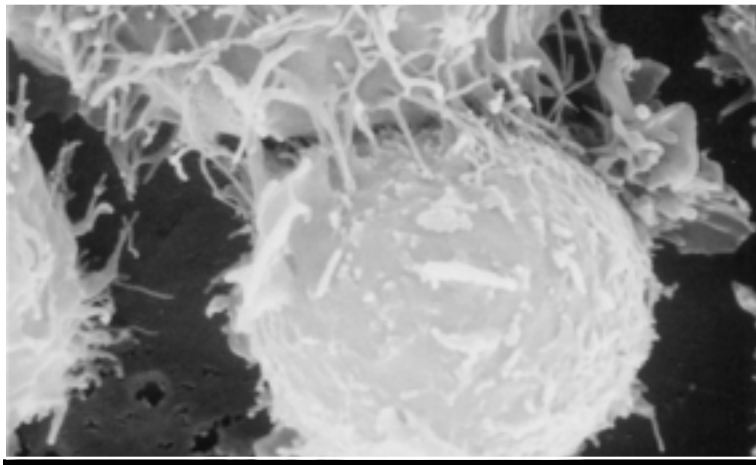
chapter 14

Cell-Mediated Effector Responses



Big CTL Attacks Little Tumor Cell

THE CELL-MEDIATED AND HUMORAL BRANCHES OF the immune system assume different roles in protecting the host. The effectors of the humoral branch are secreted antibodies, highly specific molecules that can bind and neutralize antigens on the surface of cells and in the extracellular spaces. The primary domain of antibody protection lies outside the cell. If antibodies were the only agents of immunity, pathogens that managed to evade them and colonize the intracellular environment would escape the immune system. This is not the case. The principal role of cell-mediated immunity is to detect and eliminate cells that harbor intracellular pathogens. Cell-mediated immunity also can recognize and eliminate cells, such as tumor cells, that have undergone genetic modifications so that they express antigens not typical of normal cells.

Both antigen-specific and -nonspecific cells can contribute to the cell-mediated immune response. Specific cells include $CD8^+$cytotoxic T lymphocytes (T_C cells or CTLs) and cytokine-secreting $CD4^+$ T_H cells that mediate delayed-type hypersensitivity (DTH). The discussion of DTH reactions and the role of $CD4^+$ T cells in their orchestration appears in Chapter 16. Nonspecific cells include NK cells and nonlymphoid cell types such as macrophages, neutrophils, and eosinophils. The activity of both specific and nonspecific components usually depends on effective local concentrations of various cytokines. T cells, NK cells, and macrophages are the most important sources of the cytokines that organize and support cell-mediated immunity. Finally, although humoral and cell-mediated immunity have many distinctive features, they are not completely independent. Cells such as macrophages, NK cells, neutrophils, and eosinophils can use antibodies as receptors to recognize and target cells for killing. Also, chemotactic peptides generated by the activation of complement in response to antigen-antibody complexes can contribute to assembling the cell types required for a cell-mediated response.

In the preceding chapters, various aspects of the humoral and cell-mediated effector responses have been described. This chapter addresses cytotoxic effector mechanisms mediated by T_C cells, NK cells, antibody-dependent cell-mediated cytotoxicity (ADCC), and the experimental assay of cytotoxicity.

Effector Responses

The importance of cell-mediated immunity becomes evident when the system is defective. Children with DiGeorge syndrome, who are born without a thymus and therefore lack the T-cell component of the cell-mediated immune system, generally are able to cope with infections of extracellular bacteria, but they cannot effectively eliminate intracellular pathogens. Their lack of functional cell-mediated immunity results in repeated infections with viruses, intracellular bacteria, and fungi. The severity of the cell-mediated immunodeficiency in these children is such that even the attenuated virus present in a vaccine, capable of only limited growth in normal individuals, can produce life-threatening infections.

Cell-mediated immune responses can be divided into two major categories according to the different effector populations that are mobilized. One group comprises effector cells

that have direct cytotoxic activity. These effectors eliminate foreign cells and altered self-cells by mounting a cytotoxic reaction that lyses their target. The various cytotoxic effector cells can be grouped into two general categories: one comprises antigen-specific cytotoxic T lymphocytes (CTLs) and nonspecific cells, such as natural killer (NK) cells and macrophages. The target cells to which these effectors are directed include allogeneic cells, malignant cells, virus-infected cells, and chemically conjugated cells. The other group is a subpopulation of effector $CD4^+$ T cells that mediates delayed-type hypersensitivity reactions (see Chapter 16). The next section reviews the general properties of effector T cells and how they differ from naive T cells.

General Properties of Effector T Cells

The three types of effector T cells—$CD4^+$, T_H1 and T_H2 cells, and $CD8^+$ CTLs—exhibit several properties that set them apart from naive helper and cytotoxic T cells (Table 14-1). In particular, effector cells are characterized by their less stringent activation requirements, increased expression of cell-adhesion molecules, and production of both membrane-bound and soluble effector molecules.

The Activation Requirements of T Cells Differ

T cells at different stages of differentiation may respond with different efficiencies to signals mediated by the T-cell receptor and may consequently require different levels of a second set of co-stimulatory signals. As described in Chapter 10, activation of naive T cells and their subsequent proliferation and differentiation into effector T cells require both a primary signal, delivered when the TCR complex and CD4 or CD8 coreceptor interact with a foreign peptide–MHC molecule complex, and a co-stimulatory signal, delivered by interaction between particular membrane molecules on the T cell and the antigen-presenting cell. In contrast, antigen-experienced effector cells and memory cells (as opposed to naive T cells) are able to respond to TCR-mediated signals with little, if any co-stimulation.

The reason for the different activation requirements of naive and activated T cells is an area of continuing research, but some clues have been found. One is that many populations of naive and effector T cells express different isoforms of CD45, designated CD45RA and CD45RO, which are produced by alternative splicing of the RNA transcript of the CD45 gene. This membrane molecule mediates TCR signal transduction by catalyzing dephosphorylation of a tyrosine residue on the protein tyrosine kinases Lck and Fyn, activating these kinases and triggering the subsequent steps in T-cell activation (see figures 10-10 and 10-11). The CD45RO isoform, which is expressed on effector T cells, associates with the TCR complex and its coreceptors, CD4 and CD8, much better than does the CD45RA isoform, which is expressed by naive T cells. Memory T cells have both isoforms, but the CD45RO is predominant. As a result, effector and memory T cells are more sensitive to TCR-mediated activation by a peptide-MHC complex. They also have less stringent requirements for co-stimulatory signals and therefore are able to respond to peptide-MHC complexes displayed on target cells or antigen-presenting cells that lack the co-stimulatory B7 molecules.

Cell-Adhesion Molecules Facilitate TCR-Mediated Interactions

CD2 and the integrin LFA-1 are cell-adhesion molecules on the surfaces of T cells that bind, respectively, to LFA-3 and ICAMs (*i*ntracellular *c*ell-*a*dhesion *m*olecules) on antigen-presenting cells and various target cells (see Figure 9-13). The level of LFA-1 and CD2 is twofold to fourfold higher on effector T cells than on naive T cells, enabling the effector T cells to bind more effectively to antigen-presenting cells and to various target cells that express low levels of ICAMs or LFA-3.

As Chapter 9 showed, the initial interaction of an effector T cell with an antigen-presenting cell or target cell is weak, allowing the TCR to scan the membrane for specific peptides

TABLE 14-1 Comparison of naive and effector T cells

Property	Naive T cells	Effector T cells
Co-stimulatory signal (CD28-B7 interaction)	Required for activation	Not required for activation
CD45 isoform	CD45RA	CD45RO
Cell-adhesion molecules (CD2 and LFA-1)	Low	High
Trafficking patterns	HEVs* in secondary lymphoid tissue	Tertiary lymphoid tissues; inflammatory sites

*HEV = high endothelial venules, sites in blood vessel used by lymphocytes for extravasation.

TABLE 14-2 Effector molecules produced by effector T cells

Cell type	Soluble effectors	Membrane-bound effectors
CTL	Cytotoxins (perforins and granzymes), IFN-γ, TNF-β	Fas ligand (FASL)
T_H1	IL-2, IL-3, TNF-β, IFN-γ, GM-CSF (high)	Tumor necrosis factor β (TNF-β)
T_H2	IL-3, IL-4, IL-5, IL-6, IL-10, IL-13, GM-CSF (low)	CD40 ligand

presented by self-MHC molecules. If no peptide-MHC complex is recognized by the effector cell, it will disengage from the APC or target cell. Recognition of a peptide-MHC complex by the TCR, however, produces a signal that increases the affinity of LFA-1 for ICAMs on the APC or target-cell membrane, prolonging the interaction between the cells. For example, T_H1 effector cells remain bound to macrophages that display peptide–class II MHC complexes; T_H2 effector cells remain bound to B cells that display peptide–class II MHC complexes; and CTL effector cells bind tightly to virus-infected target cells that display peptide–class I MHC complexes.

Effector T Cells Express a Variety of Effector Molecules

Unlike naive T cells, effector T cells express certain effector molecules, which may be membrane bound or soluble (Table 14-2). The membrane-bound molecules belong to the tumor necrosis factor (TNF) family of membrane proteins and include the Fas ligand (FASL) on $CD8^+$ CTLs, TNF-β on T_H1 cells, and the CD40 ligand on T_H2 cells. Each of the effector T-cell populations also secretes distinct panels of soluble effector molecules. CTLs secrete cytotoxins (perforins and granzymes) as well as two cytokines, IFN-γ and TNF-β. As described in Chapter 12, the T_H1 and T_H2 subsets secrete largely nonoverlapping sets of cytokines.

Each of these membrane-bound and secreted molecules plays an important role in various T-cell effector functions. The Fas ligand, perforins, and granzymes, for example, mediate target-cell destruction by the CTL; membrane-bound TNF-β and soluble IFN-γ and GM-CSF promote macrophage activation by the T_H1 cell; and the membrane-bound CD40 ligand and soluble IL-4, IL-5, and IL-6 all play a role in B-cell activation by the T_H2 cell.

Cytotoxic T Cells

Cytotoxic T lymphocytes, or CTLs, are generated by immune activation of T cytotoxic (T_C) cells. These effector cells have lytic capability and are critical in the recognition and elimination of altered self-cells (e.g., virus-infected cells and tumor cells) and in graft-rejection reactions. In general, CTLs are $CD8^+$ and are therefore class I MHC restricted, although in rare instances $CD4^+$ class II–restricted T cells have been shown to function as CTLs. Since virtually all nucleated cells in the body express class I MHC molecules, CTLs can recognize and eliminate almost any altered body cell.

