CASE 27

Acute Infectious Mononucleosis

Cytotoxic T cells terminate viral infection.

All viruses, and some bacteria, multiply inside infected cells; indeed, viruses are highly sophisticated parasites that do not have a complete biosynthetic or metabolic apparatus of their own and, in consequence, must replicate inside a living cell. Once inside a cell, a pathogen is not accessible to antibodies and has to be eliminated by other means.

Some intracellular bacteria live and multiply in membrane-bound phagosomes within macrophages and are killed by antibacterial agents released into these vacuoles after macrophage activation by CD4 T_H1 cells (see for example Case 30). Viruses, in contrast, together with those bacteria that live in the cytosol, can be eliminated only by destruction of the infected cell itself. This role in host defense is fulfilled by the cytotoxic CD8 T cells of adaptive immunity and the natural killer (NK) cells of innate immunity.

CD8 cytotoxic T cells kill infected cells by recognizing foreign, pathogen-derived peptides that are transported to the cell surface bound to MHC class I molecules (see Fig. 17.1). The peptides carried by MHC class I molecules come from the degradation of proteins in the cytosol and so cytotoxic T cells act against pathogens whose proteins are found in the cytosol of the host

Topics bearing on this case:
Activation of cytotoxic T cells
Cell killing by cytotoxic T cells
Processing and presentation of cytosolic antigens

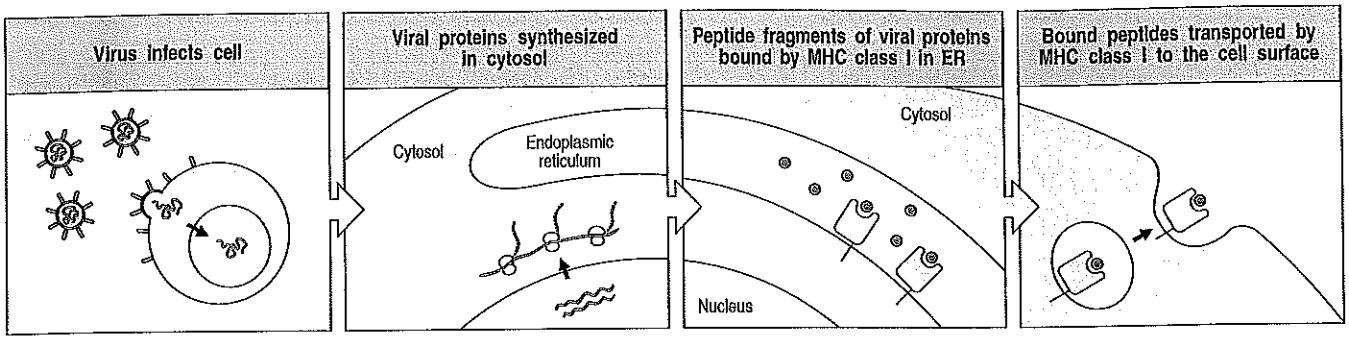
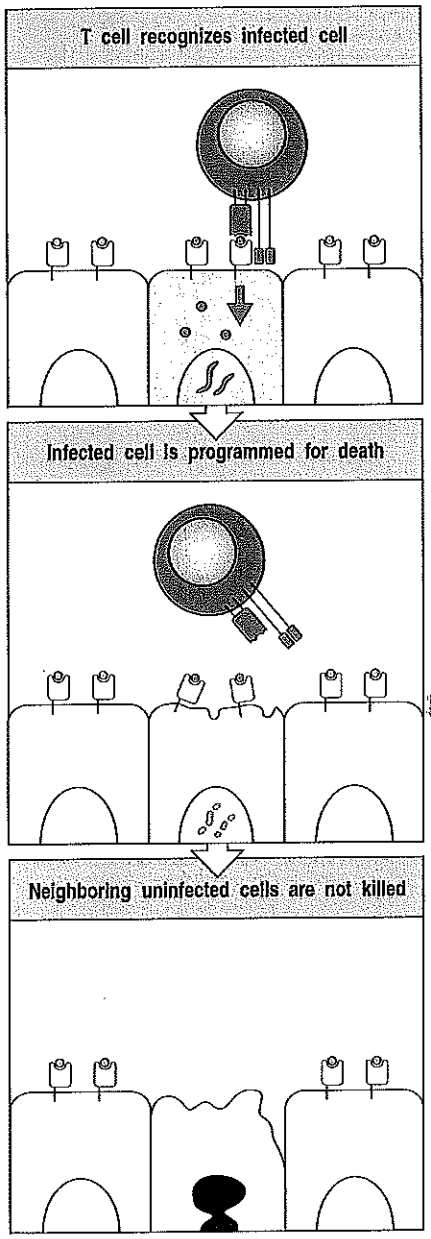


