Appendix III CLINICAL CASES

This appendix presents five clinical cases illustrating various diseases involving the immune system. These cases are not meant to teach clinical skills but rather show how the basic science of immunology contributes to our understanding of human diseases. Each case illustrates typical ways in which a disease manifests, what tests are used in diagnosis, and common modes of treatment. The appendix was compiled with the assistance of Dr. Richard Mitchell, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts, and Dr. James Faix, Department of Pathology, Stanford University School of Medicine, Palo Alto, California.

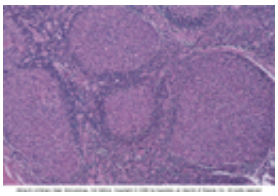
E.B. was a 38-year-old chemical engineer who had been well all of his life. One morning, he noticed a lump in his left groin while showering. It was not tender, and the overlying skin appeared normal. After a few weeks, he began to worry about it because it did not "go away," and he finally made an appointment with a doctor after 2 months. On physical examination, the physician noted a subcutaneous firm, movable nodule, about 3 cm in diameter, in the left inguinal region. The doctor asked E.B. if he had recently noticed any infections of his left foot or leg (which E.B. hadn't). The doctor also found some slightly enlarged lymph nodes in E.B.'s right neck. Otherwise, the physical examination findings were normal. The doctor explained that the nodule probably was a lymph node that was enlarged as a result of a reaction to some infection. However, he advised E.B. to see a surgeon, who would remove the lymph node so that a pathologist could examine it to be sure that it was not malignant.
The lymph node was removed, and histologic examination revealed an expansion of the node by follicular structures composed of monotonous collections of enlarged, activated ("lymphoblastoid") cells (Fig. A-1). Immunohistochemical studies revealed that these cells expressed B cell surface molecules. Also, polymerase chain reaction (PCR) analysis of DNA from the lymph node showed a clonal rearrangement of the immunoglobulin (Ig) heavy chain gene. On this basis, the diagnosis of follicular lymphoma was made.

Why does the presence of a clonal rearrangement of Ig heavy chain genes in the lymph node indicate a neoplasm rather than a response to an infection?
E.B.'s lymphoma was treated with chemotherapy. The lymphadenopathy in his neck (which was due to his lymphoma) regressed, but unfortunately, a new enlarged lymph node appeared in his left cervical area about a year later. This lymph node was removed, and it showed follicular lymphoma, with the same histologic features as those of the original.

If an anti-idiotypic antibody was developed against the surface Ig present on E.B.'s original lymphoma cells, it might not recognize the cells responsible for his recurrence. Why not?
The oncologist caring for E.B. is now planning to administer chemotherapy and radiation therapy to kill all the tumor cells, followed by bone marrow transplantation.

Why would it be necessary to perform the bone marrow transplantation, and what will be the status of the patient's immune system after the recommended treatment?

Lymph node biopsy with follicular lymphoma. The microscopic appearance of the patient's inguinal lymph node is shown. The follicular structures are abnormal, composed of a monotonous collection of neoplastic cells. By contrast, a lymph node with reactive hyperplasia would have follicles with germinal center formation, containing a heterogeneous mixture of cells.

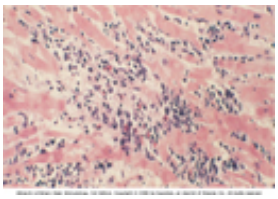


C.M., a computer software salesman, was 48 years old when he came to his primary care physician because of fatigue and shortness of breath. He had not seen a doctor on a regular basis before this visit and felt well up until 1 year ago, when he began experiencing difficulty climbing stairs or playing basketball with his children. Over the past 6 months he had had trouble breathing when he lay down in bed. He did not remember ever experiencing significant chest pain and had no family history of heart disease. He did recall that about 18 months ago he had to take 2 days off from work because of a severe flulike illness.
On examination, he had a pulse of 105 beats per minute, a respiratory rate of 32 breaths per minute, and a blood pressure of 100/60 mm Hg and was afebrile. His doctor heard rales (evidence of abnormal fluid accumulation) in the bases of both lungs. His feet and ankles were swollen. A chest x-ray film showed pulmonary edema and pleural effusions and a significantly enlarged left ventricle. C.M. was admitted to the cardiology service of the University Hospital. On the basis of further tests, including coronary angiography and echocardiography, a diagnosis of dilated