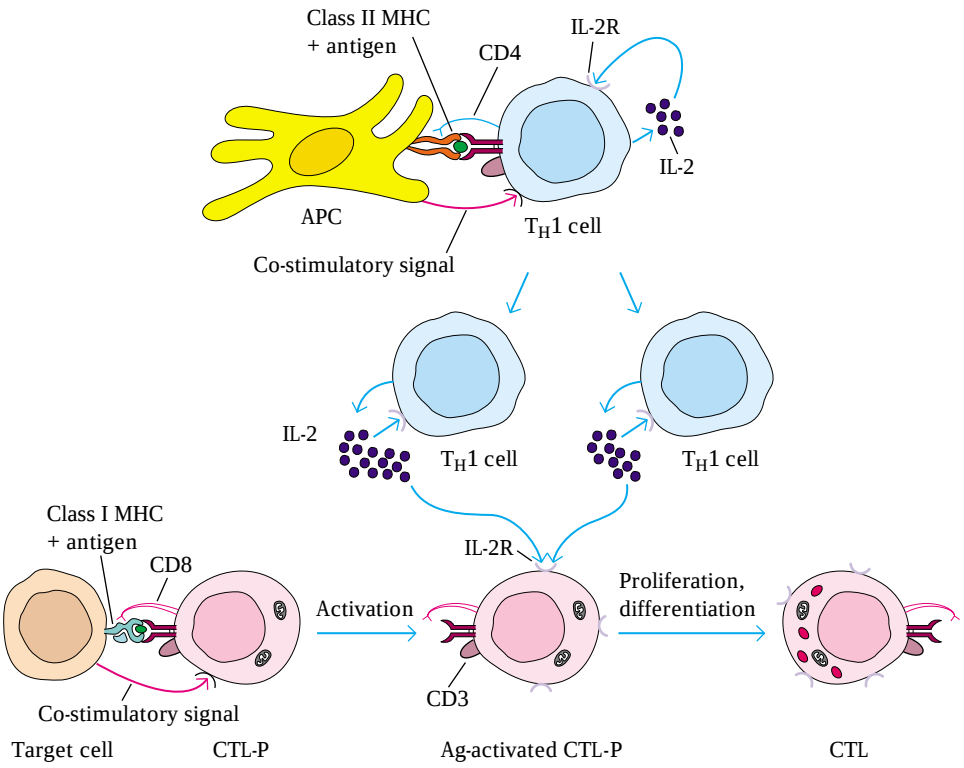
The CTL-mediated immune response can be divided into two phases, reflecting different aspects of the response. The first phase activates and differentiates naive T_C cells into functional effector CTLs. In the second phase, effector CTLs recognize antigen–class I MHC complexes on specific target cells, which leads them to destroy the target cells.

Effector CTLs Are Generated from CTL Precursors

Naive T_C cells are incapable of killing target cells and are therefore referred to as CTL precursors (CTL-Ps) to denote their functionally immature state. Only after a CTL-P has been activated will the cell differentiate into a functional CTL with cytotoxic activity. Generation of CTLs from CTL-Ps appears to require at least three sequential signals (Figure 14-1):

- An antigen-specific signal 1 transmitted by the TCR complex upon recognition of a peptide–class I MHC molecule complex
- A co-stimulatory signal transmitted by the CD28-B7 interaction of the CTL-P and the antigen-presenting cell
- A signal induced by the interaction of IL-2 with the high-affinity IL-2 receptor, resulting in proliferation and differentiation of the antigen-activated CTL-P into effector CTLs

Unactivated CTL-Ps do not express IL-2 or IL-2 receptors, do not proliferate, and do not display cytotoxic activity. Antigen activation induces a CTL-P to begin expressing the IL-2 receptor and to a lesser extent IL-2, the principal cytokine required for proliferation and differentiation of activated CTL-Ps into effector CTLs. In some cases, the amount of IL-2 secreted by an antigen-activated CTL-P may be sufficient to induce its own proliferation and differentiation; this



IL-2R expression	–	+	+
IL-2 expression	–	±	±
Proliferation	–	–	+
Effector cytotoxic function	–	–	+

FIGURE 14-1 Generation of effector CTLs. Upon interaction with antigen–class I MHC complexes on appropriate target cells, CTL-Ps begin to express IL-2 receptors (IL-2R) and lesser amounts of IL-2. Proliferation and differentiation of antigen-activated CTL-Ps generally require additional IL-2 secreted by T_H1 cells resulting from antigen activation and proliferation of $CD4^+$ T cells. In the subsequent effector phase, CTLs destroy specific target cells.

is particularly true of memory CTL-Ps, which have lower activation requirements than naive cells do (Figure 14-2a).

In general, though, most activated CTL-Ps require additional IL-2 produced by proliferating T_H1 cells to proliferate and differentiate into effector CTLs. The fact that the IL-2 receptor is not expressed until after a CTL-P has been activated by antigen plus a class I MHC molecule favors the clonal expansion and acquisition of cytotoxicity by only the antigen-specific CTL-Ps.

The proliferation and differentiation of both antigen-activated T_H1 cells and CTL-Ps depend on IL-2. In IL-2 knockout mice, the absence of IL-2 has been shown to abolish CTL-mediated cytotoxicity. After clearance of antigen, the level of IL-2 declines, which induces T_H1 cells and CTLs to undergo programmed cell death by apoptosis. In this way, the immune response is rapidly terminated, lessening the likelihood of nonspecific tissue damage from the inflammatory response.

The role of T_H1 cells in the generation of CTLs from naive CTL-Ps is not completely understood, and it is unlikely that a T_H1 cell and CTL-P interact directly. However, IL-2 and co-stimulation are important in the transformation of naive CTL-Ps into effector cells, and T_H1 cells can be mediators in the provision of these essential requirements. As shown in Figure 14-2b, the interaction of helper cells with antigen-presenting cells can result in production of IL-2 by the T_H1 cell. The paracrine action of this cytokine on nearby naive CTL-Ps whose TCRs are engaged can cause them to proliferate and differentiate into active CTLs. Additionally, T_H1 can induce the up-regulation of co-stimulatory molecules on the

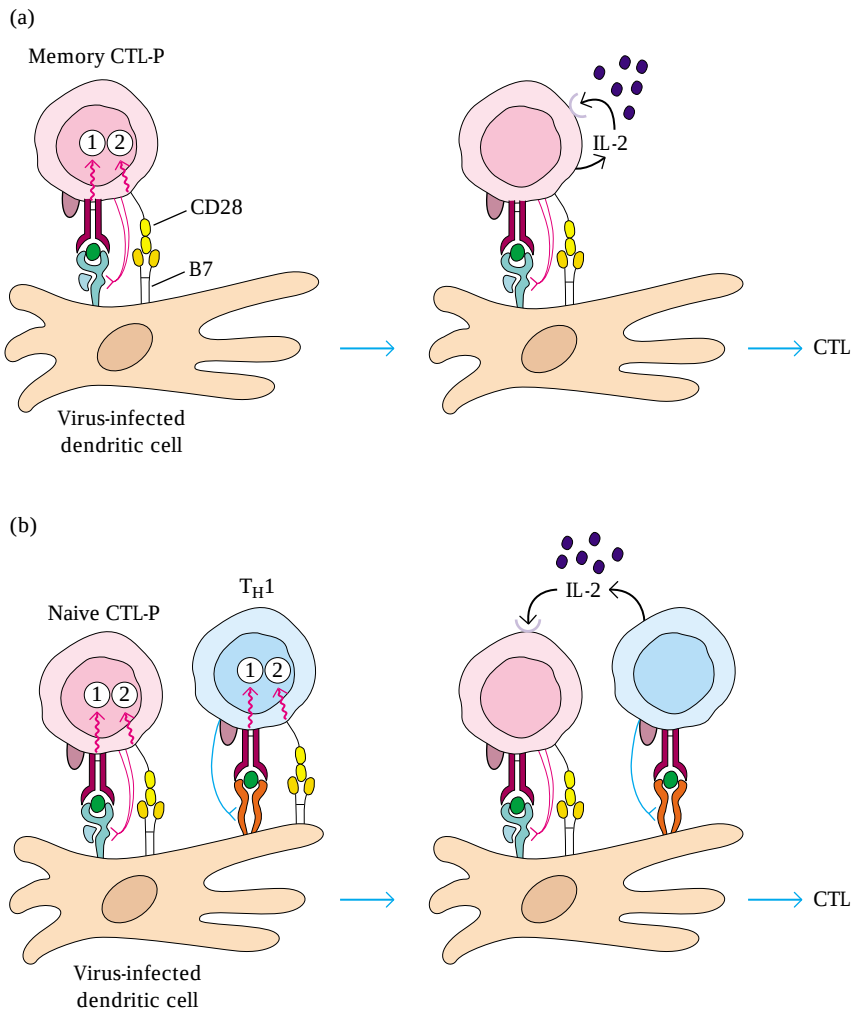


FIGURE 14-2 Proliferation of memory CTL-Ps may not require help from T_H cells. (a) Antigen-activated memory CTL-Ps appear to secrete sufficient IL-2 to stimulate their own proliferation and differentiation into effector CTLs. They also may not require the CD28-B7 co-stimulatory signal for activation. (b) A T_H cell may provide the IL-2 necessary for proliferation of an antigen-activated naive CTL-P when it binds to the same APC as the CTL-P. Also, T_H cells may alter the behavior of APCs in a number of ways, such as increasing the display of co-stimulatory molecules by the APC.

surface of antigen-presenting cells. In this manner, T_H1 cells help CTL-P division and differentiation by causing the generation of adequate levels of co-stimulation.

$CD8^+$ CTLs Can Be Tracked with MHC Tetramer Technology

MHC tetramers are laboratory-generated complexes of four MHC class I molecules bound to a specific peptide and linked to a fluorescent molecule. A given MHC-tetramer–peptide complex binds only $CD8^+$ T cells that have TCRs specific for the particular peptide-MHC complex that makes up the tetramer. Thus, when a particular tetramer is added to a cell population containing T cells (spleen cells or lymph-node cells, for example), cells that bear TCRs specific for the tetramer become fluorescently labeled (Figure 14-3). Using flow cytometry, it is then possible to determine the proportion of cells in a population that have TCRs specific for a particular antigen by counting the number of fluorescently labeled cells in a cell population. This very sensitive approach can detect antigen-specific T cells even when their frequency in the $CD8^+$ population is as low as 0.1%. Furthermore, one can directly measure the increase in antigen-specific $CD8^+$ T cells in response to exposure to pathogens such as viruses or cancer-associated antigens. In a related application, researchers infected mice with vesicular stomatitis virus (VSV) and systematically examined the distribution of $CD8^+$ cells specific for a VSV-derived peptide-MHC complex throughout the entire body. This study demonstrated that during acute infection with VSV, the distribution of

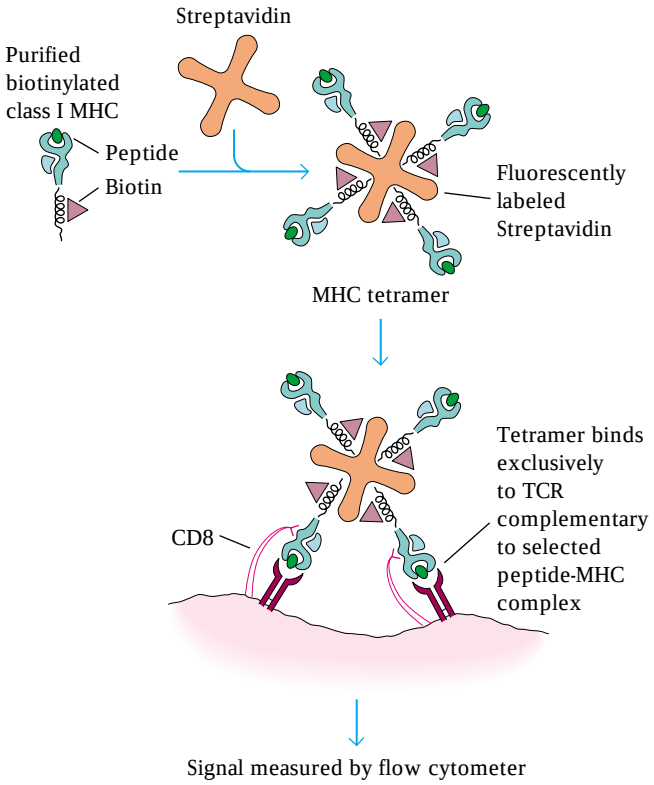


FIGURE 14-3 MHC tetramers. A homogeneous population of peptide-bound class I MHC molecules (HLA-A1 bound to an HIV-derived peptide, for example) is conjugated to biotin and mixed with fluorescently labeled Streptavidin. Four biotinylated MHC-peptide complexes bind to the high affinity binding sites of Streptavidin to form a tetramer. Addition of the tetramer to a population of T cells results in exclusive binding of the fluorescent tetramer to those $CD8^+$ T cells with TCRs complementary to the peptide-MHC complexes of the tetramer. This results in the labeling of the subpopulation of T cells that are specific for the target antigen, making them readily detectable by flow cytometry. *[Adapted in part from P. Klenerman, V. Cerundolo, and P. R. Dunbar, 2002,* Nature Reviews/Immunology ***2:****264.]*

VSV-specific $CD8^+$ cells is far from uniform (Figure 14-4); large populations of antigen-specific cells are not limited to the lymphoid system, but can be found in the liver and kidney, too.

