10 Cell-mediated cytotoxicity

- **Cytotoxic T cells recognize antigen presented on MHC molecules**. Most cytotoxic T cells are $CD8^+$ and recognize antigenic peptides presented on MHC class I.
- **Natural killer (NK) cells react against cells which do not express MHC class I.** They can interact with these cells using a variety of receptors.
- **NK cells express two major classes of inhibitory receptors for MHC molecules.** These are lectin-like receptors of the CD94 family and immunoglobulin superfamily molecules (KIRs).
- **Cytotoxicity is mediated by combinations of direct cell–cell interactions, cytokines and the release of granule proteins**. Fas ligand and tumour necrosis factors can signal apoptosis to the target cell. Granules containing perforin and granzymes contribute to target cell damage.
- **Ligation of Fas or the type-1 tumour necrosis factor (TNF) receptor on the target cell leads to the activation of caspases.** The caspases are the ultimate mediators of apoptosis in the target.
- **Myeloid cells induce damage in targets principally by the release of toxic molecules.** This is a reflection of their normal function of killing pathogens.

The last two chapters dealt with the types of immune reaction which are controlled by T helper (T_H) cells, namely antibody responses directed by T_H2 cells and cell-mediated immunity mediated by macrophages and T_H1 cells. This chapter is concerned with cytotoxicity, the ways in which leucocytes recognize and destroy other cells. Cell-mediated cytotoxicity is an essential defence against intracellular pathogens, including viruses, some bacteria and parasites. Tumour cells, eukaryotic pathogens and even cells of the body, may also become the target of cytotoxic cells. Addtionally, the process is important in the destruction of allogeneic tissue grafts. Several types of cell can execute this activity including T-cytotoxic (T_C) cells, NK cells and sometimes myeloid cells. The mechanisms of recognition and killing used by the lymphoid cells are quite distinct from those of the myeloid cells, and will be considered first.

Cytotoxic T cells and NK cells are complementary elements in the immune defence against virally infected cells

Cytotoxic T cells and NK cells recognize their targets in different ways (*Fig. 10.1*).

Cytotoxic T cells recognize specific antigens (e.g. viral peptides on infected cells) presented by MHC molecules. Most T_C cells are $CD8^+$ and recognize antigen presented on MHC class I, but about 10% of MHC-restricted cytotoxic T cells are $CD4^+$ and recognize antigen presented on class II molecules.

NK cells recognize cells which fail to express MHC class I molecules. These cells also use a variety of receptors to recognize their targets positively. For example, they can bind to antibody already attached to antigen on a target cell, using their Fc receptors (CD16): this is known as antibody-dependent cell-mediated cytotoxicity (ADCC), or killer cell (K cell) activity.

The most important role of T_C cells is the elimination of cells infected with virus (see Chapter 14). Nearly all nucleated cells express MHC class I molecules and if they become infected they can therefore present antigen to $CD8^+$ T_C cells. The detailed mechanisms of antigen presentation to Tc cells are discussed in Chapters 5 and 6 and are summarized briefly here. Cellular molecules that have been partly degraded by proteasomes are transported to the endoplasmic reticulum to become associated with MHC class I molecules and are then transported to the cell surface. Thus each cell samples its own molecules and presents them for review by $CD8^+$ T_C cells. Both the cell's own molecules and those of intracellular pathogens will be presented in this way.

Additional interactions may be required to stabilize the bond between the T_C cell and the target (*Fig. 10.2*), and can even help to trigger the killing event. For example, by adding antibodies against CD3 or CD2 on the Tc cell *in vitro*, it is possible to trigger the killing of target cells that are bound to the T_C cell. It is probable that binding of physiological ligands to these molecules can also trigger T_C cells in this way.

Recognition of target cells by Tc cells and NK cells

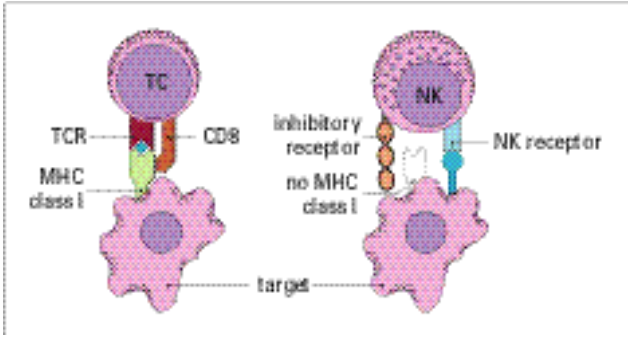

Fig. 10.1 Cytotoxic T cells recognize processed antigen presented on the target cell by MHC molecules using their T-cell receptor (TCR). Most Tc cells are $CD8^+$ and recognize antigen presented by MHc class I, but a minority are $CD4^+$ and recognize antigen presented by MHC class II. By contrast, NK cells have receptors that recognize MHC class I on the target and signal inhibition of cytotoxicity. They use a number of different receptors (NK receptors) to identify their targets positively for killing), including CD2, CD69, or antibody bound to their Fc receptor (CD16).

Interactions between Tc cells and target cells

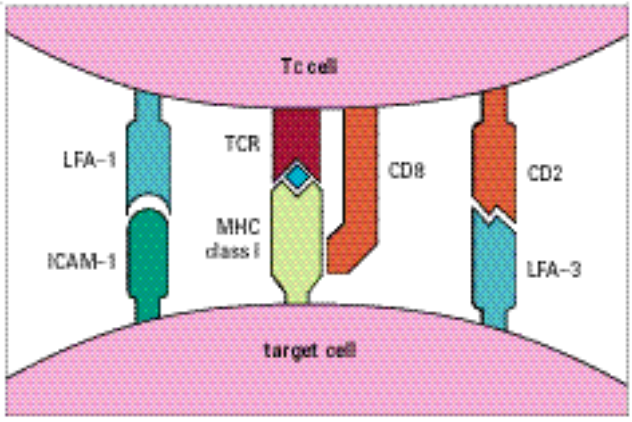

Fig. 10.2 Some of the ligands involved in the interaction between cytotoxic T cells and their targets.