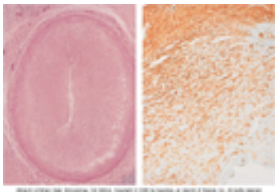
cardiomyopathy was made. The doctors explained to the patient that his heart muscle had been damaged. The cause may have been an episode of inflammation as a complication of a viral infection some time ago, but they could not be sure. The only lifesaving treatment for his condition would be to receive a heart transplant.
A panel-reactive antibody (PRA) test was performed on C.M.'s serum to determine whether he had been previously sensitized to alloantigens. This test showed the patient had no circulating antibodies against human leukocyte antigens (HLA), and no further immunologic testing was performed. Two weeks later in a nearby city, a donor heart was removed from a victim of a construction-site accident. The donor had the same ABO blood group type as C.M.'s. The transplant surgery, performed 4 hours after the donor heart was removed, went well, and the allograft was functioning properly postoperatively.

What problems might arise if the transplant recipient and the donor have different blood types, or if the recipient has high levels of anti-HLA antibodies?
C.M. was placed on immunosuppressive therapy the day after transplantation, which included daily doses of cyclosporine, mycophenolic acid, and prednisone. Endomyocardial biopsy was performed 1 week after surgery and showed no evidence of myocardial injury or inflammatory cells. He was sent home 10 days after surgery, and within a month he was able to do light exercise without problems. On routinely scheduled endomyocardial biopsy performed within the first 3 months after transplantation, findings were normal, but a biopsy performed 14 weeks after surgery showed the presence of numerous lymphocytes within the myocardium and a few apoptotic muscle fibers (Fig. A-2). The findings were interpreted as evidence of acute allograft rejection.

Endomyocardial biopsy showing acute cellular rejection. The heart muscle is infiltrated by lymphocytes, and necrotic muscle fibers are present. (Courtesy of Dr. Richard Mitchell, Department of Pathology, Brigham and Women's Hospital, Boston.)



Coronary artery with transplant-associated arteriosclerosis. This histologic section was taken from a coronary artery of a cardiac allograft that was removed from a patient 5 years after transplantation because of graft failure. The lumen is markedly narrowed by the presence of intimal smooth muscle cells. (Courtesy of Dr. Richard Mitchell, Department of Pathology, Brigham and Women's Hospital, Boston.)



What was the patient's immune system responding to, and what were the effector mechanisms in the acute rejection episode?
C.M.'s serum creatinine level, an indicator of renal function, was high (2.2 mg/dL; normal, less than 1.5 mg/dL). His doctors therefore did not want to increase his cyclosporine dose because this drug can be toxic to the kidneys. He was given three additional doses of a steroid drug over 18 hours, and a repeat endomyocardial biopsy 1 week later showed only a few scattered macrophages and a small focus of healing tissue. C.M. went home feeling well, and he was able to live a relatively normal life, taking cyclosporine, mycophenolic acid, and prednisone daily.

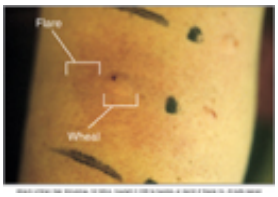
What is the goal of the immunosuppressive drug therapy?
Coronary angiograms performed yearly since the transplant showed a gradual narrowing of the lumens of the coronary arteries. In the sixth year after transplantation, C.M. began experiencing some shortness of breath after mild exercise and showed some left ventricular dilatation on radiographic examination. An intravascular ultrasound examination demonstrated significant thickening of the walls and narrowing of the lumen of the coronary arteries (Fig. A-3). An endomyocardial biopsy showed areas of ischemic necrosis. C.M. and his physicians are now considering the possibility of a second cardiac transplant.

What process has led to failure of the graft after 6 years?

I.E. was a 10-year-old girl who was brought to her pediatrician's office in November because of frequent coughing for the past 2 days, wheezing, and a feeling of tightness in her chest. Her symptoms had been especially severe at night. In addition to her routine checkups, she had visited the doctor in the past for occasional ear and upper respiratory tract infections but had not previously experienced wheezing or chest tightness. She had eczema, but otherwise, she was in good health and was developmentally normal. Her immunizations were up to date. She lived at home with her mother, father, and two sisters, ages 12 and 4,

and a pet cat. Both of her parents smoked cigarettes, her father suffered from hay fever, and her older sister had a history of sinus infections.
At the time of her examination, I.E. had a temperature of 37° C (98.6° F), blood pressure of 105/65 mm Hg, and a respiratory rate of 28 breaths per minute. She did not appear short of breath. There were no signs of ear infection or pharyngitis. Auscultation of the chest revealed diffuse wheezing in both lungs without signs of congestive heart failure (rales). There was no evidence of pneumonia. The doctor made a presumptive diagnosis of bronchospasm and referred I.E. to a pediatric allergist-immunologist who was associated with his physicians' group. In the meantime, the patient was given a prescription for a short-acting β_2-adrenergic agonist bronchodilator inhaler, and the child was instructed to administer the drug every 6 hours to relieve symptoms. This drug binds to β_2-adrenergic receptors on bronchial smooth muscle cells and causes them to relax, resulting in dilatation of the bronchioles.