CTLs Kill Cells in Two Ways

The effector phase of a CTL-mediated response involves a carefully orchestrated sequence of events that begin with the embrace of the target cell by the attacking cell (Figure 14-5). Long-term cultures of CTL clones have been used to identify many of the membrane molecules and membrane events involved in this process. As described below, studies with mouse strains carrying mutations that affect the ability of CTLs to induce death have led to the identification of the necessary molecules.

The primary events in CTL-mediated death are conjugate formation, membrane attack, CTL dissociation, and target-cell destruction (Figure 14-6). When antigen-specific CTLs are incubated with appropriate target cells, the two cell types interact and undergo conjugate formation. Formation of a CTL–target cell conjugate is followed within several minutes by a Ca^{2+}-dependent, energy-requiring step in which the CTL programs the target cell for death. The CTL then dissociates from the target cell and goes on to bind to another target cell. Within a variable period of time (up to a few hours) after CTL dissociation, the target cell dies by apoptosis. Each of the steps in this process has been studied in detail with cloned CTLs.

The TCR-CD3 membrane complex on a CTL recognizes antigen in association with class I MHC molecules on a target cell. After this antigen-specific recognition, the integrin

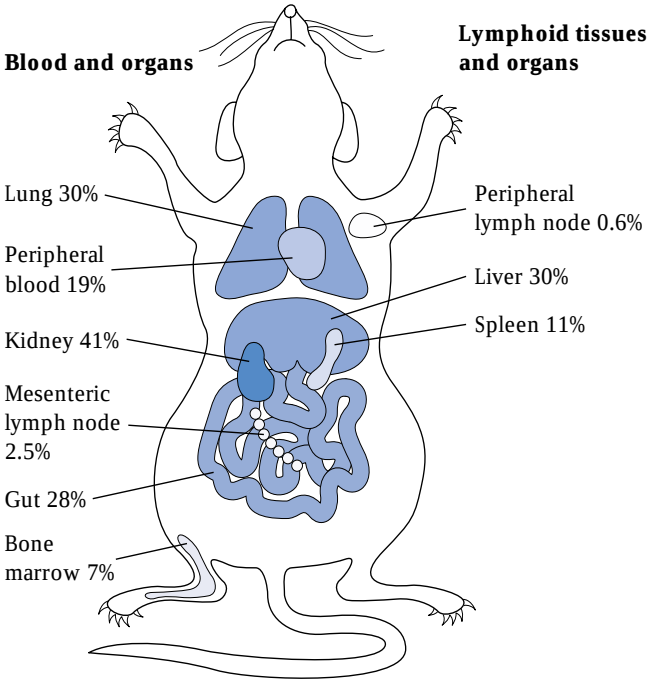


FIGURE 14-4 Localizing antigen specific $CD8^+$ T-cell populations in vivo. Mice were infected with vesicular stomatitis virus (VSV) and during the course of the acute stage of the infection, cell populations were isolated from the tissues indicated in the figure and incubated with tetramers containing VSV-peptide/MHC complexes. Flow cytometric analysis allowed determination of the percentages of $CD8^+$ T cells that were VSV-specific in each of the populations examined. *[Adapted from P. Klenerman, V. Cerundolo, and P. R. Dunbar, 2002,* Nature Reviews/Immunology ***2:****269.]*

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Cell Death

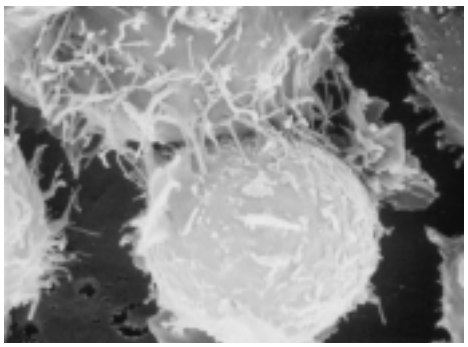


FIGURE 14-5 Scanning electron micrograph of tumor-cell attack by a CTL. The CTL makes contact with a smaller tumor cell. *[From J. D. E . Young and Z. A. Cohn, 1988,* Sci. Am. ***258****(1):38.]*

receptor LFA-1 on the CTL membrane binds to ICAMs on the target-cell membrane, resulting in the formation of a conjugate. Antigen-mediated CTL activation converts LFA-1 from a low-avidity state to a high-avidity state (Figure 14-7). Because of this phenomenon, CTLs adhere to and form conjugates only with appropriate target cells that display antigenic peptides associated with class I MHC molecules. LFA-1 persists in the high-avidity state for only 5–10 min after antigen-mediated activation, and then it returns to the low-avidity state. This downshift in LFA-1 avidity may facilitate dissociation of the CTL from the target cell.

Electron microscopy of cultured CTL clones reveals the presence of intracellular electron-dense storage granules. These granules have been isolated by fractionation and shown to mediate target-cell damage by themselves. Analysis of their contents revealed 65-kDa monomers of a pore-forming protein called **perforin** and several serine proteases called **granzymes** (or **fragmentins**). CTL-Ps lack cytoplasmic granules and perforin; upon activation, cytoplasmic granules appear, bearing newly expressed perforin monomers.

Immediately after formation of a CTL–target cell conjugate, the Golgi stacks and storage granules reorient within the cytoplasm of the CTL to concentrate near the junction with the target cell (Figure 14-8). Evidence suggests that perforin monomers and the granzyme proteases are then released from the granules by exocytosis into the junctional space between the two cells. As the perforin monomers contact the target-cell membrane, they undergo a conformational change, exposing an amphipathic domain that inserts into the target-cell membrane; the monomers then polymerize (in the presence of

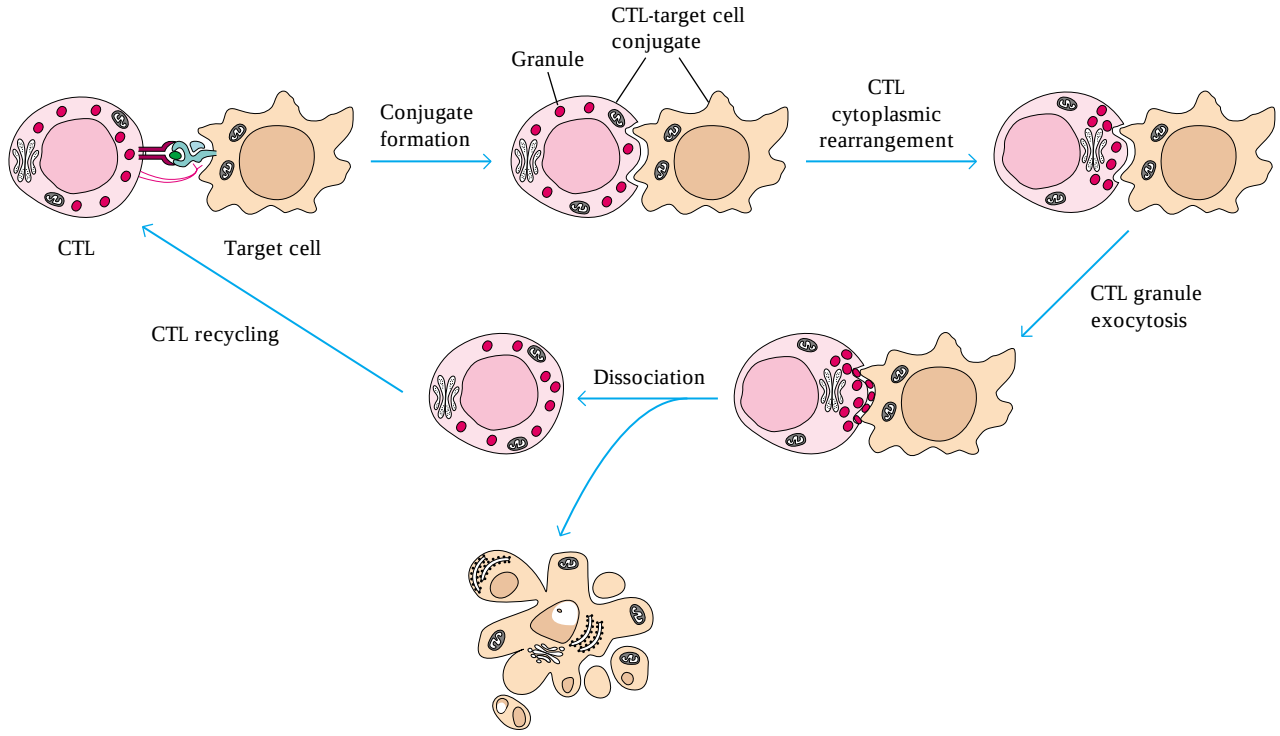


FIGURE 14-6 Stages in CTL-mediated killing of target cells. T-cell receptors on a CTL interact with processed antigen-class I MHC complexes on an appropriate target cell, leading to formation of a CTL/target-cell conjugate. The Golgi stacks and granules in the CTL reorient towards the point of contact with the target cell, and the granule's contents are released by exocytosis. After dissociation of the conjugate, the CTL is recycled and the target cell dies by apoptosis. *[Adapted from P. A. Henkart, 1985,* Annu. Rev. Immunol. ***3****:31.]*

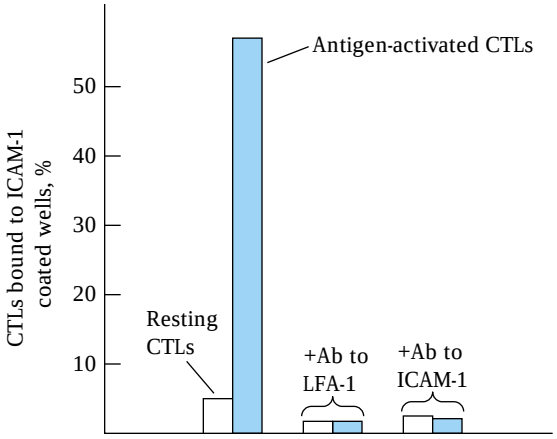


FIGURE 14-7 Effect of antigen activation on the ability of CTLs to bind to the intercellular cell-adhesion molecule ICAM-1. Resting mouse CTLs were first incubated with anti-CD3 antibodies. Crosslinkage of CD3 molecules on the CTL membrane by anti-CD3 has the same activating effect as interaction with antigen–class I MHC complexes on a target cell. Adhesion was assayed by binding radiolabeled CTLs to microwells coated with ICAM-1. Antigen activation increased CTL binding to ICAM-1 more than 10-fold. The presence of excess monoclonal antibody to LFA-1 or ICAM-1 in the microwell abolished binding, demonstrating that both molecules are necessary for adhesion. *[Based on M. L. Dustin and T. A. Springer, 1989,* Nature ***341:****619.]*

Ca^{2+}) to form cylindrical pores with an internal diameter of 5–20 nm (Figure 14-9a). A large number of perforin pores are visible on the target-cell membrane in the region of conjugate formation (Figure 14-9b). Interestingly, perforin exhibits some sequence homology with the terminal C9 component of the complement system, and the membrane pores formed by perforin are similar to those observed in complement-mediated lysis. The importance of perforin to CTL-mediated killing is demonstrated by perforin-deficient knockout mice, which are unable to eliminate lymphocytic choriomeningitis virus (LCMV) even though they mount a significant $CD8^+$ immune response to the virus.

