not allowing T cells to polarize to a Th1 response. Anti-IL-4 treatment would likely polarize T cells away from a Th2 response and towards a Th1 response.

- b. Which treatment group (anti-IFN-gamma or anti-IL-4) should result in macrophages likely to be effective in a mycobacterial infection?

The anti-IL-4 group, with its ability to polarize T cells to a Th1 response, should develop fully functional activated macrophages.