Several viruses (particularly herpes viruses) have evolved mechanisms to avoid recognition by Tc cells. They reduce the expression of MHC molecules or even produce proteins which divert MHC molecules out of the endoplasmic reticulum. This reduces the likelihood that processed viral peptides will be presented at the cell surface. Because NK cells specifically recognize cells which have lost their MHC class I molecules, we can see that Tc cells and NK cells act in a complementary way to protect the body. In effect, the NK cells check that cells of the body are carrying their identity card (MHC class I) while the Tc cell checks the specific identity (antigen specificity) on that card.

Experiments involving the micromanipulation of individual cells have shown that a single cytotoxic T cell can kill several target cells sequentially. To function in this manner, Tc must be resistant to their own killing mechanisms and able to detach effectively from dying target cells.

Cytokine activated killer (LAK) cells are related to NK cells

Immunologists have experimented with several potential treatments for cancer. One approach has been to activate the patient's own lymphocytes *in vitro* with IL-2, and then to reinfuse them. These cells, which are initially derived from blood or spleen, are called cytokine activated killer cells or LAKs. Such cells show enhanced MHC non-restricted cytotoxicity, and they appear to be largely derived from precursors that are indistinguishable from NK cells. Thus LAK cells probably do not represent a separate lineage, but rather a consequence of activation. This type of cell is undergoing trials for the treatment of cancer in humans.

NATURAL KILLER CELL RECEPTORS

NK cells are mostly derived from 'large granular lymphocytes' (LGLs), which comprise about 5% of human peripheral blood lymphocytes. The majority of NK cells are $CD3^{-}CD16^{+}CD56^{+}CD94^{+}$ (see Appendix 2), and do not contain productive rearrangements of the T-cell receptor genes. Initial experiments on the specificity of NK cells showed that MHC class I expression protected cells from NK-cell mediated cytotoxicity and that particular allotypes of HLA-C were dominant genes for producing resistance. This lead to a search for receptors on NK cells which could inhibit cytotoxicity and which might be expressed in a variety of different forms, capable of reacting with MHC class I and signalling its presence to the NK cell. Two major types of molecules were identified and termed 'killer inhibitory receptors' or KIRs. One group of molecules was identified as type-2 membrane glycoproteins (C-terminus outside) with a C-type lectin domain (Ca^{2+}-dependent). The other group of molecules were members of the Ig superfamily. Subsequently, it was realized that while some members of each group did indeed act as killer inhibitory receptors, others could actually activate the killer cell. So, it was not correct to call all members of both groups 'inhibitory receptors'. Therefore it was proposed that the term 'KIR' now meaning 'killer immunoglobulin-like receptor', should be used for just the second group of molecules.

The lectin-like receptor CD94 interacts with HLA-E

The lectin-like receptor CD94 is a characteristic marker of human NK cells, but is also found on a subset of Tc cells. It covalently assembles with different members of another group of type-2 membrane molecules called NKG2, and the dimers are expressed on the cell membrane (*Fig. 10.3*). There are at least six members of the NKG2 family (NKG2A–NKG2F). The dimer of CD94-NKG2A is an inhibitory receptor, which blocks NK cell-mediated cytotoxicity. By contrast, CD94-NKG2C is an activating receptor. Although these two molecules are very similar, they differ in their intracellular segments, and this determines whether the receptor is inhibitory or activating (*Fig. 10.3*). *In vivo*, the role of the inhibitory version is clear, but that of the activating variant is not. Possibly, it may act as one of the receptors by which NK cells carrying CD94-NKG2C actively engage their targets.

CD94 is distantly related to the mouse molecule Ly-49, which is present in the NK cell gene complex (NKC). Indeed, it was partly this homology of CD94 to mouse NK cell receptors, that alerted researchers to the possibility that CD94 was an NK cell receptor in man.

HLA-E presents peptides from other MHC class I molecules

It is now known that the ligand for both CD94-NKG2A and CD94-NKG2C is the HLA-E molecule. The HLA-E gene locus encodes an MHC class I-like molecule. These are sometimes called class 1b molecules, to distinguish them from the classical MHC molecules which present antigen to Tc cells. The extraordinary function of HLA-E is to present peptides from other MHC class I molecules. The leader peptides from other MHC molecules are released in the endoplasmic reticulum, and these are required to stabilize functional HLA-E molecules (*Fig. 10.4*). Cells lacking classical MHC class I molecules do not express HLA-E at the cell surface. Hence an inhibitory signal is not passed to the NK cell. In effect HLA-E is a sensitive

Lectin-like receptors of NK cells

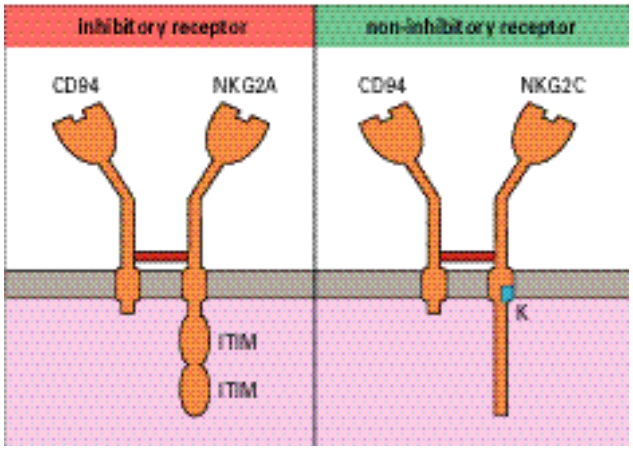

Fig. 10.3 The inhibitory receptors consist of the lectin-like CD94 disulphide bonded (red) to peptides from the NKG2 locus, such as NKG2A which have intracellular domains carrying ITIM motifs (immunoreceptor tyrosine inhibitory motif). The non-inhibitory receptors, such as CD94/NKG2C, lack ITIMs, but have a charged lysine (K) in the transmembrane segment which allows them to interact with signal transducing molecules.