Fig. 27.1 MHC class I molecules present antigen derived from proteins in the cytosol. In cells infected with viruses, viral proteins are synthesized in the cytosol. Peptide fragments of viral proteins are transported into the endoplasmic reticulum, where they are bound by MHC class I molecules, which then deliver the peptides to the cell surface.



cell at some stage in their life-cycle (Fig. 27.1). The critical role of cytotoxic CD8 T cells in host defense is seen in the increased susceptibility of animals artificially depleted of cytotoxic T cells to many viral and intracytosolic bacterial infections. Mice and humans lacking the MHC class I molecules that present antigen to CD8 cells are also more susceptible to such infections.

Cytotoxic T cells kill their infected targets with great precision and neatness, by inducing apoptosis in the infected cell while sparing adjacent normal cells; this strategy minimizes tissue damage (Fig. 27.2). CD8 cytotoxic T cells release two types of preformed cytotoxin—the fragmentins or granzmes, which seem able to induce apoptosis in any type of target cell, and the protein perforin, which is thought to act as a translocator protein to enable granzymes to cross the membrane of the target cell (Fig. 27.3). A membrane-bound molecule, the Fas ligand, which is expressed on CD8 T cells as well as on some CD4 T cells can also induce apoptosis by binding to Fas on a limited range of target cells. Together, these properties allow the cytotoxic T cell to attack and destroy virtually any infected cell. Cytotoxic CD8 T cells also produce the cytokine interferon (IFN)-γ; this cytokine inhibits viral replication, induces MHC class I expression, and also activates macrophages. As well as combating infection by viruses and intracytosolic bacteria, CD8 T cells are important in controlling some protozoal infections; they are crucial, for example, in host defense against *Toxoplasma gondii*, an intracellular protozoan.

The importance of cytotoxic T cells in the control of viral replication is highlighted by many aspects of Epstein–Barr virus (EBV) infection, which is described in this case study. EBV (also known as human herpesvirus 4) is a member of the virus family Herpesviridae. It has a double-stranded linear DNA genome enclosed in an icosahedral capsid and a lipid envelope and replicates its DNA genome in the host cell nucleus. EBV infects only humans and is one of the most successful infective agents on its obligate host. It can even be thought of as a commensal that only seldom causes injury to the host; anywhere from 60–98% of healthy adults show serological evidence of infection with EBV. The virus infects mainly B cells and epithelial cells.

Fig. 27.2 Cytotoxic T cells kill target cells bearing specific antigen while sparing neighboring uninfected cells. All the cells in a tissue are susceptible to the induction of apoptosis by the cytotoxins of armed effector CD8 T cells but only infected cells are killed. Specific recognition by the T-cell receptor identifies which target cell to kill, and the polarized release of cytotoxic granules (see Fig. 27.3) ensures that neighboring cells are spared.

