

An effector caspase is:

- A. Caspase 1
- B. Caspase 2
- C. Caspase 3
- D. Caspase 4
- E. Caspase 5

Answer: C. Caspase 3

Reference: Elmore S. Toxicol Pathol 35:495-516, 2007

Submitter: Ackermann

Their research suggests that DNA repair is activated early in the *p53*-induced apoptotic process and that this DNA repair may be involved in reversing the cell death pathway in some circumstances.

### Mechanisms of Apoptosis

The mechanisms of apoptosis are highly complex and sophisticated, involving an energy-dependent cascade of molecular events (Figure 3). To date, research indicates that there are two main apoptotic pathways: the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway. However, there is now evidence that the two pathways are linked and that molecules in one pathway can influence the other (Igney and Krammer, 2002). There is an additional pathway that involves T-cell mediated cytotoxicity and perforin-granzyme-dependent killing of the cell. The perforin/granzyme pathway can induce apoptosis via either granzyme B or granzyme A. The extrinsic, intrinsic, and granzyme B pathways converge on the same terminal, or execution pathway. This pathway is initiated by the cleavage of caspase-3 and results in DNA fragmentation, degradation of cytoskeletal and nuclear proteins, cross-linking of proteins, formation of apoptotic bodies, expression of ligands for phagocytic cell receptors and finally uptake by phagocytic cells. The granzyme A pathway activates a parallel, caspase-independent cell death pathway via single stranded DNA damage (Martinvalet et al., 2005).

**Biochemical Features:** Apoptotic cells exhibit several biochemical modifications such as protein cleavage, protein cross-linking, DNA breakdown, and phagocytic recognition that together result in the distinctive structural pathology described previously (Hengartner, 2000). Caspases are widely expressed in an inactive proenzyme form in most cells and

once activated can often activate other pro-caspases, allowing initiation of a protease cascade. Some pro-caspases can also aggregate and autoactivate. This proteolytic cascade, in which one caspase can activate other caspases, amplifies the apoptotic signaling pathway and thus leads to rapid cell death.

Caspases have proteolytic activity and are able to cleave proteins at aspartic acid residues, although different caspases have different specificities involving recognition of neighboring amino acids. Once caspases are initially activated, there seems to be an irreversible commitment towards cell death. To date, ten major caspases have been identified and broadly categorized into initiators (caspase 2, 8, 9, 10), effectors or executioners (caspase 3, 6, 7) and inflammatory caspases (caspase 1, 4, 5) (Cohen, 1997; Rai et al., 2005). The other caspases that have been identified include caspase-11, which is reported to regulate apoptosis and cytokine maturation during septic shock, caspase-12, which mediates endoplasmic-specific apoptosis and cytotoxicity by amyloid- $\beta$ , caspase-13, which is suggested to be a bovine gene, and caspase-14, which is highly expressed in embryonic tissues but not in adult tissues (Hu et al., 1998; Nakagawa et al., 2000; Koenig et al., 2001; Kang et al., 2002).

Extensive protein cross-linking is another characteristic of apoptotic cells and is achieved through the expression and activation of tissue transglutaminase (Nemes et al., 1996). DNA breakdown by  $\text{Ca}^{2+}$ - and  $\text{Mg}^{2+}$ -dependent endonucleases also occurs, resulting in DNA fragments of 180 to 200 base pairs (Bortner et al., 1995). A characteristic "DNA ladder" can be visualized by agarose gel electrophoresis with an ethidium bromide stain and ultraviolet illumination.

Another biochemical feature is the expression of cell surface markers that result in the early phagocytic recognition of apoptotic cells by adjacent cells, permitting quick

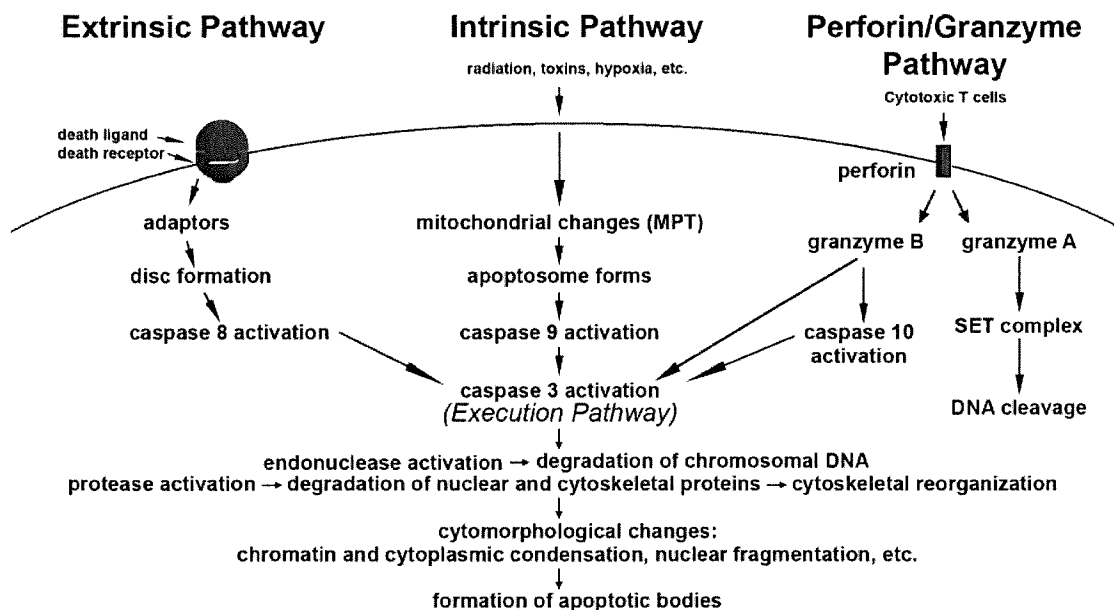


FIGURE 3.—Schematic representation of apoptotic events. The two main pathways of apoptosis are extrinsic and intrinsic as well as a perforin/granzyme pathway. Each requires specific triggering signals to begin an energy-dependent cascade of molecular events. Each pathway activates its own initiator caspase (8, 9, 10) which in turn will activate the executioner caspase-3. However, granzyme A works in a caspase-independent fashion. The execution pathway results in characteristic cytomorphological features including cell shrinkage, chromatin condensation, formation of cytoplasmic blebs and apoptotic bodies and finally phagocytosis of the apoptotic bodies by adjacent parenchymal cells, neoplastic cells or macrophages.