HLA-E presents peptides of other MHC class I molecules

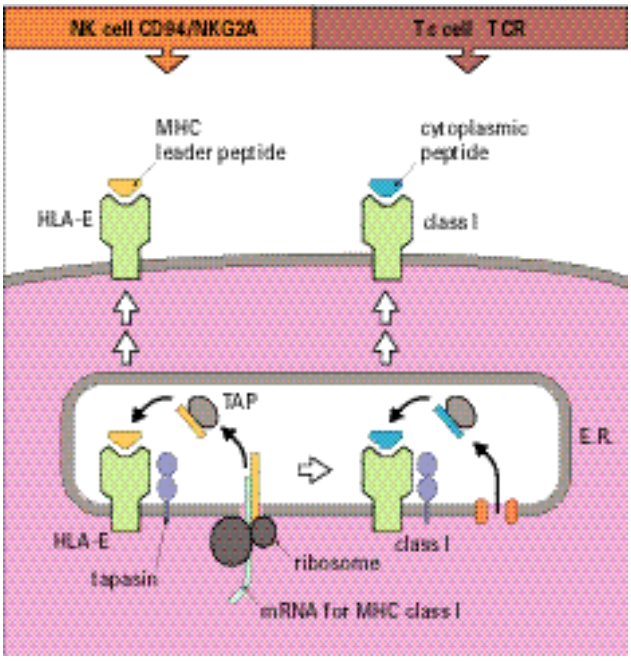

Fig. 10.4 Leader peptides from MHC class I molecules are loaded onto HLA-E molecules in the endoplasmic reticulum, a process which requires TAP transporters and tapasin to assemble functional HLA-E molecules. These are presented at the cell surface for review by the CD94 series of receptors on NK cells (left). The MHC class I molecules meanwhile present antigenic peptides from cytoplasmic proteins which have been transported into the endoplasmic reticulum. These complexes are presented to the TCR on $CD8^+$ cytotoxic T cells.

mechanism for monitoring whether viruses or tumours have downregulated MHC class I expression in a cell.

Although CD94 has a lectin domain, the recognition of the HLA-E/peptide occurs via interaction with residues from the peptide and the α_1 and α_2 domains of the MHC molecule. The lectin domain could however reinforce this interaction by binding to carbohydrate associated with the MHC molecule.

KIRs are members of the immunoglobulin superfamily

The second group of NK cell receptors are members of the immunoglobulin superfamily. They fall into two subsets, having either two or three Ig domains (*Fig. 10.5*). A set of approximately 12 such genes has been identified as a cluster on chromosome 19q13.4. The two-domain members (KIR2D) are defined as CD158, while the three-domain members (KIR3D) were originally described as p70, based on their molecular weights. Two of the two-domain members have been shown to bind to allelic variants of HLA-C, and these isoforms have inibitory cytoplasmic domains (see below). The specificity of these receptors can partly explain why particular HLA-C allotypes inhibited some NK cells, as described above. It also explains why these allotypes produce dominant resistance – if a cell expresses a particular HLA-C allotype, then that is sufficient to inhibit a K cell which has engaged it.

Other isoforms of the KIRs have activating domains. These generally engage HLA molecules with a lower affinity than the inhibitory forms, and like the activating forms of CD40-NKG2, their physiological functions are uncertain.

There is also a third group of receptors clustered in the same region as the KIRs, which have either two or four

Killer immunoglobulin-like receptors

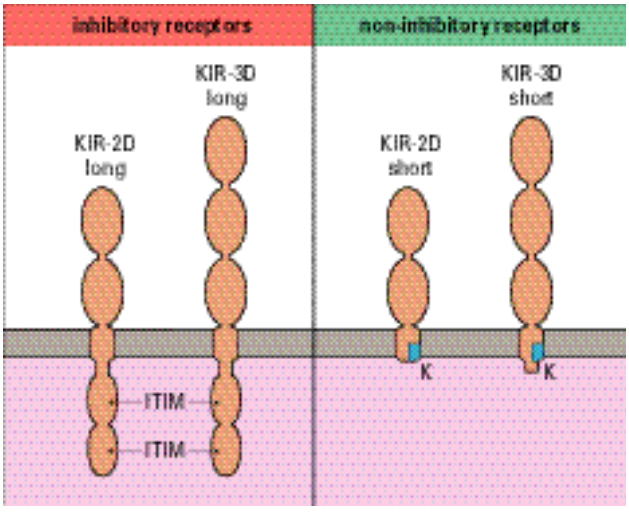

Fig. 10.5 These receptors consist of either two or three extracellular Ig superfamily domains. The inhibitory forms are longer and have intracellular ITIMs, while the non-inhibitory forms have the charged residue in the membrane comparable to the non-inhibitory forms of CD94/NKG2.

Ig-like domains. They are called immunoglobulin-like transcripts or ILTs and they have a wider cellular distribution than the other NK cell receptors, Some of these interact with a broad spectrum of MHC molecules, and others with none at all, hence their functions are still uncertain.

HLA-G inhibits NK cell action against the placenta

An intriguing recent discovery is that the HLA-G molecule, which is expressed only on placental trophoblasts, is a dominant NK-cell inhibitor that confers resistance to all types of NK cells (HLA-G is another MHC class 1b molecule). Trophoblast cells are derived from the fetus and invade the maternal circulation as the placenta is established. They are therefore allogeneic in the mother, because they contain paternal MHC genes. However, all conventional MHC genes are downregulated in these cells, and the HLA-G expression is therefore required to protect the placenta from attack by NK cells. There is some debate as to which of the inhibitory receptors recognize HLA-G. Both CD94 and several of the better-defined KIRs have been excluded, but a likely candidate is ILT2.