A positive result on skin testing for environmental antigens. Small doses of the antigens are injected intradermally. If mast cells are present with bound IgE specific for the test antigen, the antigen will cross-link the Fc receptors to which the IgE is bound. This induces degranulation of the mast cells and the release of mediators that cause the wheal-and-flare reaction.



Asthma is an example of *atopy*. What are the different ways in which atopy may manifest clinically?
One week later, I.E. was seen by the allergist. He auscultated her lungs and confirmed the presence of wheezing. I.E. was instructed to blow into a flowmeter, and the doctor determined that her peak expiratory flow rate was 65% of normal, indicating airway obstruction. The doctor then administered a nebulized bronchodilator, and 10 minutes later performed the test again. The repeat flow rate was 85% of normal, indicating reversibility of the airway obstruction. Blood was drawn and sent for total and differential blood cell count and determination of IgE levels. In addition, a skin test was performed to determine hypersensitivity to various antigens and showed a positive result for cat dander and house dust (Fig. A-4). The patient was instructed to begin using an inhaled corticosteroid and to use her bronchodilator only as needed for respiratory symptoms. Her parents were instructed to make a return appointment 2 weeks later for reevaluation of I.E. and discussion of blood test results.

What is the immunologic basis for a "positive" skin test?
At I.E.'s return appointment 2 weeks later, laboratory tests revealed that she had a serum IgE level of 1200 IU/mL (normal range, 0-180) and a total white blood cell count of 7000/mm^3 with 3% eosinophils (normal, less than 0.5%). When she returned to the allergist's office another week later, her respiratory status on physical examination was significantly improved, with no audible wheezing. I.E.'s peak expiratory airflow had improved to 90% of predicted. The family was told that I.E. had reversible airway obstruction, possibly triggered by a viral illness and possibly related to cat and dust allergies. The doctor advised that the cat should either be given to a friend or at least kept out of I.E.'s bedroom. The mother was told that smoking in the house probably was contributing to I.E.'s symptoms. The doctor recommended that I.E. continue to use the short-acting inhaler for acute episodes of wheezing or shortness of breath. I.E. was asked to return in 3 months, and sooner if she used the inhaler more than twice per month.

What is the mechanism for the increased IgE levels seen in patients who suffer from allergic symptoms?
The family cat was given to a neighbor, and I.E. did well on the therapy for about 6 months, experiencing only mild wheezing a few times. The next spring, she began to have more frequent episodes of coughing and wheezing. During a soccer game one Saturday, she became very short of breath, and her parents brought her to the emergency department of the local hospital. After confirming that she was experiencing marked upper airway constriction, the emergency department physician treated her with a nebulized β_2-agonist bronchodilator and an oral corticosteroid. After 6 hours, her symptoms resolved, and she was sent home. I.E. was brought to her allergist the next week, who changed her maintenance medication to a different inhaled corticosteroid. She has subsequently been well, with occasional mild "attacks" that are cleared by the bronchodilator inhaler.

What are the therapeutic approaches to allergic asthma?

N.Z. was a 25-year-old unmarried woman who presented to her primary care physician with complaints of joint pain involving her wrists, fingers, and ankles. When seen in the physician's office, N.Z. had normal body temperature, heart rate, blood pressure, and respiratory rate. There was a noticeable red rash on her cheeks, most marked around her nose, and on questioning she said the redness got worse after being out in the sun for 1 or 2 hours. The joints of her fingers and her wrists were swollen and tender. The remainder of the findings on the physical examination were unremarkable.
Her doctor took a blood sample for various tests. Her hematocrit was 35% (normal, 37% to 48%). The total white blood cell count was 9800/mm^3 (within normal range) with a normal differential count. The erythrocyte sedimentation rate was 40 mm per hour (normal, 1-20). Her serum antinuclear antibody (ANA) test was positive at 1 : 256 dilution (normally, negative at 1 : 8 dilution). Other laboratory findings were unremarkable.

On the basis of these findings, a diagnosis of systemic lupus erythematosus (SLE) was made. N.Z.'s physician prescribed oral prednisone, a corticosteroid, and with this treatment, her joint pain subsided.