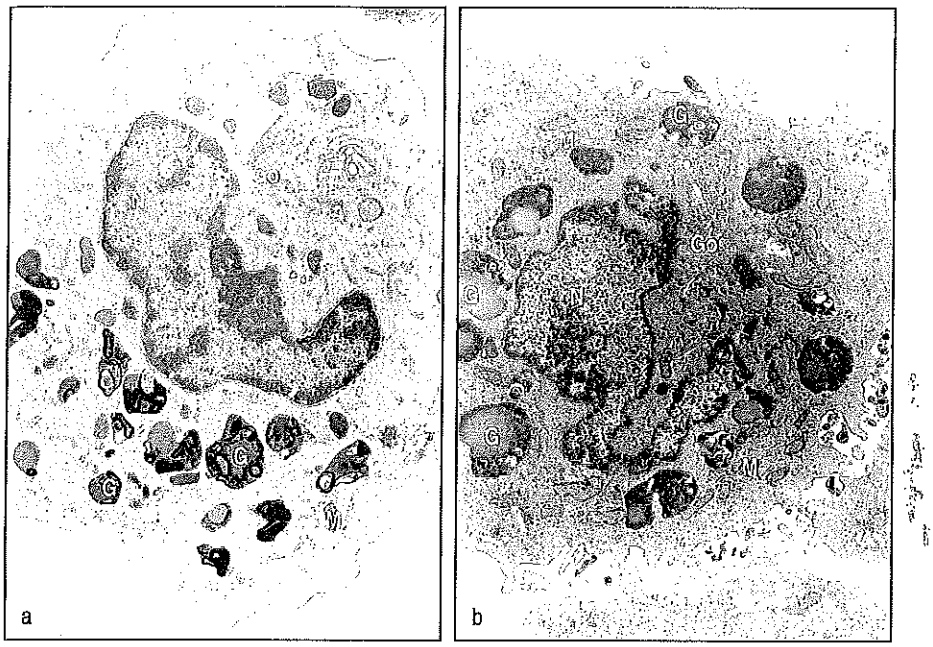


Fig. 27.3 The cytotoxic granules of cytotoxic T cells are released in a polarized fashion. Perforin molecules, as well as several other effector molecules, are contained in the granules of cytotoxic T cells (panel a: G, granules; N, nucleus; M, mitochondria; Go, Golgi apparatus). When a CD8 cytotoxic T cell recognizes its target, the granules are released onto the target cell (panel b, bottom right quadrant). Photographs courtesy of E. Podack.

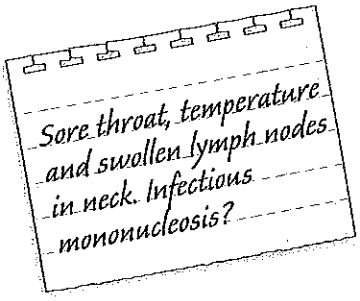
The case of Emma Bovary: a bad sore throat from a B-cell infection.

Emma Bovary was a healthy 15-year-old when she suddenly developed a very sore throat accompanied by fever and malaise. Her throat was so swollen she had difficulty swallowing. Over the next few days the fever waxed and waned, her sore throat became worse and she became progressively more tired and anorectic (lost her appetite). On the third day of illness her pediatrician noted severe pharyngitis and took a throat culture for β-hemolytic streptococci; the culture proved negative.

Emma's symptoms persisted, and she was unable to eat as she could hardly swallow. She said she had no difficulty breathing but that her left upper abdomen felt slightly uncomfortable. Emma's 1-year-old brother became ill at the same time, but did not have such severe symptoms. He was merely listless and felt warm. He had no particular physical symptoms, and seemed to recover completely after a few days.

On physical examination on the tenth day of illness, Emma appeared very ill. She had a high temperature (38.2 °C), pulse rate of 84, respiratory rate of 18, and blood pressure 85/55. Her mouth was dry and her tonsils were red and enlarged. They met in the midline, leaving a passage of only 2 × 2 cm approximately. Palatal petechiae (very small hemorrhages under the mucosa) could be seen. Her anterior and posterior cervical lymph nodes were swollen and tender (lymphadenopathy); the largest nodes were 2 × 2 cm. Her abdomen felt soft and the liver was enlarged, the edge being palpable 2 cm below the right costal margin. The spleen was also enlarged; the tip was easily palpable under the left costal margin.

A blood test gave a white blood cell count of 18,590 μl^{-1} with 39% neutrophils, 27% lymphocytes, 22% atypical lymphocytes (very high), and 11% monocytes (high); her



hematocrit was 45% and the platelet count 397,000 μl^{-1}. Serum electrolytes were normal. Another throat culture was obtained and blood tests for Epstein–Barr virus (EBV) were ordered.

In the meantime a presumptive diagnosis of acute infectious mononucleosis was made with complications including partial pharyngeal obstruction and mild dehydration. Emma was admitted to the hospital and received 1 liter normal saline intravenously followed by 20 mg methylprednisolone (a corticosteroid) intravenously every 12 hours.

Her throat culture again proved negative for streptococcus but her blood serum was positive for IgM and IgG antibodies against EBV capsid antigen. Emma improved quickly with the symptomatic treatment and was discharged on the second day after admission to complete her recovery at home.