NK cells and K cells use several different receptors to positively identify their targets

NK cells may engage their targets using a variety of receptors, including CD2, CD16, CD69 and receptors related to those which inhibit cytotoxicity. The Fc receptor, CD16, binds antibody bound to target cells and mediates ADCC (*Fig. 10.6*). It is customary to refer to this as killer (K) cell activity, but this function may also be performed by several other cell types with Fc receptors, including T cells. Myeloid cells expressing Fc receptors can also show K-cell activity, but probably use killing mechanisms different from those of T cells and NK cells (discussed later).

Potential targets for K-cell action include viral antigens on cell surfaces, MHC molecules and some epitopes present on tumours. Thus monocytes and (according to some controversial reports) polymorphs may also be active against antibody-coated tumour targets. Some myeloid cells (monocytes and eosinophils) are certainly important effectors of damage to antibody-coated schistosomulae (see Chapter 18).

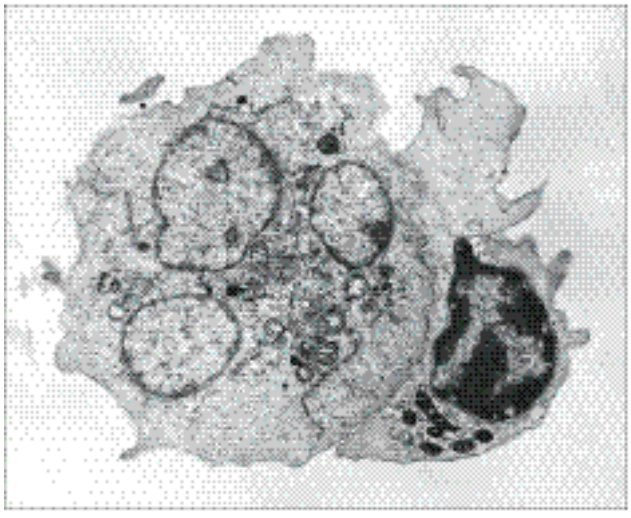

Fig. 10.6 K-cell activity. Electron micrograph of a lymphocyte (right) engaging a target cell sensitized with antibody (left). × 2500. (Courtesy of Dr P. Penfold.)

Intracellular signalling pathways coordinate inhibitory and activating signals

The next question is how a NK cell decides between cytotoxic action or inaction. This decision is thought to depend on the coordination of intracellular signalling pathways, and may involve the balance between activating and inhibitory signals. Both the lectin-like receptor and the KIRs occur as inhibitory or activating molecules. The key difference is the presence of 'immunoreceptor tyrosine inhibitory motifs' or ITIMs. If these motifs become phosphorylated, they can recruit phosphatases which downregulate the activity of the NK cell (*Fig. 10.7*).

By contrast, other KIRs which lack the ITIMs can associate with a molecule (DAP12) which is related to the ζ chain of the T-cell receptor complex. This molecule has 'immunoreceptor tyrosine activation motifs' (ITAMs), which allow it to phosphorylate and recruit tyrosine kinases including ZAP-70 (*Fig. 10.7*, and compare with figure 6.21), which lead to cell activation. At present, it is not known how the balance of activation and inhibition is resolved. In particular it is uncertain how a ligand such as HLA-C will act when it can bind to both inhibitory and activating receptors on the same cell.

Activation and inhibition by NK cell receptors

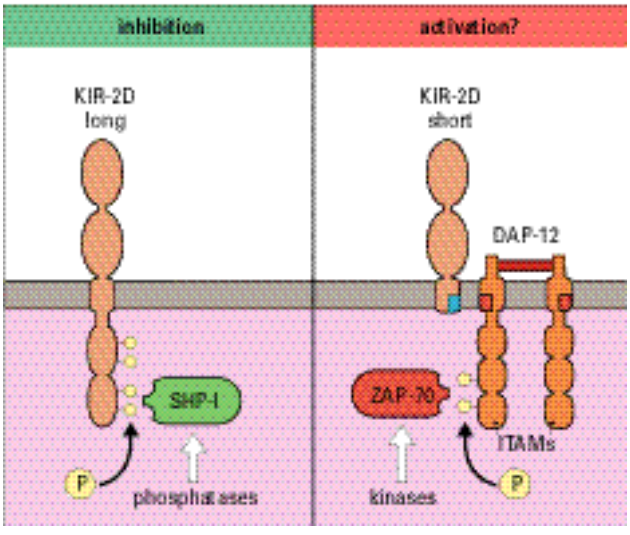

Fig. 10.7 Following phosphorylation of its ITIMs, the inhibitory receptors of NK cells can bind to phosphatases, including SHP-1 and SHP-2, which inhibit killing. The non-inhibitory forms of the receptor associate with a dimeric molecule DAP12, via the complementary charged residues in their membanes. DAP12 has activation motifs (ITAMs). When phosphorylated this recruits kinases of the syk family or ZAP-70 (cf. T-cell activation, Chapter 6). Whether this leads to NK cell activation, or whether it modulates the inhibitory signals is not known.

MECHANISMS OF CYTOTOXICITY

Cytotoxicity is effected by direct cellular interactions, cytokines and granule exocytosis

Cytotoxic T cells, NK cells and K cells use a variety of different mechanisms to kill their targets. These include direct cell–cell signalling via surface molecules and indirect signalling via cytokines. In addition, many $CD8^+$ cytotoxic T cells and large granular lymphocytes (NK cells and K cells) have granules which contain proteins that damage target cells if they are released directly against the target cell plasma membrane. Exactly which combination of these three mechanisms is used depends on the Tc cell involved.