What is the significance of the positive result for the ANA test?
Three months later, N.Z. began feeling unusually tired and thought that she had the "flu." For about a week she had noticed that her ankles were swollen, and she had difficulty putting on her shoes. She returned to her primary care physician. Her ankles and feet showed severe edema (swollen as a result of extra fluid in the tissue). Her abdomen appeared slightly distended, with a mild shifting dullness to percussion (a sign of an abnormally high amount of fluid in the peritoneal cavity). Her physician ordered several laboratory tests. Her ANA test result was still positive, with a titer of 1 : 256, and her erythrocyte sedimentation rate was 120 mm per hour. Serum albumin was 0.8 g/dL (normal, 3.5-5.0). Measurement of serum complement proteins revealed a C3 of 42 mg/dL (normal, 80-180) and a C4 of 5 mg/dL (normal, 15-45). Urinalysis showed 4+ proteinuria, both red and white blood cells, and numerous hyaline and granular casts. A 24-hour urine sample contained 4 g of protein.

What is the likely reason for the decreased complement levels and the abnormalities in blood and urinary proteins?
Because of the abnormal urinalysis findings, the doctor recommended that a renal biopsy be taken. This was performed a week later in the outpatient surgery department of the community hospital next door to the doctor's office. The biopsy specimen was examined by routine histologic methods, immunofluorescence, and electron microscopy (Fig. A-5).

What is the explanation for the pathologic changes seen in the kidney?
The physician made the diagnosis of proliferative lupus glomerulonephritis and prescribed a higher dose of prednisone than what N.Z. was taking previously. The proteinuria and edema subsided over a 2-week period, and serum C3 levels returned to normal. Her corticosteroid dose was tapered down to a lower amount. Over the next few years, she has had intermittent flare-ups of her disease, with joint aches and tissue swelling and laboratory tests indicating depressed C3 levels and proteinuria. These have been effectively managed with corticosteroids, and she has been able to lead an active life.

Some autoimmune diseases are thought to be caused by lymphocytes specific for microbes that are activated by an infection and that cross-react with self antigens. Why is this not likely to be a valid explanation for how SLE develops?

Glomerulonephritis with immune complex deposition in systemic lupus erythematosus. A, A light micrograph of a renal biopsy specimen in which neutrophilic infiltration in a glomerulus can be seen. **B,** An immunofluorescence micrograph showing granular deposits of immunoglobulin G (IgG) along the basement membrane. (In this technique, called immunofluorescence microscopy, a frozen section of the kidney is incubated with a fluorescein-conjugated antibody against IgG, and the site of deposition of the IgG is defined by determining where the fluorescence is located.) **C,** An electron micrograph of the same tissue revealing immune complex deposition. (Courtesy of Dr. Helmut Rennke, Department of Pathology, Brigham and Women's Hospital, Boston.)

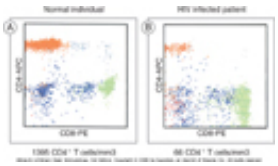
J.C. was a 28-year-old assistant carpenter with a history of human immunodeficiency virus (HIV) infection who came to the emergency department of his local hospital complaining of difficulty breathing and chills. The patient had a history of intravenous heroin abuse, with an admission to the same hospital 7 years earlier because of a drug overdose. At that time he had tested positive for both anti-HIV and anti-hepatitis B virus antibodies by enzyme-linked immunosorbent assay (ELISA). On discharge from the hospital, he was referred to an HIV clinic, where Western blot testing confirmed the presence of anti-HIV antibodies. A reverse transcriptase PCR test for viral RNA in the blood revealed 15,000 copies/mL of viral genome. His $CD4^+$ T cell count was 800/mm^3 (normal, 500 to 1500/mm^3). There was no evidence of opportunistic infections at that time.

What major risk factor did this patient have for acquiring HIV infection? What are other risk factors for HIV infection?
J.C. began taking anti-HIV medications including two nucleoside reverse transcriptase inhibitors and one viral protease inhibitor. He also attended a drug abuse rehabilitation program (and has not used illegal drugs since the time of his overdose). He became steadily employed and acquired health insurance benefits. After a year of his triple-drug therapy, J.C.'s $CD4^+$ T cell count remained about 800/mm^3, and a viral load test indicated less than 100 copies/mL. Over the next 5 years, however, his $CD4^+$ T cell count gradually declined to 300/mm^3. He assured his doctors that he rarely missed a dose of his medication, which was changed to different reverse transcriptase inhibitors three times, and a different protease inhibitor once, in an attempt to stop the decline in his $CD4^+$ count. He felt well and was able to work regularly, with the only sign of his HIV disease being multiple enlarged lymph nodes. He was started on antibiotic prophylaxis for *Pneumocystis jiroveci* pneumonia 3 years after his initial diagnosis.