Pore formation in the cell membrane of the target is one way that perforin mediates granzyme entry; another is the perforin-assisted pathway. Many target cells have a molecule known as the mannose 6-phosphate receptor on their surface that also binds to granzyme B. Granzyme B/mannose 6-phosphate receptor complexes are internalized and appear inside vesicles. In this case, perforin is necessary for releasing granzyme B from the vesicle into the cytoplasm of the target cell.

Once it enters the cytoplasm of the target cell, granzyme B initiates a cascade of reactions that result in the fragmentation of the target-cell DNA into oligomers of 200 bp; this type of DNA fragmentation is typical of apoptosis. Since granzymes are proteases, they cannot directly mediate DNA fragmentation. Rather, they activate an apoptotic pathway within the target cell. This apoptotic process does not require mRNA or protein synthesis in either the CTL or the target cell. Within 5 min of CTL contact, target cells begin to exhibit DNA fragmentation. Interestingly, viral DNA within infected target cells has also been shown to be fragmented during this process. This observation shows that CTL-mediated killing not only kills virus-infected cells but can also destroy the viral DNA in those cells. It has been suggested that the rapid onset of DNA fragmentation after CTL contact may prevent continued viral replication and assembly in the period before the target cell is destroyed.

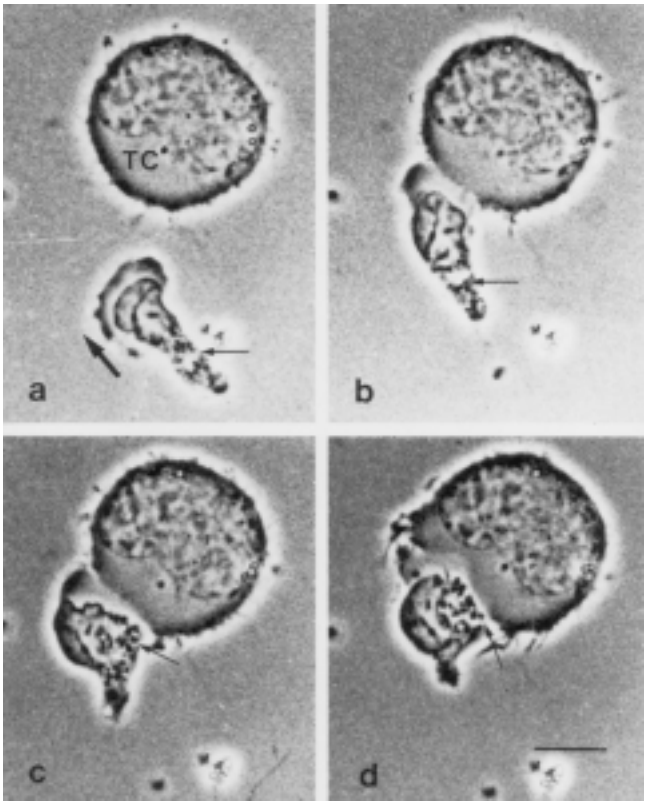


FIGURE 14-8 Formation of a conjugate between a CTL and target cell and reorientation of CTL cytoplasmic granules as recorded by time-lapse cinematography. (a) A motile mouse CTL (thin arrow) approaches an appropriate target cell (TC). The thick arrow indicates direction of movement. (b) Initial contact of the CTL and target cell has occurred. (c) Within 2 min of initial contact, the membrane-contact region has broadened and the rearrangement of dark cytoplasmic granules within the CTL (thin arrow) is under way. (d) Further movement of dark granules toward the target cell is evident 10 min after initial contact. *[From J. R. Yanelli et al., 1986,* J. Immunol. ***136:****377].*

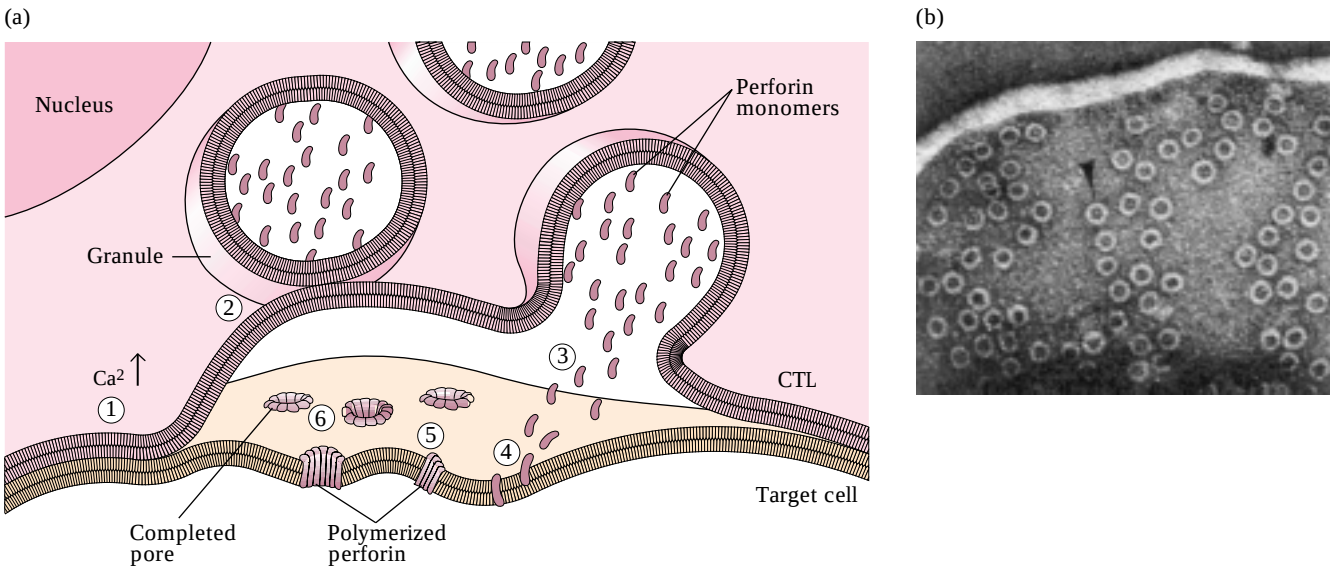


FIGURE 14-9 CTL-mediated pore formation in target-cell membrane. (a) In this model, a rise in intracellular Ca^{2+} triggered by CTL-target cell interaction (1) induces exocytosis, in which the granules fuse with the CTL cell membrane (2) and release monomeric perforin into the small space between the two cells (3). The released perforin monomers undergo a Ca^{2+}-induced conformational change that allows them to insert into the target-cell membrane (4). In the presence of Ca^{2+}, the monomers polymerize within the membrane (5), forming cylindrical pores (6). (b) Electron micrograph of perforin pores on the surface of a rabbit erythrocyte target cell. *[Part (a) adapted from J. D. E. Young and Z. A. Cohn, 1988,* Sci. Am. ***258****(1):38; part (b) from E. R. Podack and G. Dennert, 1983,* Nature ***301:****442.]*

Some potent CTL lines have been shown to lack perforin and granzymes. In these cases, cytotoxicity is mediated by Fas. As described in Chapter 10, this transmembrane protein, which is a member of the TNF-receptor family, can deliver a death signal when crosslinked by its natural ligand, a member of the tumor necrosis family called Fas ligand (see Figure 10-19). Fas ligand (FasL) is found on the membrane of CTLs, and the interaction of FasL with Fas on a target cell triggers apoptosis.

Key insight into the role of perforin and the Fas-FasL system in CTL-mediated cytolysis came from experiments with mutant mice. These experiments used two types of mutant mice, the perforin knockout mice mentioned above and a strain of mice known as lpr (Figure 14-10). Mice that are homozygous for the lpr mutation express little or no Fas and, consequently, cells from these mice cannot be killed by interaction with Fas ligand. If lymphocytes from normal H-2^b mice are incubated with killed cells from H-2^k mice, anti-H-2^k CTLs are generated. These H-2^b CTLs will kill target cells from normal H-2^k mice or from H-2^k animals that are homozygous for the lpr mutation. Incubation of H-2^b cells of perforin knockout mice with killed cells from H-2^k mice resulted in CTLs that killed wild-type target cells but failed to induce lysis in target cells from H-2^k mice homozygous for the lpr mutation.

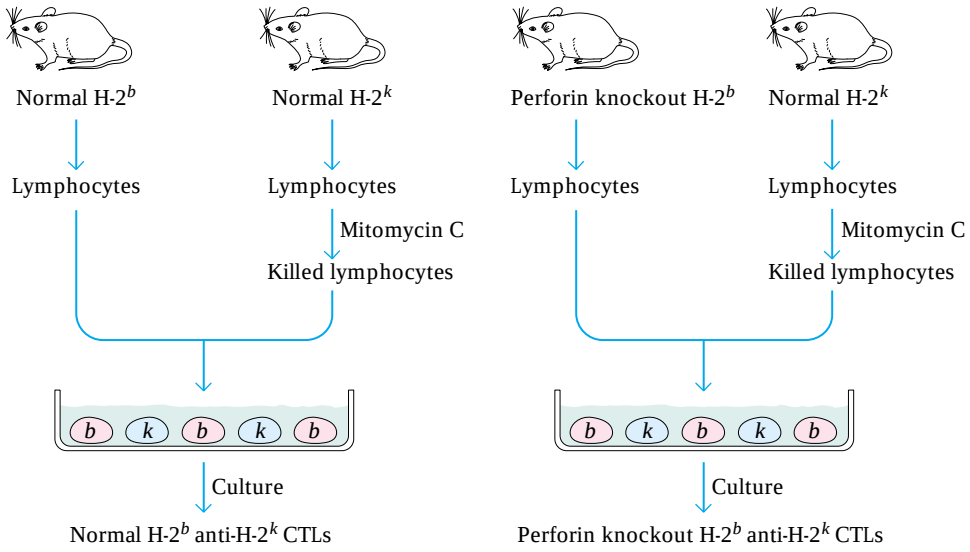
The results of these experiments taken together with other studies allowed the investigators to make the following interpretation. CTLs raised from normal mice can kill target cells by a perforin-mediated mechanism, by a mechanism involving engagement of target-cell Fas with Fas ligand displayed on the CTL membrane, or, in some cases perhaps, by a combination of both mechanisms. Such CTLs can kill target cells that lack membrane Fas by using the perforin mechanism alone. On the other hand, CTLs from perforin-knockout mice can kill only by the Fas-FasL mechanism. Consequently, CTLs from perforin-knockout mice can kill Fas-bearing normal target cells but not lpr cells, which lack Fas. These workers also concluded that all of the CTL-mediated killing they observed could be traced to the action of perforin-dependent killing, Fas-mediated killing, or a combination of the two. No other mechanism was detected.