Cytotoxic T cells and NK cells induce apoptosis in their targets

Cells can die in two principle ways – by necrosis or apoptosis. Apoptosis is a highly ordered process in which cells are systematically disassembled. The cells detach from their neighbours and the cytoplasm and nucleus condense. Mitochondria lose their membrane potential and leak cytochrome c into the cytoplasm. As the chromatin condenses, it is cleaved into regular-sized fragments by endonucleases. Finally the cell membrane starts to form blebs and the cell may fragment into condensed apoptotic bodies. Dead cells attract mononuclear phagocytes and are rapidly taken up by phagocytosis to be broken down in phagolysomes. Apoptosis is a feature of normal physiology. For example, T cells which fail thymic selection die by apoptosis (see Chapters 2, 12), as do B cells which are not selected during affinity maturation in germinal centres (see Chapter 8). This type of cell death also occurs extensively during organ development, particularly in the central nervous system. In each case the final stages of cell death involve the activation of a group of enzymes called caspases, but the events which trigger apoptosis are different for each process.

By comparison necrosis is a less orderly event, in which dying cells fall apart releasing their contents. This tends to provoke macrophage activation and inflammation. Another difference is that apoptosis requires energy (ATP) while necrosis does not. The damage caused by pathogens and the colateral damage caused by granulocytes and macrophages acting against them induce cell death by necrosis.

Caspases mediate cell death by apoptosis

Caspases are a group of proteases which have the unusual property of cleaving their substrates on the C-terminal side of an aspartate residue. More than 10 caspases have been identified in man. They are produced in a pro-enzyme form and become activated by cleavage into two or three subunits. The caspases have very wide-ranging effects within a cell. They can affect cell structure, intracellular signalling, cell cycle control, DNA integrity and repair, and intercellular adhesion. Studies with caspase gene knockout mice have shown that different caspases are associated with particular forms of apoptosis. For example, caspase-3-deficient mice die shortly after birth due to a failure of normal CNS development, but the mechanisms of cell death induced by cytotoxic cells are unaffected. Notably, however, caspase-8-deficient mice are not susceptible to cell killing by the mechanisms outlined below.

Cytotoxicity may be signalled via Fas or a TNF receptor on a target cell

Cytotoxic T cells signal to their targets using members of the TNF receptor group of molecules. These include Fas (CD95) and the type 1 TNF receptor, TNFR-1, which are widely distributed in the body (*Fig. 10.8*). Other members of the group are CD30 and CD40 which are involved in lymphocyte differentiation The ligand for Fas (FasL) is expressed on mature $CD4^+$ and $CD8^+$ T cells after activation. Ligation of Fas induces trimerization of the Fas molecules on the cell surface, which causes them to associate with a transducing molecule which recruits and activates caspases 8 or 10 (*Fig. 10.9*). Note that cell killing mediated by Fas also occurs as part of the normal processes of lymphocyte selection, during development. For cytotoxic lymphocytes which lack granules the Fas pathway is thought to be the principle means of signalling to the target.

Most $CD8^+$ Tc cells, NK cells (and macrophages) have vesicles containing TNF and lymphotoxin which can be released onto a target cell. TNF acts in a very similar way to the Fas ligand. It causes trimerization of the TNFR-1 so that the receptor associates with adaptor proteins which recruit caspases. Both TNFR-1 and Fas contain intracytoplasmic domains (death domains) which are found on a number of proteins involved in cell survival. (Note however that a different form of TNF receptor, TNFR-2, lacks these intracytoplasmic segments and therefore does not transduce signals for apoptosis.)

TNF receptor family molecules

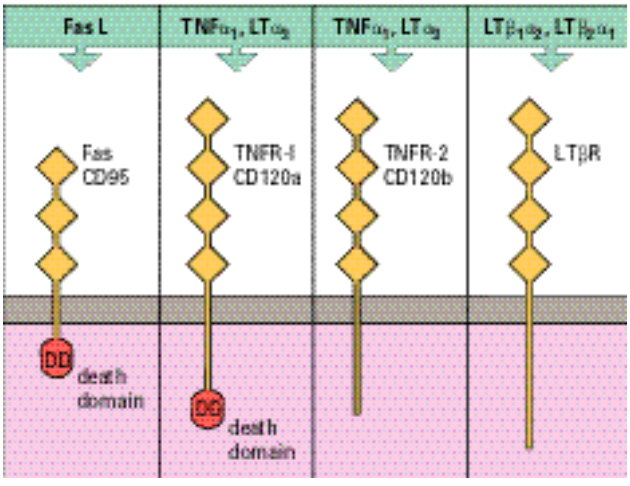

Fig.10.8 The molecule Fas (CD95), the two TNF receptors and the lymphotoxin receptor are illustrated diagramatically. The extracellular domains are similar to those found in the NGF receptor. Both Fas and TNFR-1 have death domains which are involved in the recruitment of caspases. The ligands for these receptors are indicated at the top. Lymphotoxin-α can form homotrimers or heterotrimers with lymphotoxin-β. Up to 25 other members of these families have been identified by data-base searching.