Preformed mediators include all EXCEPT:

- A. IL-1
- B. Serotonin
- C. Histamine
- D. Bradykinin
- E. Substance P

Answer: A. IL-1

Reference: McGavin and Zachary, Chap 3, pg. 122

Submitter: Ackermann

(free and fixed macrophages), spleen (free and fixed macrophages), bone marrow (fixed macrophages), serous fluids (pleural and peritoneal macrophages [free macrophages]), and skin (histiocytes [fixed macrophages]). As indicated, during inflammatory conditions monocytes are recruited by inflammatory mediators, such as chemokines, to leave the circulatory system, enter sites of inflammation, and become activated macrophages. This process can occur virtually anywhere in the body and often sets the stage for the development of chronic inflammation.

Functionally, macrophages are a component of the innate immune system in terms of their role in phagocytosis and cytokine release during the acute inflammatory response. However, macrophages are one of the main triggers of the adaptive immune response because of their ability to process and present antigen and regulate T-cell activity. Monocytes, macrophages, dendritic cells, and other cell types of the chronic inflammatory response will be discussed in greater detail in Chapter 4.

### CHEMICAL MEDIATORS OF THE ACUTE INFLAMMATORY RESPONSE

Chemical mediators of the acute inflammatory response (Fig. 3-3, Table 3-3, Appendix 3-1) are often produced as preformed or synthesized molecules in the liver and in neutrophils, basophils, macrophages/monocytes, platelets, mast cells, endothelial cells, smooth muscle cells, fibroblasts, and most epithelial cells. Preformed molecules, such as histamine are transcribed, translated, processed, and stored, often in granules or vacuoles within inflammatory cells. They can be released immediately upon cellular activation and are therefore active in seconds. Other molecules, such as most cytokines, adhesion molecules, and prostaglandins, are largely synthesized after an inflammatory cell becomes activated. Endothelial cells, for example, often express low, basal levels of the adhesion molecule ICAM-1, but after the cell becomes activated (by cytokines such as IL-1), they rapidly transcribe the ICAM-1 gene generating ICAM-1 mRNA that is translated into ICAM-1 protein, which is processed, transported, and expressed on the cell surface. This process is somewhat rapid, resulting in ICAM-1 expression within hours; however, it is not nearly as rapid as the release of histamine. Inflammatory mediators originating from plasma proteins, such as kinin, and the coagulation and complement system proteins are constantly secreted by the liver in precursor forms that must be activated via proteolytic cleavage in the circulatory system to their active forms; however, once the proteolytic cleavage is initiated, kinin and complement activity is immediate, similar to histamine.

Inflammatory mediators, whether preformed, synthesized, or derived from plasma, generally bind to receptors on target cells and often activate target cells or cause the target cell to secrete additional inflammatory mediators. In the latter case, the mediators may amplify or suppress secretion by target cells of additional mediators. Once activated and released or secreted, most inflammatory mediators:

- Have short half-lives and quickly decay
- Are enzymatically destroyed by kinases
- Are scavenged by protective mechanisms, such as antioxidants
- Are blocked by endogenous inhibitors such as complement inhibitors

This arrangement provides a check and balance system on the severity of the acute inflammatory response and also can be exploited in the development of drugs to inhibit excessive inflammatory responses. Inflammatory mediators, if excessively unregulated, have the potential to cause severe injury to tissue in and surrounding the acute inflammatory response.

Preformed inflammatory proteins include histamine, serotonin, bradykinin, and tachykinins (substance P and neurokinins). These substances are released during the initial phases of the acute inflammatory response. Mast cells and basophils are the principal sources of histamine and serotonin. Bradykinin is released by leukocytes and vascular endothelial cells, and substance P is released by mast cells, basophils, and C-reactive (sensory) nerve fibers. As indicated, the mediators are rapidly active (in seconds to minutes) and contribute to increased vascular permeability that lasts from minutes to hours.

Histamine rapidly enhances vascular permeability and is one of the earliest recognized mediators of inflammation. Experiments by Sir Thomas Lewis in 1927 and Dale and Laidlaw in 1911 indicated the potential role of histamine and other local mediators in acute inflammation. Histamine is derived from the amino acid histidine through the action of histidine decarboxylase. This enzyme catalyzes the decarboxylation of histidine to histamine and carbon dioxide. Histamine is stored (preformed) in granules of mast cells, basophils, and platelets.

As a mediator of inflammation, major effects of histamine are: (1) vasodilation (active hyperemia) via both  $H_1$  and  $H_2$  receptors, (2) increased microvascular permeability via predominantly the  $H_1$  receptor, (3) neural reflexes, vagal reflexes, bronchial constriction, (4) release of  $PGF_{2\alpha}$ , (5) pain and itching, (6) tachycardia, and (7) eosinophil chemotaxis. The acute vascular effects of histamine are immediate (within minutes) and transient (last about 30 to 90 minutes). Whether histamine has a role in chronic inflammation is speculative, but it may act to modulate the inflammatory response and the reactivity of various leukocytes, including lymphocytes.