Acute infectious mononucleosis.

Emma shows many of the clinical features characteristic of acute infectious mononucleosis (IM) induced by the Epstein–Barr virus, a disease also known as glandular fever in some countries. She had severe pharyngitis with petechiae on the palate, swollen lymph nodes in the neck, enlarged liver and spleen, and large numbers of atypical lymphocytes (the mononucleosis after which the disease is named) in her blood. These cells, also known as Downey–McKinlay cells, are large cells with foamy basophilic cytoplasm and fenestrated nuclei. They are mostly T cells, with a preponderance of CD8 cytotoxic T cells, and are present in 90% of patients with IM, where they sometimes constitute the majority of blood leukocytes.

It is these cells that control the acute infection by destroying EBV-infected B cells. The atypical lymphocytosis in IM is a reflection of the increased CD8 T cell cytotoxic activity. In the vast majority of individuals, the infection is brought under control but not eradicated, because the viral genome persists latently in many B cells. In the latent phase some viral antigens are produced and peptides derived from them are presented by MHC I molecules at the surface of the infected cell, thus enabling latently infected cells to be recognized and destroyed by EBV-specific cytotoxic T cells. Some of the latently infected B lymphocytes become transformed; they are able to propagate themselves indefinitely if removed from the presence of EBV-specific cytotoxic T cells, and are potentially malignant. In healthy people after infection, approximately 1 in 10^6 B cells is transformed. In patients with immune deficiency or who are immunosuppressed, infected B cells can grow unchecked. In immunodeficient patients, EBV infection can cause immunoblastic lymphoma and B-cell lymphoma.

EBV has a long incubation period: the time between primary EBV infection and the onset of illness is 30–50 days. Infection in infancy or early childhood is almost always asymptomatic, or results in mild disease, as evidenced by Emma's younger brother, who probably also had a primary EBV infection. In developed countries, primary infection is delayed in about half of the population to adolescence and early adulthood, so that 30–50% of primary EBV infection results in acute IM.

EBV enters B cells by binding to the B-cell surface molecule CD21 (also called complement receptor 2 (CR2) because it acts as a receptor for the complement fragment C3dg). This receptor is also present on a small subpopulation of T cells and on various types of epithelial cell in the nasopharynx, parotid gland duct, female cervix, and male urethra. After an active phase of viral multiplication, latency is established in B cells and, in some cases, epithelial cells. The EBV DNA is maintained during latency as an extrachromosomal DNA within the nucleus. Virus production is reactivated from time to time and periodic shedding of infectious virus in oral secretions of healthy infected people is common and lifelong. Adolescents like Emma often catch the disease through kissing; although Emma was not yet sexually active, she had dated several boys in her class.

Definitive diagnosis of EBV infection is best made by serological or molecular biological tests. Infected B cells are stimulated to secrete immunoglobulin, producing, among other antibodies, a so-called heterophile IgM antibody whose detection is one of the most widely used diagnostic tests for EBV infection. This heterophile antibody is not specific for EBV antigens but binds to antigens present on heterologous red blood cells (that is, those of other animals, such as sheep or goat) and agglutinates them. In addition, specific antibody responses are generated against several EBV-specific antigens, and the appearance of different antibodies is informative as to the time of infection and the pattern of virus replication (Fig. 27.4). The presence of IgM antibody against the EBV capsid antigen (VCA) indicates that the infection is acute; this antibody declines gradually in the convalescent phase. Because of the long incubation period of the virus, anti-VCA IgG antibody is also detectable at the onset of illness. The continued presence of EBV antigens in the host maintains antibody production throughout life.

Antibodies to so-called early antigens (EA) are produced mainly in the convalescent phase (1–6 months after disease onset); they then disappear. The absence of EA antibody indicates that the virus is mostly quiescent, and is not undergoing replication on a large scale. If the infection is reactivated, EA antibody titers rise again. Antigens expressed later in the viral life cycle, during its latent phase, are the EBNAs (Epstein–Barr nuclear antigens). Appearance of EBNA antibody indicates that the virus has been present in the body for at least a few months.