Mechanisms of cell killing

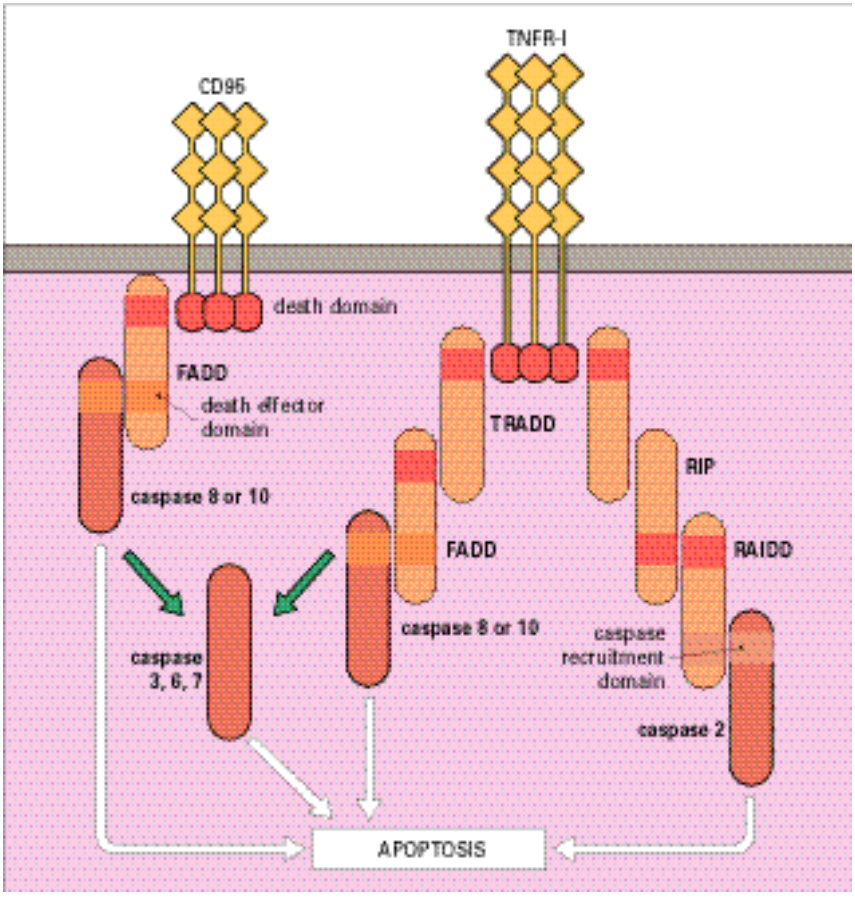

Fig. 10.9 Ligation of CD95 or TNFR-1 causes trimerization of the receptors. Death domains in the cytoplasmic portion of CD95 bind to the adaptor protein FADD (=MORT-1), which recruits caspase 8 or 10. TNFR-1 can activate either caspase 8 or 10, via TRADD and FADD, or caspase 2 via RIP and RAIDD. Caspase 8 can further activate other caspases, and these in concert lead to apoptosis of the target cell.

Activated caspase 8 can cleave and activate other caspases, in addition to its own direct actions in the pathways of apoptosis (*Fig. 10.9*).

Granules of cytotoxic T cells contain perforin and granzymes

It was originally thought that all cytotoxicity was caused by the release of granule proteins onto the target cell. Indeed the processes described above were only identified when it was realized that cells which lack granules could still kill targets. The specific granules of NK cells and Tc cells contain several proteins, including perforin and granzymes (granule-associated enzymes). After binding to its target, the Tc cell directs its granules towards the membrane adjoining the target. Then, in a Ca^{2+}-dependent phase, the granule contents are discharged into the cleft between the two cells. This process can be seen in time-lapse video microscopy (*Fig. 10.10*).

Perforin is a monomeric pore-forming protein that is related both structurally and functionally to the complement component, C9. The vesicles also contain a serine esterase that may be involved in the assembly of the lytic complex. In the presence of Ca^{2+}, the perforin monomers bind to the target cell membrane and polymerize to form transmembrane channels. Although in close contact with the perforin, the Tc cell survives and can continue to kill further targets. It is thought to be protected from autodestruction by a proteoglycan (chondroitin sulphate A) which is also present in the vesicles, and which may bind to and inactivate the perforin. Perforin-knockout mice have Tc cells which display reduced but still functional cytotoxicity, implying that perforin cannot be the only mechanism used by these cells.

Granzymes are a collection of serine esterases (enzymes) which are also released upon granule exocytosis and become active after release. They are not essential for cytotoxicity, as cells lacking granzymes may still be cytotoxic. Some of the granzymes may interact with intracellular pathways in the target cell to activate mechanisms which trigger apoptosis and DNA degradation. In fact, it is notable that granzyme B has the same unusual specificity as the caspases (see above). In order to activate apoptosis pathways in the target cell, the granzymes need to gain access to the cytoplasm. It has been proposed that perforin and granzymes act synergistically; the granzymes enter the target cell via pores created by perforin. The ways in which granule proteins can contribute to cytotoxicity are shown in *Figure 10.11*.

To summarize, $CD8^+$ Tc cells use both FasL and granule release to kill their targets, $CD4^+$ Tc cells use principally FasL, and NK cells use primarily their granules. TNF may contribute to the cytotoxic damage produced by any of these cells.