This experiment taken together with a number of other studies shows that two mechanisms are responsible for initiating all CTL-mediated apoptotic death of target cells:

- Directional delivery of cytotoxic proteins (perforin and granzymes) that are released from CTLs and enter target cells
- Interaction of the membrane-bound Fas ligand on CTLs with the Fas receptor on the surface of target cells

Either of these initiating events results in the activation of a signaling pathway that culminates in the death of the target cell

(a) Generation of CTLs



(b) Interaction of CTLs with Fas^+ and Fas^- targets

CTLs	Target cells: Normal H-2^k	Target cells: lpr mutant H-2^k (no Fas)
Normal H-2^b anti-H-2^k	Killed	Killed
Perforin knockout H-2^b anti-H-2^k	Killed	Survive

FIGURE 14-10 Experimental demonstration that CTLs use Fas and perforin pathways. (a) Generation of CTLs. Lymphocytes were harvested from mice of H-2^b and H-2^k MHC haplotypes. H-2^k haplotype cells were killed by treatment with mitomycin C and co-cultured with H-2^b haplotype cells to stimulate the generation of H-2^k CTLs. If the H-2^b lymphocytes were derived from normal mice, they gave rise to CTLs that had both perforin and Fas ligand. If the CTLs were raised by stimulation of lymphocytes from perforin knockout (KO) mice, they expressed Fas ligand but not perforin. (b) Interaction of CTLs with Fas^+ and Fas^- targets. Normal H-2^b anti-H-2^k CTLs that express both Fas ligand and perforin kill normal H-2^k target cells and H-2^k lpr mutant cells, which do not express Fas. In contrast, H-2^b anti-H-2^k CTLs from perforin KO mice kill Fas^+ normal cells by engagement of Fas with Fas ligand but are unable to kill the lpr cells, which lack Fas.

by apoptosis (Figure 14-11). A feature of cell death by apoptosis is the involvement of the caspase family of cysteine proteases, which cleave after an aspartic acid residue. The name **caspase** incorporates all of these elements (*c*ysteine, *asp*artate prote*ase*). Normally, caspases are present in the cell as inactive proenzymes—procaspases—that require proteolytic cleavage for conversion to the active forms. More than a dozen different caspases have been found, each with its own specificity. Cleavage of a procaspase produces an active initiator caspase, which cleaves other procaspases, thereby activating their proteolytic activity. The end result is the systematic and orderly disassembly of the cell that is the hallmark of apoptosis.

CTLs use granzymes and Fas ligand to initiate caspase cascades in their targets. The granzymes introduced into the target cell from the CTL mediate proteolytic events that activate an initiator caspase. Similarly, the engagement of Fas on a target cell by Fas ligand on the CTL causes the activation of an initiator caspase in the target cell. Fas is associated with a protein known as FADD (*F*as-*a*ssociated protein with *d*eath *d*omain), which in turn associates with a procaspase form of caspase 8. Upon Fas crosslinking, procaspase 8 is converted to caspase 8 and initiates an apoptotic caspase cascade. The end result of both the perforin/granzyme and Fas-mediated pathways is the activation of dormant death pathways that are present in the target cell. As one immunologist has so aptly put it, CTLs don't so much kill target cells as persuade them to commit suicide.

Natural Killer Cells

Natural killer cells were discovered quite by accident when immunologists were measuring in vitro activity of tumor-

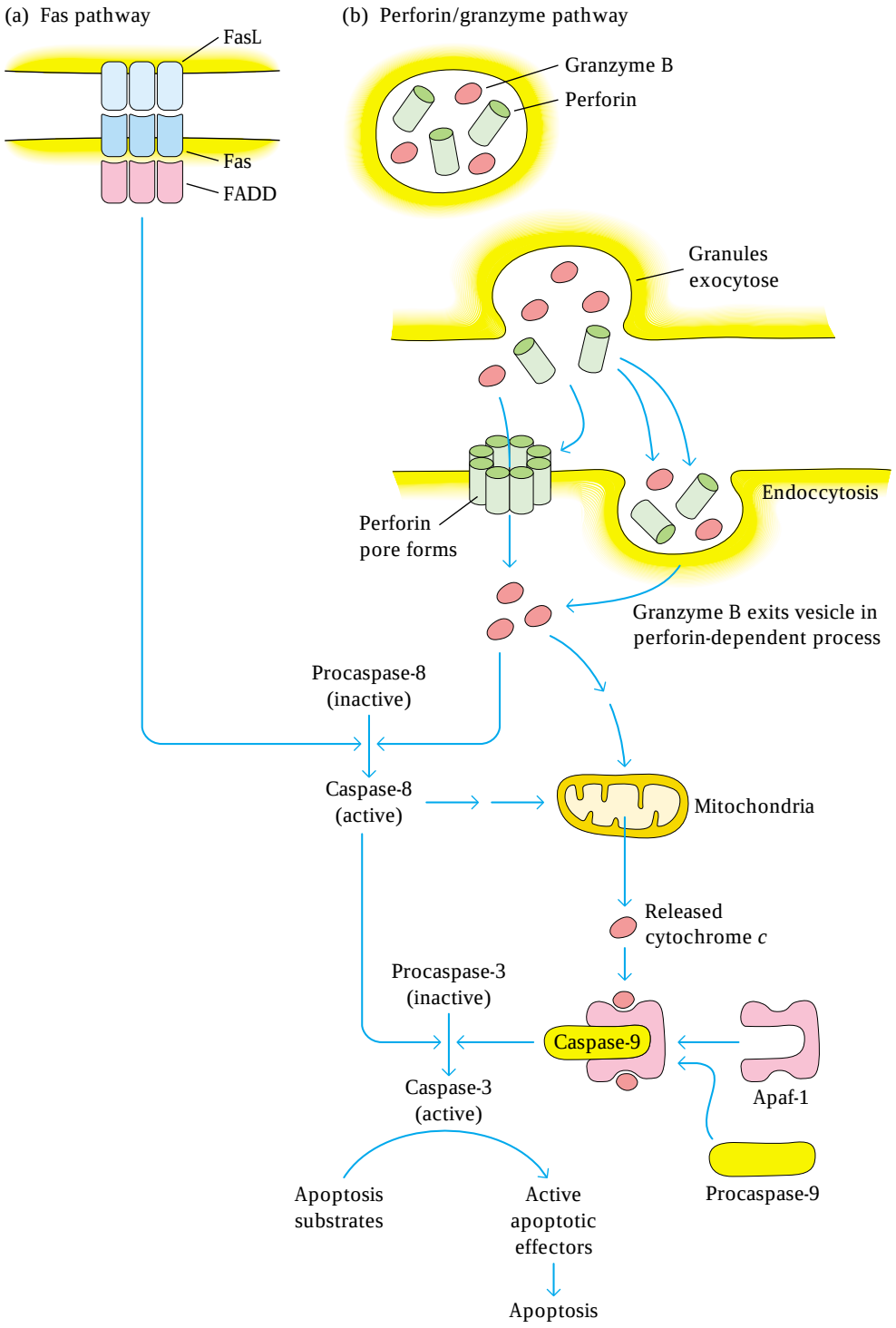


FIGURE 14-11 Two pathways of target-cell apoptosis stimulated by CTLs. (a) The Fas pathway. Ligation of trimeric Fas units by CTL-borne Fas ligand leads to the association of the death domains of Fas with FADD, which in turn results in a series of reactions leading to apoptosis of the target cell. (b) The perforin/granzyme pathway. Granule exocytosis releases granzymes and perforin from the CTL into the space between the CTL and the target cell. Granzyme B enters the target cell in two ways: via perforin-generated pores, or by binding to mannose 6-phosphate receptors that are subsequently endocytosed. Granzyme B is then released into the cytoplasm in a perforin-dependent process. Cleavage of procaspase 8 by granzyme B activates a caspase cascade that results in the apoptotic death of the cell, and interaction of granzyme B with other targets can invoke mitochondrially mediated death pathways. *[Adapted from M. Barry and C. Bleackley, 2002,* Nature Reviews Immunology ***2:****401.]*

specific cells taken from mice with tumors. Normal unimmunized mice and mice with unrelated tumors served as negative controls. Much to the consternation of the investigators, the controls showed significant lysis of the tumor cells, too. Characterization of this nonspecific tumor-cell killing revealed that a population of large granular lymphocytes was responsible. The cells, which were named natural killer (NK) cells for their nonspecific cytotoxicity, make up 5%–10% of the recirculating lymphocyte population. These cells are involved in immune defenses against viruses and tumors. Because NK cells produce a number of immunologically important cytokines, they play important roles in immune regulation and influence both innate and adaptive immunity. In particular, IFN-γ production by NK cells can affect the participation of macrophages in innate immunity by activation of the phagocytic and microbicidal activities. IFN-γ derived from NK cells can influence the T_H1 versus T_H2 commitment of helper T cell populations by its inhibitory effects on T_H2 expansion, and stimulate T_H1 development via induction of IL-12 by macrophages and dendritic cells. The Chediak-Higashi syndrome described in the Clinical Focus illustrates the disastrous consequences of a lack of NK cells.

NK cells are involved in the early response to infection with certain viruses and intracellular bacteria. NK activity is stimulated by IFN-α, IFN-β, and IL-12. In the course of a viral infection, the level of these cytokines rapidly rises, followed closely by a wave of NK cells that peaks in about 3 days (Figure 14-12). NK cells are the first line of defense against virus infection, controlling viral replication during the time required for activation, proliferation, and differentiation of CTL-P cells into functional CTLs at about day 7. The importance of NK cells in defense against viral infections is illustrated by the case of a young woman who completely lacked these cells. Even though this patient had normal T- and B-cell counts, she suffered severe varicella virus infections and a life-threatening cytomegalovirus infection.

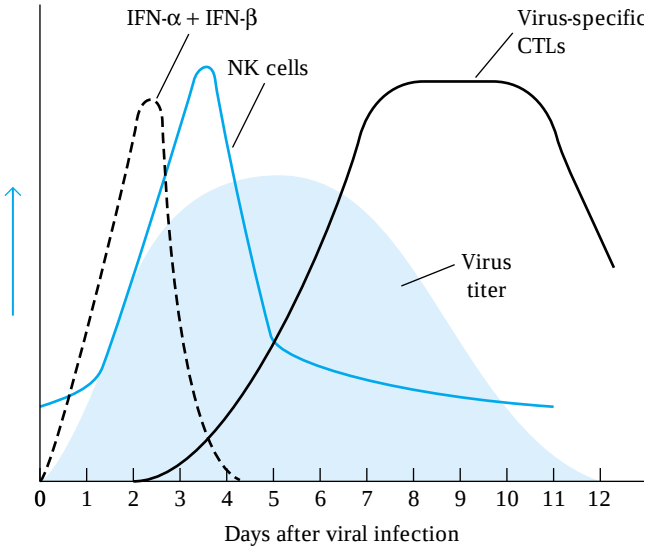


FIGURE 14-12 Time course of viral infection. IFN-α and IFN-β (dashed curve) are released from virus-infected cells soon after infection. These cytokines stimulate the NK cells, quickly leading to a rise in the NK-cell population (blue curve) from the basal level. NK cells help contain the infection during the period required for generation of CTLs (black curve). Once the CTL population reaches a peak, the virus titer (blue area) rapidly decreases.

