Gene Therapy: The Ex Vivo Approach
(Textbook Figure 15.23)

Introduction
In the ex vivo approach to gene therapy, the somatic cells of an individual are modified outside the body in a laboratory setting and then transplanted back again. During this procedure, a copy of the normal allele of the defective gene is inserted into a viral vector, which is used to infect cultured somatic cells. The virus enters the cells and inserts the gene of interest into the somatic cells’ chromosomal DNA. Following infection, the somatic cells containing the normal allele are cultured and then injected back into the patient. If the procedure is successful, the normal allele is expressed, and symptoms of the disease are alleviated by expression of the normal allele. This approach has been used with some measure of success to treat several genetic disorders and cancers. Importantly, gene therapy procedures are not without risk. While viral vectors remain the preferred method of gene delivery, they can pose a variety of problems to the patient. In particular, cell toxicity, immune and inflammatory responses, as well as gene control and targeting issues have all surfaced during gene therapy trials. More recent studies, however, have identified potential alternative non-viral methods for use in gene therapy. Examples include RNA silencing techniques to deactivate faulty genes and lipid-based systems for gene delivery. The success of such methods remains to be seen.

Original Paper
Links
(For additional links on this topic, refer to the Chapter 15 Experiment Links.)

The Human Genome Project: Gene Therapy

Access Excellence: Gene Therapy—An Overview

Medical News Today: Discovery In “Bubble Boy” Disease Gene Therapy

Institute of Science in Society: Predicted Hazard of Gene Therapy a Reality
http://www.i-sis.org.uk/PHGT.php

University of Stanford: New gene therapy technique sharply reduces risks

Analyze the Data

Patient 1 was a 4-year-old girl. She had been receiving injections of the enzyme adenosine deaminase (ADA), which she does not produce due to a genetic mutation. Patient 2 was a 9-year-old girl who had also received ADA injections. Because both were developing immune deficiency due to a lack of functional T cells (an immune system cell; see Chapter 18 of the textbook), they were treated using gene therapy. As shown in Figure 15.23 of the textbook, T cells were removed from the patients, the gene for normal ADA inserted into the T cells in the laboratory via a retroviral vector, the cells expanded for a week, and then re-infused into the patients on day 0. T-cell counts and ADA levels were measured; the results are shown in Figure 1.

Figure 1
**Question 1**  
Examine the T-cell levels in Figure 1. Did the addition of genetically engineered T cells improve overall T-cell levels over time (that is, did the T cells “take and grow in the patients”)?

**Question 2**  
What was the effect of the addition of gene therapy on ADA levels?

**Figure 2**

**Question 3**  
PCR was used to detect the presence of gene therapy-derived T cells. The PCR primers recognized the vector used. Figure 2 shows the results of PCR on blood samples taken after gene therapy began; D stands for days after gene therapy began. What do the results indicate about the persistence of the gene therapy-derived cells in the patient?
An important immunologic response to the presence of T cells is their response to certain molecules (antigens). In some cases, specific T cells respond to the presence of antigen by proliferating. When T cells from a patient treated with gene therapy were challenged with antigen, the results were as shown in Figure 3. (The dotted line is the T-cell count in the patient.) By what percentage did the T-cell response improve in the patient after gene therapy?