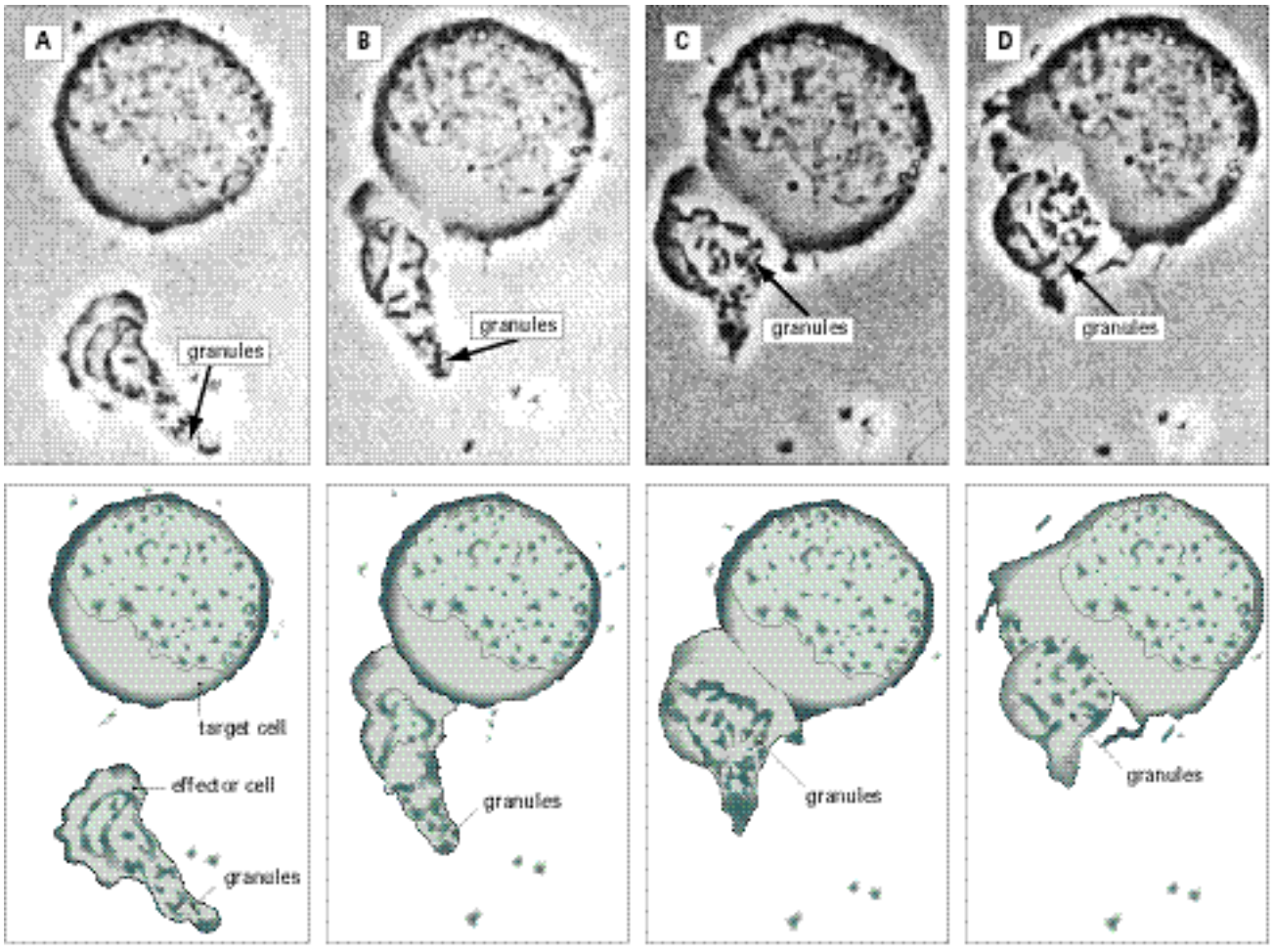

Fig. 10.10 Intracellular reorganizations during effector–target cell interaction. Early events in the interaction of Tc with specific targets were studied with high-resolution cinematographic techniques. Four frames (together with interpretative drawings) are shown, taken at different times, of a Tc interacting with its target. The location of the granules within the effector cell is indicated in each case. Before contact with the target (a), the effector had granules located in a uropod at the rear, and was seen to move randomly by extending pseudopods from the organelle-free, broad leading edge of the cell. Within 2 minutes of contacting the target (b), the Tc had begun to round up and initiate granule reorientation (c). After 10 minutes (d), the granules occupied a position in the zone of contact with the target, where they appear to be in the process of emptying their contents into the i ntercellular space between the two cells. (Courtesy of Dr V.H. Engelhard.)

Granule-associated killing mechanisms

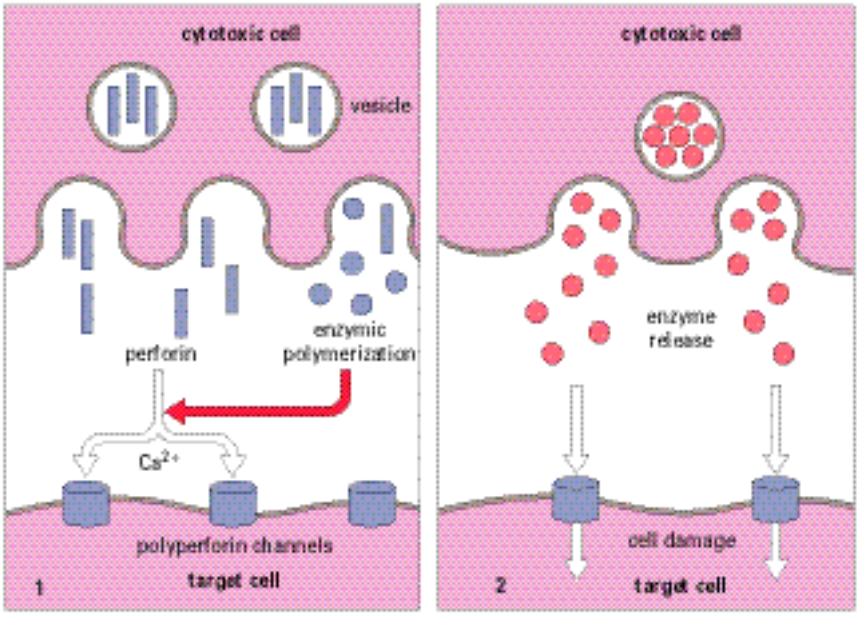

Fig. 10.11 The cytotoxic lymphoid cell degranulates, releasing perforin and various enzymes (granzymes) into the immediate vicinity of the target cell membrane. In the presence of Ca^{2+} there is enzymic polymerization of the perforin to form polyperforin channels on the target cell (**1**). Enzymes which activate the apoptosis pathways, degradative enzymes or other toxic substances released from the cytotoxic cell may pass through the channels on the target and cause cell damage or killing (**2**).

NON-LYMPHOID CYTOTOXIC EFFECTORS

Macrophages can damage targets using their non-specific toxic effector systems or via cytokines

A number of non-lymphoid cells may be cytotoxic to other cells or invading microorganisms, such as bacteria or parasites. Cytotoxicity may be triggered specifically to a target by antibody-dependent cell mediated cytotoxicity (ADCC) or may involve a range of non-specific toxic mediators. For example macrophages and neutrophils both express FcγRI and FcγRII which allows them to engage tumours by ADCC.