In very young, immunosuppressed, or immunodeficient patients, antibody formation can be so impaired that serologic diagnosis is impossible. In these cases EBV antigens can be detected by immunofluorescence microscopy on

	Viral capsid antigen; IgM	Viral capsid antigen; IgG	Early antigen	Epstein–Barr virus nuclear antigen
Never exposed	–	–	–	–
Acute infection	+	+	+/–	–
Recent infection	+/–	+	+/–	+/–
Past infection	–	+	–	+
Reactivated or chronic infection	–	+	+/–	+/–

Fig. 27.4 Serologic diagnosis of EBV infection.

blood or tissue (e.g., lymph node) specimens or by *in situ* hybridization for small EBV RNAs (EBERs). Alternatively, viral DNA can be amplified from infected cells and tissue by the polymerase chain reaction with oligonucleotide primers specific for EBV DNA.

EBV infection is mitogenic for B cells, overriding the normal regulatory mechanisms preventing them from dividing. Activation of B cells by virus infection also leads to immunoglobulin production, as noted above. Several EBV genes are critical for B-cell activation. One is the viral gene *BCRF-1*, which encodes a protein, also called VIL-10, very similar to human interleukin-10 (IL-10). The BCRF-1 protein enhances the activation and proliferation of EBV-infected cells. EBV infection also stimulates endogenous synthesis of IL-10 and IL-6, with further autocrine stimulatory effects on B cells. IL-10 also inhibits the T-cell production of IL-2 and IFN-γ, and enhances the production of B-cell stimulatory cytokines such as IL-4. IL-6 may inhibit the ability of NK cells to destroy EBV-infected cells.

In most patients IM is a self-limited disease for which supportive therapy suffices. Nucleoside analogues such as acyclovir or ganciclovir, or the DNA polymerase inhibitor foscarnet, have limited ability to inhibit the replication of EBV *in vitro*. The clinical usefulness of these drugs is so far unproven. They are frequently administered to patients with fulminant disease, or to immunosuppressed patients. Corticosteroids are often prescribed as a palliative measure, especially when airway obstruction is a potential concern. In the most extreme cases, when respiratory distress is present, tonsillectomy can be required. Corticosteroids reduce virus shedding and provide some symptomatic relief due to their anti-inflammatory effects. They do not significantly alter the course of the disease.

The ability of EBV to transform B cells is an extremely useful laboratory tool. When peripheral blood B cells are cultured with EBV, they become immortalized at a relatively high frequency and can be propagated indefinitely *in vitro*. This allows the general study of various aspects of B-cell biology, as well as providing material for the study of individual patients.

The virus-encoded Epstein–Barr virus nuclear antigen 2 (EBNA-2) is critical for transformation. EBNA-2 protein interacts with transcription factors leading to the activation of several host genes such as those encoding B-cell activating molecules such as CD21 (the EBV receptor) and CD23 (FcεRII, the low-affinity receptor for IgE), and viral genes such as LMP-1 (latent membrane protein-1, the primary oncogene of EBV). EBNA-3, -4, -5, and -6 also have a role in B-cell transformation.

Acute IM is only one of the possible outcomes of EBV infection. The Epstein–Barr virus is in fact named after two workers who studied Burkitt's lymphoma in Africa in the 1960s and first cultured the virus from these patients. This B-cell lymphoma is strongly associated with EBV infection in Africa, but not in other parts of the world; the high rate of EBV infection in early infancy together with the high incidence of malaria in Africa seem to be the predisposing factors. EBV infection is also strongly associated with nasopharyngeal carcinoma in Southeast Asia. This may perhaps be due to a particular strain of EBV that no longer possesses the epitope provoking an immunodominant response in people with a certain HLA class I allele that is present in a high proportion of people in Southeast Asia. Such people are therefore not so efficient at clearing cells infected with this EBV strain, making transformation and eventual malignancy more likely.

Questions.

1 *Patients with humoral immunodeficiency (an impaired antibody response) are susceptible to infection with some viruses such as poliomyelitis or enteric viruses but they have no problems with EBV. Why?*

2 *There is a high risk of EBV-induced lymphoproliferative malignancy after T-cell depleted bone marrow transplantation. Why?*

3 *In vitro transformation by EBV of B cells from umbilical cord blood rarely fails. Transformation of B cells in blood cultures from some adults is difficult. Why?*

4 *Why is heterophile antibody produced during EBV infection?*

5 *Males with X-linked agammaglobulinemia (XLA) never get infected with EBV. How do you explain this?*