NK Cells and T Cells Share Some Features

NK cells are lymphoid cells derived from bone marrow that share a common early progenitor with T cells, but their detailed lineage remains to be worked out. They express some membrane markers that are found on monocytes and granulocytes, as well as some that are typical of T cells. Different NK cells express different sets of membrane molecules. It is not known whether this heterogeneity reflects subpopulations of NK cells or different stages in their activation or maturation. Among the membrane molecules expressed by NK cells are CD2, the 75-kDa β subunit of the IL-2 receptor, and, on almost all NK cells, CD16 (or FcγRIII), a receptor for the Fc region of IgG. Cell depletion with monoclonal anti-CD16 antibody removes almost all NK-cell activity from peripheral blood.

Despite some similarities of NK cells to T lymphocytes, they do not develop exclusively in the thymus. Nude mice, which lack a thymus and have few or no T cells, have functional NK-cell populations. Unlike T cells and B cells, NK cells do not undergo rearrangement of receptor genes. This is demonstrated by the observation that NK cells develop in mice in which the recombinase genes *RAG-1* or *RAG-2* have been knocked out. Furthermore, while no T or B cells are found in SCID mice, functional populations of NK cells can be readily demonstrated. The power of NK cells and other protective mechanisms of innate immunity to protect animals totally lacking in adaptive immunity is nicely illustrated by the family of RAG-1 knockout mice shown in Figure 14-13.

Killing by NK Cells Is Similar to CTL-Mediated Killing

Natural killer cells appear to kill tumor cells and virus-infected cells by processes similar to those employed by CTLs. NK cells bear FasL on their surface and readily induce death in Fas-bearing target cells. The cytoplasm of NK cells contains numerous granules containing perforin and granzymes. Unlike CTLs, which need to be activated before granules appear, NK cells are constitutively cytotoxic, always having large granules in their cytoplasm. After an NK cell adheres to a target

cell, degranulation occurs with release of perforin and granzymes at the junction of the interacting cells. The roles of perforin and granzymes in NK-mediated killing of target cells by apoptosis are believed to be similar to their roles in the CTL-mediated process.

Despite these similarities, NK cells differ from CTLs in several significant ways. First, NK cells do not express antigen-specific T-cell receptors or CD3. In addition, recognition of target cells by NK cells is not MHC restricted; that is, in many cases the same levels of NK-cell activity are observed with syngeneic and allogeneic tumor cells. Moreover, although prior priming enhances CTL activity, NK-cell activity does not increase after a second injection with the same tumor cells. In other words, the NK-cell response generates no immunologic memory.

NK Cells Have Both Activation and Inhibition Receptors

Given that NK cells do not express antigen-specific receptors, the mechanism by which NK cells recognize altered self-cells and distinguish them from normal body cells baffled immunologists for years. The solution to the problem emerged with the realization that NK cells employ two different categories of receptors, one that delivers inhibition signals to NK cells, and another that delivers activation signals. Initially, it was thought that there were two receptors, one that activated and another that inhibited NK cells—the so-called two-receptor model. It is now clear that there are many different cell-surface receptors for activation signals and a number of different kinds for inhibitory ones. Consequently, it is more appropriate to think in terms of an *opposing-signals model* rather than a *two-receptor model*. It is the balance between activating signals and inhibitory signals that is believed to enable NK cells to distinguish healthy cells from infected or cancerous ones. It is important to be aware that additional NK-activating signals can be delivered by soluble factors. These include cytokines such as α and β interferons, TNF-α, IL-12, and IL-15.

FIGURE 14-13 Family of RAG-1 KO mice. These mice have no adaptive immunity because they lack T and B cells. However, NK cells and other mechanisms of innate immunity provide sufficient protection against infection that, if maintained in clean conditions, these mice can reproduce and raise healthy offspring. However, they are more susceptible to infection than normal mice and have reduced lifespans. *[From the laboratory of R. A. Goldsby.]*

The exact nature of the membrane-bound receptors on NK cells that produce activation is not completely clear. Antibody crosslinking of many molecules found on the surface of NK cells can activate these cells artificially, but the natural ligands for many of these putative activation receptors (ARs) are not known. Some of the candidate ARs are members of a class of carbohydrate-binding proteins known as **C-type lectins,** so named because they have calcium-dependent carbohydrate-recognition domains. NKR-P1 is an example of a C-type lectin found on NK cells that has activation properties. In addition to lectins, other molecules on NK cells might be involved in activation, including CD2 (receptor for the adhesion molecule LFA-3), and the FcγIII receptor, CD16. Although CD16 is responsible for antibody-mediated recognition and killing of target cells by NK cells, it is probably not involved in non-antibody-dependent killing. In addition to the molecules already mentioned, three additional proteins, NKp30, NKp44, and NKp46, appear to play significant roles in the activation of human NK cells.

Clues to the sources of inhibitory signals came from studies of the killing of tumor cells and virus-infected cells by NK cells. It was noticed that the preferential killing of mouse tumor cells compared with normal cells correlated with a lack of expression of MHC molecules by the tumor cells. Experiments with human cells showed that NK cells lysed a B-cell line that was MHC deficient because it had been transformed by Epstein-Barr virus. However, when this cell line was transformed with human HLA genes so that it expressed high levels of MHC molecules, NK cells failed to lyse it. These observations led to the idea that NK cells target for killing cells that have aberrant MHC expression. Since many virus-infected and tumor cells have reduced MHC expression, this model made good physiological sense. Vindication of this proposal has come from the discovery of receptors on NK cells that produce inhibitory signals when they recognize MHC molecules on potential target cells. These inhibitory receptors on the NK cell then prevent NK-cell killing, proliferation, and cytokine release.

Two major groups of inhibitory receptors have been found on NK cells. One of these is a family of *C-type-lectin–inhibitory receptors* (CLIR), and the other is a group of Ig-superfamily–inhibitory receptors (ISIR) known as the *killer-cell–inhibitory receptors* (KIR). Even though these groups are chemically quite different, they are together referred to as the *inhibitory-receptor superfamily* (IRS). In humans, the

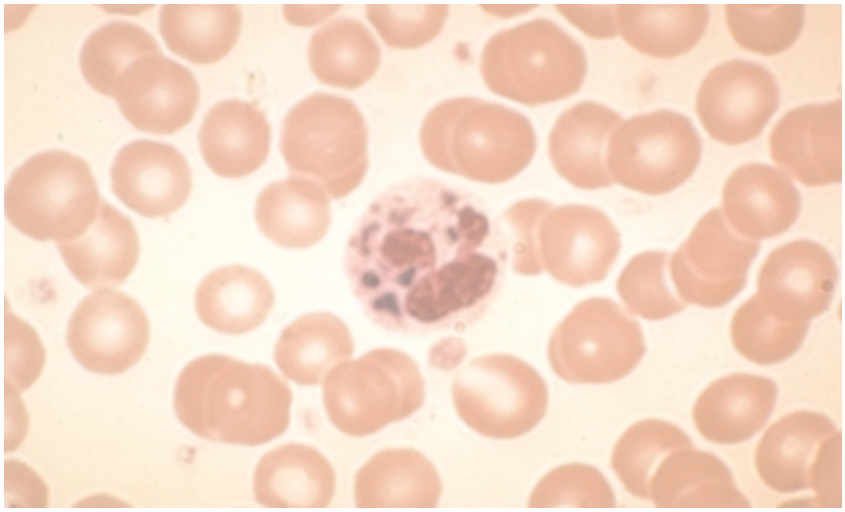
CLINICAL FOCUS

Chediak-Higashi Syndrome

The Chediak-Higashi syndrome (CHS) is a rare inherited disorder that inflicts a diversity of maladies on those afflicted by it. Identifying features of the disease include progressive neurological dysfunction, an increased tendency to develop leukemia and lymphoma, and depigmentation of hair, skin and eyes. Almost 90% of those afflicted have severe immunological deficiency, displaying defective natural-killer-cell function and deficits in neutrophil activity. These abnormalities in the leukocyte population are reflected in a greatly heightened susceptibility to infection, traceable in part to neutrophils that are deficient in chemotactic and bactericidal activities, and to dysfunctional populations of natural killer cells. The result is a greatly shortened life span; many Chediak-Higashi patients succumb to the disease in childhood. Microscopic examination of leukocytes from CHS patients reveals giant lysozomes that are characteristic of this disease.

Only those homozygous for a mutant form of a gene known as *CHS-1/LYST* (*ly*sosomal *t*rafficking regulator) develop Chediak-Higashi syndrome. A corresponding mutation has been found in beige mice and the mouse analogue of human *CHS-1/LYST.* The mouse and human homologues both encode a very large polypeptide of 2,186 amino acids. Beige mice display a pattern of symptoms very much like those seen in humans, and their granulocytes, like those of afflicted humans, display the huge cytoplasmic granules that are a morphological hallmark of the disease. Studies of the disease in beige mice complement those in humans, and have led to the conclusion that severe defects in the formation, fusion, or trafficking of intracellular vesicles probably underlie its devastating pathology.

Bone marrow transplantation (BMT) is the only effective therapy for the defective natural killer activity, aberrant macrophage activation, and susceptibility to bacterial infections that plague those afflicted with Chediak-Higashi syndrome. However, this is a risky and complex therapy. A look at the experience of 10 CHS children who underwent BMT for their disease is informative. BMT is best done with marrow from a donor whose HLA type is identical to that of the recipient. Unfortunately, it may be difficult or impossible to obtain HLA-matched bone marrow, and 3 of the patients had to settle for HLA-nonidentical marrow. After a median interval of 6.5 years post-transplantation, 6 of the 7 patients who had received marrow from HLA-identical donors were alive, but only 1 of the 3 recipients of HLA-nonidentical marrow survived. The clinical picture in the survivors was markedly improved. They were no longer hypersusceptible to bacterial infection, displayed significant NK-cell activity, and did not suffer from uncontrolled and pathological macrophage activation. However, the albinism and lack of eye pigmentation were not improved by BMT. HLA-identical BMT is thus accepted as a curative treatment for Chediak-Higashi syndrome, but reliance on HLA-nonidentical transplantation is experimental and carries very high risk.



A neutrophil with the giant lysozomes characteristic of Chediak-Higashi syndrome. *(Courtesy of American Society of Hemotology Slide Bank, 3rd edition.)*

C-type-lectin–inhibitory receptor is CD94/NKG2, a disulfide-bonded heterodimer made up of two glycoproteins, one of which is CD94 and the other a member of the NKG2 family. The CD94/NKG2 receptors recognize HLA-E on potential target cells. Because HLA-E is not transported to the surface of a cell unless it has bound a peptide derived from HLA-A, HLA-B, or HLA-C, the amount of HLA-E on the surface serves as indicator of the overall level of class I MHC biosynthesis in the cells. These inhibitory CD94/NKG2 receptors are thus not specific for a particular HLA allele and will send inhibitory signals to the NK cell, with the net result that killing of potential target cells is inhibited if they are express-

ing adequate levels of class I. In contrast, KIR receptors, of which more than 50 family members have been found, are specific for one or a limited number of polymorphic products of particular HLA loci. Unlike B and T cells, NK cells are not limited to expressing a single KIR, but may express several, each specific for a different MHC molecule or for a set of closely related MHC molecules. For example, individual clones of human NK cells expressing a CD94/NKG2 receptor and as many as six different KIR receptors have been found. Because signals from inhibitory receptors have veto power over signals from activating receptors, a negative signal from any inhibitory receptor, whether of the CD94/NKG2 or KIR type, can block the lysis of target cells by NK cells. Thus, cells expressing normal levels of unaltered MHC class I molecules tend to escape all forms of NK-cell–mediated killing.