In general, macrophages and neutrophils aim to destroy pathogens by internalizing them and subjecting them to toxic molecules and enzymes within the phagolysosome. These include the production of reactive oxygen intermediates, toxic oxidants and NO detailed in Chapter 9, as well as the secreted molecules such as neutrophil defensins, lysosomal enzymes and cytostatic proteins. If the phagocyte fails to internalize its target, then these mediators may be released into the extracellular environment and contribute to localized cell damage. This action is referred to as 'frustrated phagocytosis', and occurs when the target is engaged by surface receptors, but is too large to phagocytose. The actions of the mediators produced by the phagocyte damage the target, rather than induce apoptosis. For this reason, the cytotoxic action of these cells tends to produce necrosis and inflammation.

Nevertheless, in the case of macrophages, activated cells secrete TNF which can induce apoptosis in a similar way to NK cells and Tc cells. For this reason, macrophages can induce necrosis, apoptosis or a combination of both, depending on the state of activation of the macrophages and the target involved. The mechanisms by which myeloid cells produce cytotoxic damage are shown in *Figure 10.12*

Mechanisms which may contribute to the cytotoxicity of myeloid cells

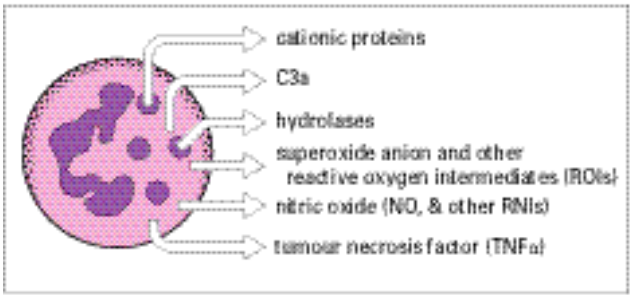

Fig. 10.12 Reactive oxygen intermediates (ROIs) and reactive nitrogen intermediates (RNIs), cationic proteins, hydrolytic enzymes and complement proteins released from myeloid cells may damage the target cell in addition to cytokine-mediated attack.

Eosinophils mediate cytotoxicity by exocytosis of their granules

Mature eosinophils are characterized by their granules, which have a crystalloid core which binds the dye eosin. Generally, eosinophils are only weakly phagocytic; they ingest some bacteria following activation, but are less efficient than neutrophils at intracellular killing. Their major function appears to be the secretion of various toxic granule constituents, following activation. They are therefore effective for the extracellular killing of microorganisms, particularly large parasites such as schistosomes (see Chapter 16).

The components of the eosinophil granule include major basic protein (MBP), eosinophil peroxidase (EPO) and eosinophil cationic protein (ECP). Major basic protein (not to be confused with myelin basic protein of oligodendrocytes) is the major component of eosinophil granules, forming the crystalloid core. It has been shown to damage, and sometimes kill, parasites, but also damages host tissue cells. Eosinophil peroxidase is a highly cationic heterodimeric 71–77-kDa haemoprotein, which is distinct from the myeloperoxidase of neutrophils and macrophages. In the presence of H_2O_2, also produced by eosinophils, EPO will oxidize a variety of substrates, including halide ions to produce hypohalite. Indeed, this may represent the eosinophils' most potent killing mechanism for some parasites. ECP is an eosinophil-specific toxin which is very potent at killing many parasites, particularly the schistosomulae of *Schistosoma mansoni*. The molecule is a ribonuclease, which because of its high charge, binds avidly to negatively charged surfaces. It is possible that it forms membrane channels, which allow other mediators access to the target organism. Other molecules produced by eosinophils are lysophospholipase and eosinophil-derived neurotoxin (EDN), which is also a ribonuclease but with strong neurotoxic activity.

Degranulation of eosinophils can be triggered in a number of ways. Binding to IgG-coated parasites via surface FcγRII triggers release of some mediators including ECP, but not EPO. By contrast, triggering via FcεRII leads to the release of EPO, but not ECP. Parasite killing may involve contact-dependent degranulation or may simply require deposition of toxins within the local tissue. Degranulation may also be triggered directly *in vitro* by several cytokines, including IL-3, IL-5, granulocyte-macrophage–colony-stimulating factor (GM-CSF), TNF, interferon-β (IFNβ) and platelet activating factor (PAF). These mediators also enhance ADCC-mediated degranulation. Eosinophils are prominent in the inflammatory lesion of a number of diseases, particularly atopic disorders of the gut, skin and respiratory tract, where they are often closely associated with fibrotic reactions. Examples are atopic exzema, asthma and inflammatory bowel disease. Although eosinophils may play some regulatory role in these conditions, such as inactivating histamine, their toxic products and cytotoxic mechanisms are a major cause of the tissue damage. For example, in asthma, eosinophil granule proteins are detectable in the blood and lungs following asthmatic attacks. MBP can kill some pneumocytes and tracheal epithelial cells while EPO kills type II pneumocytes. MBP can also induce mast cells to secrete histamine, thus exacerbating allergic inflammation.

CRITICAL THINKING • Mechanisms of cytotoxicity

Lymphocytes from a normal individual were stimulated in vitro by coculture with irradiated T lymphoma cells. (Irradiation of these stimulator cells prevents them from dividing in the coculture.) After 7 days the lymphocytes were harvested and fractionated to obtain a population of cytotoxic cells ($CD8^+$), and a population of NK cells ($CD94^+$, $CD16^+$). These effector cells were set up in a cytotoxicity assay with the tumour cells as targets. The tumour cells were labelled, in order to detect both DNA fragmentation and cell lysis. The following results were obtained in these assays.

10.1 Why do the Tc cells lyse the targets and induce DNA fragmentation? What are these cells recognizing on the tumour cell surface?

10.2 Why might the NK cells cause some damage to the tumour? Why does the presence of antibody to the tumour enhance the cytotoxic capacity of the NK cells?

10.3 Explain the result with purified perforin.

Treatment	Lysis	DNA fragmentation
No effector cells	4%	1%
$CD8^+$ Tc cells	82%	80%
NK cells	12%	11%
Anti-tumour cell antibody	5%	1%
NK cells + anti-tumour cell antibody	28%	28%
Purified perforin	95%	2%

FURTHER READING

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