What caused the gradual decline in the $CD4^+$ T cell count?
After 6 years from the time of initial diagnosis, J.C. began to lose weight. At a clinic visit around this time, he complained of a sore throat and had white plaque lesions in his mouth. Flow cytometry indicated a $CD4^+$ count of 64/mm^3 (Fig. A-6), and the viral load was more than 500,000 copies/mL. A diagnosis of acquired immunodeficiency syndrome (AIDS) was made.

Flow cytometry analysis of a human immunodeficiency virus (HIV)-infected patient's $CD4^+$ and $CD8^+$ T cells. A suspension of the patient's white blood cells was incubated with monoclonal antibodies specific for CD4 and CD8. The anti-CD4 antibody was labeled with the fluorochrome allophycocyanin (APC), and the

anti-CD8 antibody was labeled with the fluorochrome phycoerythrin (PE). These two fluorochromes emit light of different colors when excited by the appropriate wavelengths. The cell suspensions were analyzed in a flow cytometer, which can enumerate the number of cells stained by each of the differently labeled antibodies. In this way, the number of $CD4^+$ and $CD8^+$ T cells can be determined. Shown here are two-color plots of a control blood sample (**A**) and that of the patient (**B**). The $CD4^+$ T cells are shown in orange *(upper left quadrant)*, and the $CD8^+$ T cells are shown in green *(lower right quadrant)*. (These are not the colors of light emitted by the APC and PE fluorochromes.)



What is the likely reason for why the anti-HIV drugs given to this patient became ineffective over time?

Six months later, the patient came to the emergency department with a temperature of 39°C (102.2°F), blood pressure of 160/55 mm Hg, and shallow respirations, with a respiratory rate of 40 breaths per minute. He had lost 10 kg of body weight since his last clinic visit. Several red skin nodules were present on the patient's chest and arms. A chest radiograph showed a diffuse pneumonia. Intravenous antibiotics were administered for presumed *Pneumocystis jiroveci* pneumonia, and the patient was admitted to the infectious disease service. That night, a sputum sample was collected, and the following day, skin biopsy specimens were taken from his chest. Staining of the sputum sample revealed numerous *Pneumocystis jiroveci* organisms. The skin biopsy specimens showed Kaposi's sarcoma. Despite intensive care, the patient's pneumonia progressed, and he died 3 days later.

Why are patients with AIDS at high risk for developing opportunistic infections such as *Pneumocystis jiroveci* and malignancies such as Kaposi's sarcoma?

ANSWERS TO QUESTIONS FOR CASE 51. Intravenous drug use is the major risk factor for HIV infection in this patient. Shared needles among drug addicts transmit blood-borne viral particles from one infected person to others. Other major risk factors for HIV infection include sexual intercourse with an infected person, transfusion of contaminated blood products, and birth from an infected mother. (See Chapter 12.)2. After initial infection, the HIV rapidly enters various types of cells in the body, including $CD4^+$ T lymphocytes, dendritic cells, mononuclear phagocytes, and others. Once in an intracellular location, the virus is safe from antibody neutralization. The gradual decline in $CD4^+$ T cells in this patient was caused by repetitive cycles of HIV infection and death of $CD4^+$ T cells in lymphoid organs. The symptoms of AIDS do not usually occur until the blood count of $CD4^+$ T cells is below $200/mm^3$, reflecting a severe depletion of T cells in the lymphoid organs. (See Chapter 12.)3. HIV has a very high mutation rate. Mutations in the reverse transcriptase gene that render the enzyme resistant to nucleoside inhibitors occur frequently in patients receiving these drugs. Resistance to protease inhibitors may come about by similar mechanisms.4. The deficiencies in T cell-mediated immunity in patients with AIDS lead to impaired immunity to viruses, fungi, and protozoa, which otherwise are easily controlled by normal immune system. *Pneumocystis jiroveci* is a fungal organism and usually is eradicated by the action of activated $CD4^+$ T cells. Many of the malignancies that are frequent in patients with AIDS are associated with oncogenic viruses. For example, Kaposi's sarcoma is associated with human herpesvirus 8 infection. Many of the lymphomas that occur in patients with AIDS are associated with the Epstein-Barr virus, and many of the skin and cervical carcinomas that occur in these patients are associated with human papillomavirus.