In the opposing-signals model of NK-cell regulation that is emerging from studies of NK cells (Figure 14-14), activating receptors engage ligands on the target cell. These ligands may be abnormal patterns of glycosylation on the surface of tumor or virus-infected cells. Recognition of these determinants by ARs on NK cells would signal NK cells to kill the target cells. Ligand engagement by NKR-P1-type lectin receptors, or a number of other ARs, such as CD16, or in some cases CD2, generates signals that direct the NK cell to kill the target cell. Any of these killing signals can be overridden by a signal from inhibitory receptors. As we have already seen, members of the inhibitory superfamily of receptors (ISRs) provide a signal that decisively overrides activation signals when these inhibitory receptors detect normal levels of MHC class I expression on potential target cells. This prevents the death of the target cell. It also prevents NK-cell proliferation and the induction of secretion of cytokines such as IFN-γ and TNF-α. The overall consequence of the opposing-signals model is to spare cells that express critical indicators of normal self, the MHC class I molecules, and to kill cells that lack indicators of self (absence of normal levels of class I MHC).

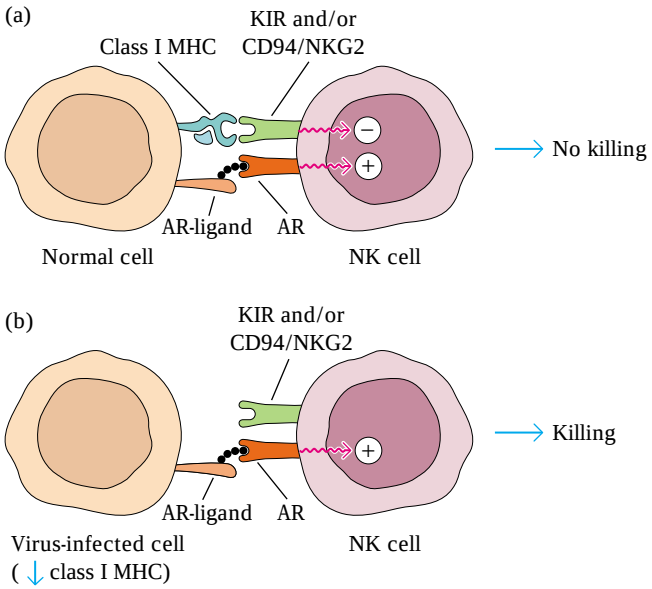


FIGURE 14-14 Opposing-signals model of how cytotoxic activity of NK cells is restricted to altered self-cells. An activation receptor (AR) on NK cells interacts with its ligand on normal and altered self-cells, inducing an activation signal that results in killing. However, engagement of inhibitory NK cell receptors such as KIR and CD94/NKG2 by class I MHC molecules delivers an inhibitory signal that counteracts the activation signal. Expression of class I molecules on normal cells thus prevents their destruction by NK cells. Because class I expression is often decreased on altered self-cells, the killing signal predominates, leading to their destruction.

Antibody-Dependent Cell-Mediated Cytotoxicity

A number of cells that have cytotoxic potential express membrane receptors for the Fc region of the antibody molecule. When antibody is specifically bound to a target cell, these receptor-bearing cells can bind to the antibody Fc region, and thus to the target cells, and subsequently cause lysis of the target cell. Although these cytotoxic cells are nonspecific for antigen, the specificity of the antibody directs them to specific target cells. This type of cytotoxicity is referred to as **antibody-dependent cell-mediated cytotoxicity (ADCC).**

Among the cells that can mediate ADCC are NK cells, macrophages, monocytes, neutrophils, and eosinophils. Antibody-dependent cell-mediated killing of cells infected with the measles virus can be observed in vitro by adding anti-measles antibody together with macrophages to a culture of measles-infected cells. Similarly, cell-mediated killing of helminths, such as schistosomes or blood flukes, can be observed in vitro by incubating larvae (schistosomules) with antibody to the schistosomules together with eosinophils.

Target-cell killing by ADCC appears to involve a number of different cytotoxic mechanisms, but not complement-mediated lysis (Figure 14-15). When macrophages, neutrophils, or eosinophils bind to a target cell by way of the Fc receptor, they become more active metabolically; as a result, the lytic enzymes in their cytoplasmic lysosomes or granules increase. Release of these lytic enzymes at the site of the Fc-mediated contact may result in damage to the target cell. In addition, activated monocytes, macrophages, and NK cells have been shown to secrete tumor necrosis factor (TNF), which may have a cytotoxic effect on the bound target cell. Since both NK cells and eosinophils contain perforin in cytoplasmic granules, their target-cell killing also may involve perforin-mediated membrane damage similar to the mechanism described for CTL-mediated cytotoxicity.

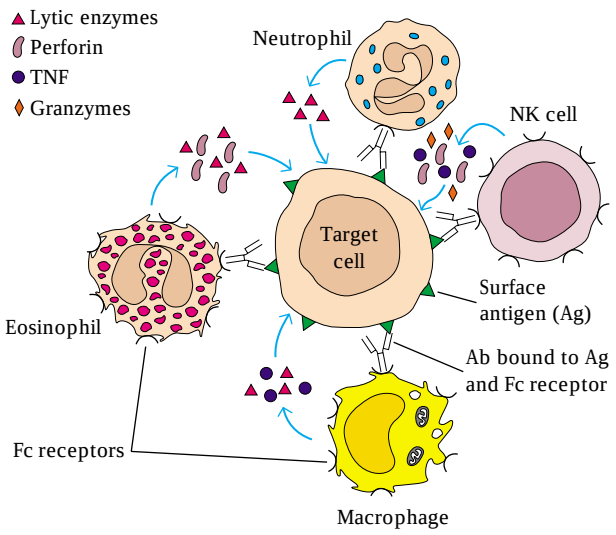


FIGURE 14-15 Antibody-dependent cell-mediated cytotoxicity (ADCC). Nonspecific cytotoxic cells are directed to specific target cells by binding to the Fc region of antibody bound to surface antigens on the target cells. Various substances (e.g., lytic enzymes, TNF, perforin, granzymes) secreted by the nonspecific cytotoxic cells then mediate target-cell destruction.

Experimental Assessment of Cell-Mediated Cytotoxicity

Three experimental systems have been particularly useful for measuring the activation and effector phases of cell-mediated cytotoxic responses. The *mixed-lymphocyte reaction* (MLR) is an in vitro system for assaying T_H-cell proliferation in a cell-mediated response; *cell-mediated lympholysis* (CML) is an in vitro assay of effector cytotoxic function; and the *graft-versus-host reaction* (GVH) in experimental animals provides an in vivo system for studying cell-mediated cytotoxicity.

Co-Culturing T Cells with Foreign Cells Stimulates MLR

During the 1960s, early in the history of modern cellular immunology, it was observed that when rat lymphocytes were cultured on a monolayer of mouse fibroblast cells, the rat lymphocytes proliferated and destroyed the mouse fibroblasts. In 1970 it was discovered that functional CTLs could also be generated by co-culturing allogeneic spleen cells in a system termed the mixed-lymphocyte reaction (MLR). The T lymphocytes in an MLR undergo extensive blast transformation and cell proliferation. The degree of proliferation can be assessed by adding [^{3}H] thymidine to the culture medium and monitoring uptake of label into DNA in the course of repeated cell divisions.

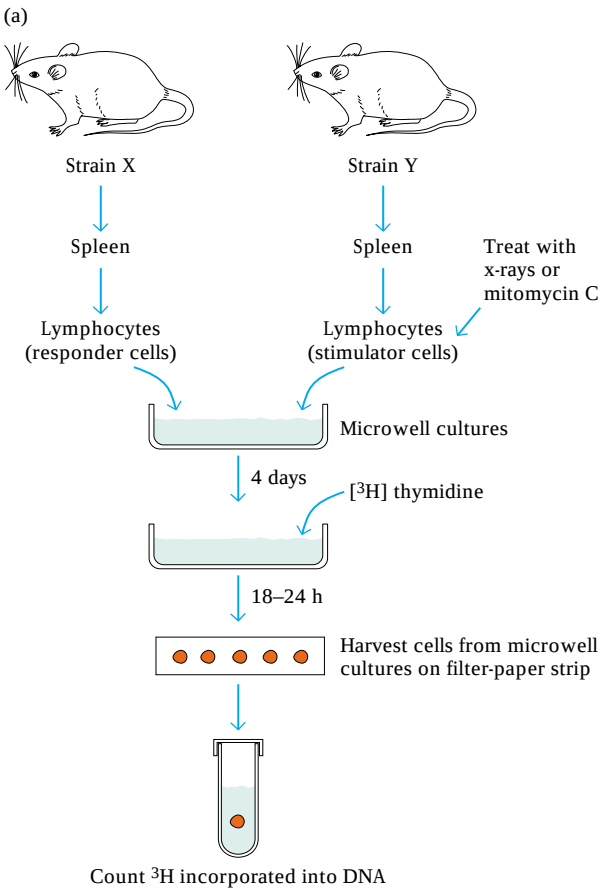
Both populations of allogeneic T lymphocytes proliferate in an MLR unless one population is rendered unresponsive by treatment with mitomycin C or lethal x-irradiation (Figure 14-16). In the latter system, called a one-way MLR, the unresponsive population provides stimulator cells that express alloantigens foreign to the responder T cells. Within 24–48 h, the responder T cells begin dividing in response to the alloantigens of the stimulator cells, and by 72–96 h an expanding population of functional CTLs is generated. With this experimental system, functional CTLs can be generated entirely in vitro, after which their activity can be assessed with various effector assays.

The significant role of T_H cells in the one-way MLR can be demonstrated by use of antibodies to the T_H-cell membrane marker CD4. In a one-way MLR, responder T_H cells recognize allogeneic class II MHC molecules on the stimulator cells and proliferate in response to these differences. Removal of the $CD4^+$ T_H cells from the responder population with anti-CD4 plus complement abolishes the MLR and prevents generation of CTLs. In addition to T_H cells, accessory cells such as macrophages also are necessary for the MLR to proceed. When adherent cells (mostly macrophages) are removed from the stimulator population, the proliferative response in the MLR is abolished and functional CTLs are no longer generated. It is now known that the function of these macrophages is to activate the class II MHC–restricted T_H cells, whose proliferation is measured in the MLR. In the absence of T_H-cell activation, there is no proliferation.

CTL Activity Can Be Demonstrated by CML

Development of the cell-mediated lympholysis (CML) assay was a major experimental advance that contributed to understanding of the mechanism of target-cell killing by CTLs. In this assay, suitable target cells are labeled intracellularly with chromium-51 (^{51}Cr) by incubating the target cells with $Na_2{}^{51}CrO_4$. After the ^{51}Cr diffuses into a cell, it binds to cytoplasmic proteins, reducing passive diffusion of the label out of the cell. When specifically activated CTLs are incubated for 1–4 h with such labeled target cells, the cells lyse and the ^{51}Cr is released. The amount of ^{51}Cr released correlates directly with the number of target cells lysed by the CTLs. By means of this assay, the specificity of CTLs for allogeneic cells, tumor cells, virus-infected cells, and chemically modified cells has been demonstrated (Figure 14-17).

The T cells responsible for CML were identified by selectively depleting different T-cell subpopulations by means of antibody-plus-complement lysis. In general, the activity of CTLs exhibits class I MHC restriction. That is, they can kill only target cells that present antigen associated with syngeneic class I MHC molecules. Occasionally, however, class II–restricted $CD4^+$ T cells have been shown to function as CTLs.



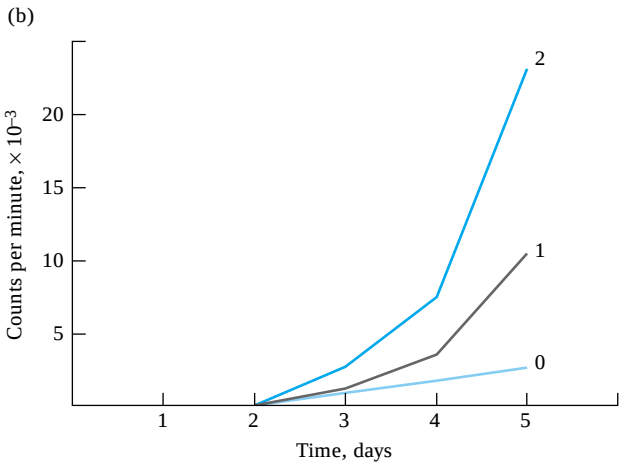


FIGURE 14-16 One-way mixed-lymphocyte reaction (MLR). (a) This assay measures the proliferation of lymphocytes from one strain (responder cells) in response to allogeneic cells that have been x-irradiated or treated with mitomycin C to prevent proliferation (stimulator cells). The amount of [^{3}H] thymidine incorporated into the DNA is directly proportional to the extent of responder-cell proliferation. (b) The amount of [^{3}H]-thymidine uptake in a one-way MLR depends on the degree of differences in class II MHC molecules between the stimulator and responder cells. Curve 0 = no class II MHC differences; curve 1 = one class II MHC difference; curve 2 = two class II MHC differences. These results demonstrate that the greater the class II MHC differences, the greater the proliferation of responder cells.

The GVH Reaction Is an Indication of Cell-Mediated Cytotoxicity

The graft-versus-host (GVH) reaction develops when immunocomponent lymphocytes are injected into an allogeneic recipient whose immune system is compromised. Because the donor and recipient are not genetically identical, the grafted lymphocytes begin to attack the host, and the host's compromised state prevents an immune response against the graft. In humans, GVH reactions often develop after transplantation of bone marrow into patients who have had radiation exposure or who have leukemia, immunodeficiency diseases, or autoimmune anemias. The clinical manifestations of the GVH reaction include diarrhea, skin lesions, jaundice, spleen enlargement, and death. Epithelial cells of the skin and gastrointestinal tract often become necrotic, causing the skin and intestinal lining to be sloughed.

Experimentally, GVH reactions develop when immunocompetent lymphocytes are transferred into an allogeneic neonatal or x-irradiated animal. The recipients, especially neonatal ones, often exhibit weight loss. The grafted lymphocytes generally are carried to a number of organs, including

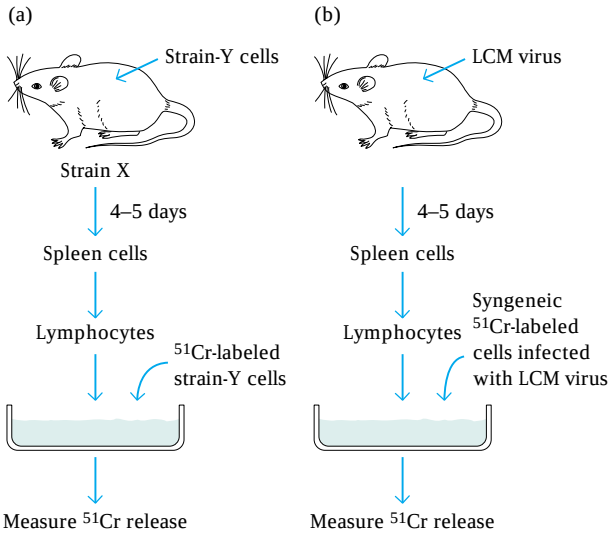


FIGURE 14-17 In vitro cell-mediated lympholysis (CML) assay. This assay can measure the activity of cytotoxic T lymphocytes (CTLs) against allogeneic cells (a) or virus-infected cells (b). In both cases the release of ^{51}Cr into the supernatant indicates the presence of CTLs that can lyse the target cells.

the spleen, where they begin to proliferate in response to the allogeneic MHC antigens of the host. This proliferation induces an influx of host cells and results in visible spleen enlargement, or splenomegaly. The intensity of a GVH reaction can be assessed by calculating the *spleen index* as follows:

$$\text{Spleen index} = \frac{\text{weight of experimental spleen/total body weight}}{\text{weight of control spleen/total body weight}}$$

A spleen index of 1.3 or greater is considered to be indicative of a positive GVH reaction. Spleen enlargement results from proliferation of both $CD4^+$ and $CD8^+$ T-cell populations. NK cells also have been shown to play a role in the GVH reaction, and these cells may contribute to some of the skin lesions and intestinal-wall damage observed.

SUMMARY

- The cell-mediated branch of the immune system involves two types of antigen-specific effector cells: cytotoxic T lymphocytes (CTLs) and CD4+ T cells that mediate DTH reactions (discussed in Chapter 17). Compared with naive T_H and T_C cells, the effector cells are more easily activated, express higher levels of cell-adhesion molecules, exhibit different trafficking patterns, and produce both soluble and membrane effector molecules.
- The first phase of the CTL-mediated immune response involves the activation and differentiation of T_C cells, called CTL precursors (CTL-Ps).
- Antigen-specific CD8+ populations can be identified and tracked by labeling with MHC tetramers.
- The second phase of the CTL-mediated response involves several steps: TCR-MHC mediated recognition of target cells, formation of CTL/target-cell conjugates, reorientation of CTL cytoplasmic granules toward the target cell, granule release, formation of pores in the target-cell membrane, dissociation of CTL from the target, and the death of the target cell.
- CTLs induce cell death via two mechanisms: the perforin-granzyme pathway and the Fas/FasL pathway.
- Various nonspecific (non-MHC dependent) cytotoxic cells (NK cells, neutrophils, eosinophils, macrophages) can also kill target cells. Many of these cells bind to the Fc region of antibody on target cells and subsequently release lytic enzymes, perforin, or TNF, which damage the target-cell membrane, a process, called antibody-dependent cell-mediated cytotoxicity (ADCC).
- NK cells mediate lysis of tumor cells and virus-infected cells by perforin-induced pore formation, a mechanism similar to one of those employed by CTLs.
- The expression of relatively high levels of class I MHC molecules on normal cells protects them against NK cell–mediated killing. NK cell killing is regulated by the balance between positive signals generated by the engagement of activating receptors (NKR-P1 and others) and negative signals from inhibitory receptors (CD94/NKG2 and the KIR family).

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USEFUL WEB SITES

http://www.cellsalive.com/ctl.htm

This Cells Alive subsite has a time-lapse video of cytotoxic T lymphocytes (CTLs) recognizing, attacking, and killing a much larger influenza-infected target.

http://www.antibodyassay.com/nkcell.htm

The AAL Reference Laboratories web site provides the rationale for clinical tests of NK cell function and tells how such tests are performed.

Study Questions

CLINICAL FOCUS QUESTION Would you expect the Chediak-Higashi syndrome to have its immediate effects on innate or adaptive immunity? Considering what was learned in Chapter 12, which T cell-mediated adaptive responses would you predict would be more significantly affected by CHS? Justify your answer.

1. Indicate whether each of the following statements is true or false. If you believe a statement is false, explain why.

   a. Cytokines can regulate which branch of the immune system is activated.

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Review and quiz of key terms

b. Both CTLs and NK cells release perforin after interacting with target cells.
c. Antigen activation of naive CTL-Ps requires a co-stimulatory signal delivered by interaction of CD28 and B7.
d. CTLs use a single mechanism to kill target cells.
e. The secretion of certain critical cytokines is the basis of the role played by T cells in DTH reactions.

2. You have a monoclonal antibody specific for LFA-1. You perform CML assays of a CTL clone, using target cells for which the clone is specific, in the presence and absence of this antibody. Predict the relative amounts of ^{51}Cr released in the two assays. Explain your answer.

3. You decide to co-culture lymphocytes from the strains listed in the table below in order to observe the mixed-lymphocyte reaction (MLR). In each case, indicate which lymphocyte population(s) you would expect to proliferate.

Population 1	Population 2	Proliferation
C57BL/6 (H-2^b)	CBA (H-2^k)	
C57BL/6 (H-2^b)	CBA (H-2^k) mitomycin C-treated	
C57BL/6 (H-2^b)	(CBA × C57BL/6) F_1 (H-$2^{k/b}$)	
C57BL/6 (H-2^b)	C57L (H-2^b)	

4. In the mixed-lymphocyte reaction (MLR), the uptake of [^{3}H]thymidine often is used to assess cell proliferation.

a. Which cell type proliferates in the MLR?
b. How could you prove the identity of the proliferating cell?
c. Explain why production of IL-2 also can be used to assess cell proliferation in the MLR.

5. Indicate whether each of the properties listed below is exhibited by T_H cells, CTLs, both T_H cells and CTLs, or neither cell type.

a. _____ Can make IFN-γ
b. _____ Can make IL-2
c. _____ Is class I MHC restricted
d. _____ Expresses CD8
e. _____ Is required for B-cell activation
f. _____ Is cytotoxic for target cells
g. _____ Is the main proliferating cell in an MLR
h. _____ Is the effector cell in a CML assay
i. _____ Is class II MHC restricted
j. _____ Expresses CD4
k. _____ Expresses CD3
l. _____ Adheres to target cells by LFA-1
m. _____ Can express the IL-2 receptor
n. _____ Expresses the αβ T-cell receptor
o. _____ Is the principal target of HIV
p. _____ Responds to soluble antigens alone
q. _____ Produces perforin
r. _____ Expresses the CD40 ligand on its surface

6. Mice from several different inbred strains were infected with LCM virus, and several days later their spleen cells were isolated. The ability of the primed spleen cells to lyse LCM-infected, ^{51}Cr-labeled target cells from various strains was determined. In the accompanying table, indicate with a (+) or (–) whether the spleen cells listed in the left column would cause ^{51}Cr release from the target cells listed in the headings across the top of the table.

Source of primed spleen cells	^{51}Cr release from LCM-infected target cells			
	B10.D2 (H-2^d)	B10 (H-2^b)	B10.BR (H-2^k)	(BALB/c × B10) F1 (H-$2^{b/d}$)
B10.D2 (H-2^d)				
B10 (H-2^b)				
BALB/c (H-2^d)				
BALB/c × B10 (H-$2^{b/d}$)				

7. A mouse is infected with influenza virus. How could you assess whether the mouse has T_H and T_C cells specific for influenza?

8. Explain why NK cells from a given host will kill many types of virus-infected cells but do not kill normal cells from that host.

9. Consider the following genetically altered mice and predict the outcome of the indicated procedures. H-2^d mice in which both perforin and Fas ligand have been knocked out are immunized with LCM virus. One week after immunization, T cells from these mice are harvested and tested for cytotoxicity on the following:

a. Target cells from normal LCM-infected H-2^b mice
b. Target cells from normal H-2^d mice
c. Target cells from H-2^d mice in which both perforin and Fas have been knocked out
d. Target cells from LCM-infected normal H-2^d mice
e. Target cells from H-2^d mice in which both perforin and FasL have been knocked out

10. You wish to determine the levels of class I–restricted T cells in an HIV-infected individual that are specific for a peptide that is generated from gp120, a component of the virus. Assume that you know the HLA type of the subject. What method would you use and how would you perform the analysis? Please be as specific as you can.